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Derivation of human Biomonitoring Guidance Values for chlorpyrifos using a physiologically based pharmacokinetic and pharmacodynamic model of cholinesterase inhibition

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ABSTRACT

A number of biomonitoring surveys have been performed for chlorpyrifos (CPF) and its metabolite (3,5,6-trichloro-2-pyridinol, TCPy); however, there is no available guidance on how to interpret these data in a health risk assessment context. To address this gap, Biomonitoring Guidance Values (BGVs) are developed using a physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model. The PBPK/PD model is used to predict the impact of age and human variability on the relationship between an early marker of cholinesterase (ChE) inhibition in the peripheral and central nervous systems [10% red blood cell (RBC) ChE inhibition] and levels of systemic biomarkers. Since the PBPK/PD model characterizes variation of sensitivity to CPF in humans, interspecies and intraspecies uncertainty factors are not needed. Derived BGVs represent the concentration of blood CPF and urinary TCPy associated with 95% of the population having less than or equal to 10% RBC ChE inhibition. Blood BGV values for CPF in adults and infants are 6100 ng/L and 4200 ng/L, respectively. Urinary TCPy BGVs for adults and infants are 2100 μg/L and 520 μg/L, respectively. The reported biomonitoring data are more than 150-fold lower than the BGVs suggesting that current US population exposures to CPF are well below levels associated with any adverse health effect.

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1. Introduction

Chlorpyrifos (CPF) (*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate) is an insecticide used to control a broad-spectrum of insect pests. CPF is not persistent in the environment and does not bioaccumulate. The compound is metabolized in both insects and mammals (USEPA, 2011). CPF was first registered in the

United States (US) in 1965 and it is used in over 100 countries to protect more than 50 different crop types against insect pests. Registered uses of CPF in the US include food crops such as fruit and nut trees and many fruits, vegetables, and grains. Non-food crop applications include forage, golf course turf, industrial sites, greenhouse and nursery production, sod farms, and wood products (USEPA, 2011). Because CPF was withdrawn from residential use in the US in 2001, the primary route of chlorpyrifos exposure to the general population is via dietary residues (USEPA, 2011).

Human exposure to CPF has been investigated in multiple biomonitoring studies of the general population and occupationally exposed subpopulations. These studies generally employed measurements of the parent CPF in blood or its major metabolite, 3,5,6-trichloro-2-pyridinol (TCPy), in urine (Alexander et al., 2006; Barr et al., 2005; Centers for Disease Control and Prevention (CDC), 2009; Farahat et al., 2011; Garabrant et al.,

2009; Thomas et al., 2010). However, methods for relating measurements of blood CPF and urinary TCPy to levels associated with potential health effects have been lacking.

This manuscript describes a novel methodology that uses a physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model to derive Biomonitoring Guidance Values (BGVs) for measurements of blood CPF and urinary TCPy. The BGVs represent the blood CPF and urinary TCPy levels predicted to occur in sensitive individuals exposed to an oral acute dose that results in 10% red blood cell (RBC) cholinesterase (ChE) inhibition (the early marker of ChE inhibition in the peripheral and central nervous systems). The BGVs are then compared to actual CPF biomonitoring data from a number of studies and margins of safety (MOSs) are generated. The MOSs can then be used to inform whether populations are at potential risk.

2. Background

2.1. CPF metabolism

Oral doses of CPF are well absorbed and undergoe extensive first pass metabolism (Bakke et al., 1976; Smith et al., 1967; Nolan et al., 1984, 1987; Timchalk et al., 2006; Griffin et al., 1999). When CPF enters the body it is initially metabolized by cytochrome (CYP) P450s, mainly in the liver, to primarily TCPy or diethylphosphate (DEP) metabolites or to the trace-level, short-lived CPF-oxon metabolite. Minor CYP metabolism also occurs in the intestine, lung, skin, and brain (Poet et al., 2014b), and CPF is known to irreversibly inhibit CYP P450s (Eaton et al., 2008). The low levels of CPF-oxon that are formed are rapidly metabolized to TCPy in both the liver and circulating blood (Fig. 1) by paraoxonase (PON1) and other esterases (Eaton et al., 2008).

In rats, CPF is primarily excreted in the urine as conjugates of TCPy (Bakke et al., 1976; Smith et al., 1967). Nolan et al. (1987) also studied CPF metabolism in the rat and found that it undergoes extensive first-pass metabolism to TCPy, with no parent compound excreted in urine. These authors reported the major urinary metabolite to be TCPy-glucuronide, with lesser amounts of TCPy-sulfate and free TCPy (Nolan et al., 1987). Sunaga et al. (1989) reported

similar findings, following intraperitoneal administration of CPF to rats, showing urinary TCPy accounted for more than 85% of the administered dose of CPF, with lower percentages of the diethylphosphate metabolites recovered. A subsequent study was conducted in rats to evaluate the time-course of blood metabolites following oral administration of 0.5–100 mg CPF/kg body weight (Timchalk et al., 2006). The authors determined 99% of blood metabolites were in the form of TCPy with only 1% as parent compound. Trace levels of CPF-oxon levels were found but were generally 100-fold lower than CPF levels. These trace levels of the CPF-oxon metabolite are consistent with the report of high first-pass metabolism of CPF by the liver (Sultatos, 1994).

The metabolism and pharmacokinetics of CPF have been evaluated in several human volunteer studies. Nolan et al. (1984) found at least 70% of a single oral dose of CPF (0.5 mg/kg) was absorbed and excreted in the urine, primarily as acid-labile conjugates of TCPy (Nolan et al., 1984). Trace levels of CPF were found in this study, with no measurements conducted for CPF-oxon (Nolan et al., 1984). Griffin et al. (1999) measured urinary DEP metabolites of CPF following oral or dermal doses, showing 93% of administered dose is excreted in urine as these DEP metabolites. In a later multi-dose level pharmacokinetic study, Kisicki et al. (1999) found that following a single oral dose of either 0.5, 1.0 or 2.0 mg CPF/kg body weight, TCPy was the major metabolite in blood, with CPF levels <1% of TCPy concentrations. No CPF-oxon was detected in blood samples from this study, with the 1 ng/mL limit of quantitation value 3- to 18-fold lower than the highest CPF concentrations observed. These CPF-oxon results are consistent with the rat pharmacokinetic results and indicate that CPF undergoes extensive first-pass metabolism in humans as well as animals.

2.2. CPF mode of action

The mode of action for CPF has been well described (Eaton et al., 2008). CPF is lipophilic with a $\log K_{ow}$ value of 4.96 (Sangster, 1994); whereas, the CPF-oxon and hydrolysis metabolites, TCPy and diethylphosphates, are substantially more polar. The parent compound; containing the P=S bond is more chemically stable than the reactive CPF-oxon, due to the P=S bond being less electronegative than
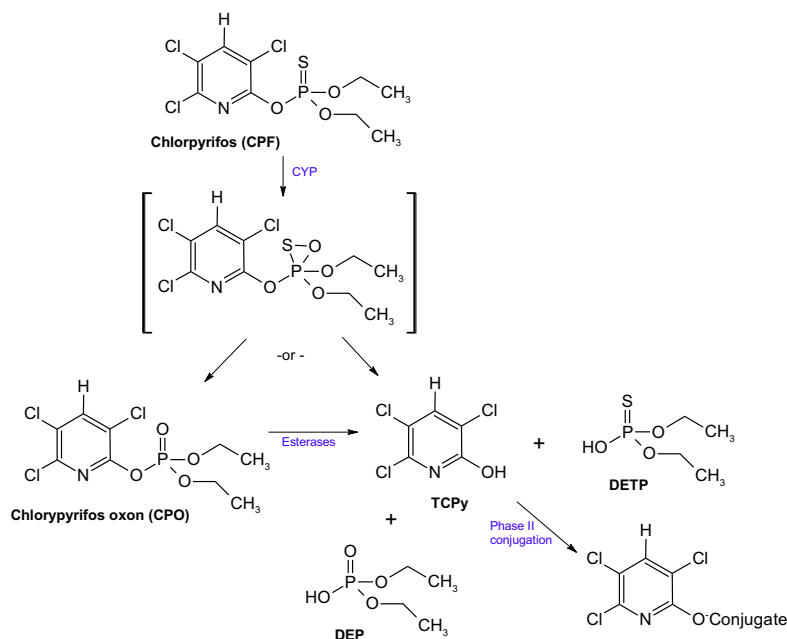

Fig. 1. Metabolic scheme of chlorpyrifos in mammals.

the corresponding P=O moiety of the CPF-oxon metabolite, which stabilizes CPF from loss of TCPy (Ballantyne and Marrs, 1992; Chambers and Levi, 1992). As a result, toxicological effects are ascribed to the more reactive CPF-oxon rather than CPF (Sultatos, 1994). Toxicological effects arising from formation of the CPF-oxon metabolite are primarily via inhibition of ChE activity, through covalent binding of CPF-oxon to the serine active site of this class of enzymes (Thompson et al., 2010). The inhibition of the enzyme results in accumulation of acetylcholine at neuronal junctions and causes hyperactivity of nervous tissues leading to a range of apical effects in multiple tissues (Eaton et al., 2008).

The USEPA has concluded that inhibition of ChE represents the most sensitive effect of CPF in animals and humans (USEPA, 2011). While USEPA Policy (USEPA, 2000) recognizes the importance of ChE inhibition in the peripheral and central nervous system in hazard evaluation, in the absence of these data, USEPA uses RBC ChE inhibition as a surrogate measure for ChE inhibition in the peripheral and central nervous systems. RBC ChE inhibition is not an adverse effect *per se*, but is readily measured and occurs at lower doses than inhibition in the peripheral and central nervous systems. It should be noted that data exists on ChE inhibition in both central and peripheral nervous systems for CPF in dogs (Marable et al., 2007). Consistent with the risk assessment for other organophosphate compounds, the USEPA has used a response level of 10% RBC ChE inhibition as the endpoint to predict the point of departure for the acute and chronic oral exposure benchmarks (i.e., acute and chronic Population Adjusted Doses) for CPF (USEPA, 2011).

2.3. Biomarkers of exposure and effect

Controlled animal toxicokinetic experiments and human volunteer studies have identified a number of biomarkers of exposure and effect (i.e., ChE inhibition), and these have been used to validate the PBPK/PD model for CPF. As shown in Table 1, these biomarkers include CPF, CPF-oxon and TCPy metabolites in blood and urine, as well as ChE inhibition in plasma, red blood cells (RBCs) and other tissues.

2.4. PBPK/PD model

CPF is a data-rich chemical with multiple *in vitro* and *in vivo* animal and human data sets that have defined CPF metabolism, the mechanism of action, and multiple markers for exposure and effect. These data have permitted the development of a robust and well-validated PBPK/PD model that quantitatively predicts the concentration of biomarkers (i.e., CPF in blood and TCPy in urine) and RBC ChE inhibition (Hinderliter et al., 2011; Juberg et al., 2011; Price et al., 2011; Poet et al., 2014a).

Timchalk et al. (2002) published the first CPF PBPK/PD model and the model has been refined in subsequent publications. The

enhancements of the model include refining estimates of ChE inhibition rates and CYP P450 metabolism rates with measured *in vivo* and *in vitro* human and animal data. The model also includes metabolism in the intestines and other non-hepatic tissues (Price et al., 2011; Smith et al., 2014; Poet et al., 2014a,b).

The model is coded in acslXtreme (Aegis Technology, Huntsville, AL). The model has been primarily parameterized and validated using human data from *in vitro* and *in vivo* studies, and by a lesser extent by fitting the model to human exposure data. Data on human physiology is based on the growth models described by Luecke et al. (2007) and Young et al. (2009). CPF specific metabolism was measured *in vitro* and describes the detoxification to TCPy and bioactivation to CPF-oxon in the liver. The model describes the pharmacokinetics of CPF and pharmacokinetics and pharmacodynamics of CPF-oxon, the active metabolite of CPF, and a more simplified single compartment with a volume of distribution and first-order urinary elimination for TCPy. The model is able to predict CPF and CPF-oxon concentrations over time in the fat, slowly perfused tissue, rapidly perfused tissue, diaphragm, brain, and liver compartments.

The submodel that describes the active oxon includes biomolecular rate constants to describe inhibition of cholinesterases (acetyl ChE and butyryl ChE) and carboxylesterase inhibition in multiple tissues. The modeling of these tissues also includes descriptions of reactivation and aging of these enzymes (Smith et al., 2014). The resultant model has been shown to predict the concentration of CPF in blood and TCPy in urine as well as inhibition of plasma butyryl ChE and RBC ChE in exposed individuals (Juberg et al., 2011).

The version of the model used to set the BGVs assesses interindividual variation in RBC ChE inhibition in populations of humans of different ages that occurs as a result of interindividual differences in physiology and metabolic capacity. The model characterizes interindividual variation in response using Monte Carlo analysis. The interindividual variation in model predictions include blood CPF and urinary TCPy concentrations as well as inhibition of plasma butyryl ChE and RBC ChE (Juberg et al., 2011; Price et al., 2011; Poet et al., 2014a).

Monte Carlo models require data on interindividual variation in the inputs to the PBPK/PD model. There are approximately 120 inputs for the PBPK/PD model used to set the BVGs (Smith et al., 2014). Many of the inputs do not vary across individuals, and other inputs vary but the model predictions are not sensitive to this variation. As a result, the Monte Carlo model does not require distributions for these inputs. The impacts of interindividual variation in model inputs on the model predictions were determined by performing a sensitivity analysis. In this analysis a small change, 1%, was made in the value of each parameter and the impact of the change on peak RBC ChE inhibition was determined. Only sixteen of the inputs were identified as having some impact on peak RBC

Table 1
Toxicokinetic and cholinesterase (ChE) inhibition biomarkers of CPF exposure or effect used to validate the PBPK/PD model (adapted from Poet et al., 2014b).

Exposure route	ChE inhibition				Toxicokinetic			
	Plasma	RBC	Diaphragm /lung	Brain	Blood CPF	Blood CPF-oxon	Blood TCPy	Urine TCPy
Oral								
Rat	•	•	•	•	•	•	•	•
Human	•	•			•	•	•	•
Dermal								
Rat	•	•		•				
Human	•	•			•		•	•
Inhalation								
Rat	•	•	•	•	•	•	•	•
Human								

Table 2
Parameters showing a sensitivity coefficient greater than 0.1 in the local sensitivity analysis after a 1 mg/kg/day dose (adapted from Poet et al., 2014a).

Parameter	Sensitivity coefficient[a] infant	Mean value for Monte Carlo distribution	Coefficient of variation	Variability references
Total blood volume	1.18	Age-dependent[b]	0.03[c]	Price et al. (2003)
Plasma paraoxonase (PON1)	1.18	Age-dependent[b]	0.53[d]	Smith et al. (2011)
Hepatic blood flow (L/h × kg tissue)	1.18	50	0.27	Materne et al. (2000)
Red blood cell (RBC) cholinesterase (ChE) inhibition rate (l/μmol × h)	1.15	100	0.17	Dimitriadis and Syrmos (2011)
Hepatic PON1 (μmol/h × kg tissue)	1.02	$1.5e^5$	0.57[d]	Smith et al. (2011)
Hematocrit (%)	0.45	0.45	0.068	Price et al. (2003)
RBC ChE degradation rate (l/h)	0.63	0.01	0.14	Chapman and McDonald (1968)
Hepatic CYP P450 bioactivation to oxon (μmol/h × kg tissue)	0.62	690	0.59	Smith et al. (2011)
Hepatic CYP P450 Detoxification to TCPy (μmol/h × kg tissue)	0.62	$1.5e^3$	0.53	Smith et al. (2011)
RBC ChE reactivation rate (l/h)	0.49	0.014	0.36	Mason et al. (2000)
Intestinal CYP P450 bioactivation to oxon (μmol/h × kg tissue)	0.48	82	0.52[e]	Obach et al. (2001)
Intestinal CYP P450 detoxification to TCPy (μmol/h × kg tissue)	0.43	53	0.52[e]	Obach et al. (2001)
Transfer rate to intestine (h^{-1})	0.41	0.31	0.26[c]	Singh et al. (2006)
Volume of the liver	0.26	Age-dependent[b]	0.03[c]	Price et al. (2003)
Hepatic carboxyl basal activity rate (l/h/kg tissue)	0.25	$1.27e^6$	0.36	Pope et al. (2005)
Hepatic carboxyl reactivation rate (l/h)	0.10	0.014	0.36	Mason et al. (2000)

Parameters are listed in descending order of sensitivity. The sensitivity of most parameters was greater in infants than adults. For clarity only infants are shown. These 16 parameters accounted for >95% of all model sensitivity.

[a] Local sensitivity analysis following a repeated 3 μg/kg/day dose in both infants and adults resulted in no parameters more sensitive than 0.001.

[b] Age-specific parameters are calculated using a polynomial equation, see Smith et al. (2014).

[c] The sensitivity of blood and liver volume were assessed based on the first term in a age-dependent calculation formula that predicts total volume normalized to body weight for an individual of a specific age. These parameters are likely more sensitive than the final parameter since small changes in the first term will be carried through the equation. Despite this, to be conservative, the total blood and liver volume were varied. The variability shown here is for the parameter prior to multiplying by body weight.

[d] Plasma and hepatic PON1 were linked in the final analyses using the same coefficient of variation.

[e] Based on intestinal metabolism of testosterone.

ChE inhibition (the criterion for including a parameter was that a 1% change in the input value results in a corresponding change in RBC ChE inhibition of at least 0.1%). These inputs account for greater than 95% of the summed sensitivities of all inputs (Table 2).

3. Methodology

3.1. Description of BGVs

The BGV developed using the PBPK/PD builds on the concept of Biomonitoring Equivalents (BEs) (Hays et al., 2007). The BE methodology uses available pharmacokinetic data and forward dosimetry to calculate levels of biomarkers associated with exposures that are consistent with general population exposure guidance values (e.g., Reference Doses, Minimal Risk Levels, or Tolerable Daily Intakes) (Hays et al., 2008). Over 100 BEs for a wide range of chemicals have been derived to date (Aylward et al., 2013). BEs are risk-based screening tools designed to help interpret human biomonitoring data in a public health risk context. The BGVs are intended to be used in the same ways as BE values; however unlike BEs, BVGs are not based on existing exposure guidance values. The approach described here for the first time instead uses a PBPK/PD model of interindividual differences in human metabolism and physiology to assess the relationship between biomarker concentrations and RBC ChE inhibition (Juberg et al., 2011; Poet et al., 2014a).

The BGVs derived here are conceptually similar to biological exposure limits established by the German Human Biomonitoring Commission using epidemiology studies. Known as human biomonitoring (HBM-I) values, they are described by Angerer et al. (2011) as "the concentration in the body matrix of a substance below which, according to the Commission's current assessment,

no adverse health effect should be expected. Thus, no action will be needed from a toxicological perspective if concentrations are below the HBM-I value". Angerer and colleagues further state that when HBM-I are based on epidemiological studies, the HBM-I levels reflect the highest biomarker concentration where adverse effects are not expected to occur.

3.2. Derivation of BGV values for CPF in blood and TCPy in urine

The methodology used in setting the BGV values for CPF in blood and TCPy in urine is as follows. The PBPK/PD model is used to predict the concentration of CPF in blood and TCPy in urine that will occur when an individual receives a single oral dose that causes 10% inhibition of RBC ChE and how these values vary across individuals as a result of interindividual differences in physiology and metabolism. The model can also address age-related differences in these relationships.

The BGV levels for CPF in blood and TCPy in urine are based on the modeling of single daily dietary exposures rather than a continuous exposure to a specific dose. Models of longitudinal dietary exposures have shown that CPF exposures of all individuals vary greatly from one day to the next, and as a result, elevated dietary exposures occur as independent and isolated events in an individual's exposure history. This variation occurs because an individual's diet typically varies from one day to the next and even when it does not, the residue levels vary from one food item to the next (Juberg et al., 2011; Price et al., 2011).

Adults 30 years of age and infants six months of age were modeled to provide age-specific BGV values that could be used to evaluate published CPF biomonitoring data for these ages. The PBPK/PD model produces an estimate of the amount of TCPy excreted in the urine over a 24-h period. These data were used

to estimate the concentration of TCPy in urine and the amount excreted per gram of creatinine using published values for age- and gender-specific urine production and creatinine elimination. The average urinary flow used for infant and adults are 79.3 mL/kg/day and 18.0 mL/kg/day, respectively (Heffernan et al., 2013). Adult creatinine clearance was assumed to be 0.201 mmol/kg/day in males and 0.182 mmol/kg/day in females based on data in adolescents (Remer et al., 2002). Data on creatinine clearance was not identified for infants; therefore, the Remer et al. (2002) data reported for 3 year-old-children was applied for infants (0.134 mmol/kg/day in male children and 0.127 mmol/kg/day in female children).

Following an oral dose, the spot urine concentration of TCPy, blood concentration of CPF, and the degree of RBC ChE inhibition varies over 24-h (Hinderliter et al., 2011). The use of the central tendency or mean concentration for spot samples of chemicals with a short half-life has been recommended (LaKind et al., 2008). Therefore, the BGV for urine and blood are defined as the average concentrations of TCPy in urine and CPF in blood that will occur in an individual over the 24-h period following a dose that causes an average of 10% inhibition of RBC ChE for the 24-h period.

For a given single oral dose, the PBPK/PD was used to estimate the 24-h average of: (1) the level of RBC ChE inhibition, (2) the concentration of CPF in blood, and (3) the concentration of TCPy in urine. This was repeated for 100,000 adults and 100,000 infants (varying model parameters as noted in Table 2) exposed to a log uniform range of randomly generated doses ranging from 0.005 mg/kg to 5 mg/kg. This range of doses was predetermined to cover the range of adult and infant sensitivities to CPF (Poet et al., 2014a).

For the concentration of TCPy in urine, the results were sorted from the lowest to highest TCPy concentration and a 1000 person running average was determined. For each set of 1000 individuals, the fractions of individuals with less than or equal to 10% RBC ChE inhibition were determined. The sets of individuals where 5%, 50%, and 95% of the individuals had RBC ChE inhibition of 10% or less were identified and the mean value of the concentration of TCPy in urine for each group was determined. The value of the average concentration of TCPy in urine for the group of 1000 individuals where 95% of the group have RBC ChE inhibition of 10% or less was used as the basis for the BGVs for TCPy in urine. The same procedure was repeated with blood CPF as the dose metric to produce the BGV for CPF in blood.

Traditional BE values derived using the point of departure from a regulatory standard are corrected using uncertainty factors determined in the underling risk assessment to account for inter-individual and interspecies PK/PD differences (Hays et al., 2007). The BGV values generated here using the PBPK/PD model; however, do not require any additional adjustment factors. The PBPK/PD model predicts the time-varying extent of response (RBC ChE inhibition) directly in humans; therefore, there is no need for an interspecies adjustment factor. The BGV levels are based on a model of the most sensitive age and on the lower per-centile of a concentration range that reflect human variation in response; therefore, there is no need for an intraspecies adjustment factor.

The urinary and blood BGV values generated in the above process are based on simulations of the relationships between single oral doses and the concentrations of CPF in blood and TCPy in urine. As a result, the values of the BGVs are specific to exposures to dietary residues and other oral sources that have a high day-to-day variability. However, the BGVs may be relevant for individuals' whose exposures could occur over longer periods of time and by other routes if those exposures resulted in the same relationships between RBC ChE inhibition and concentrations of CPF in blood and TCPy in urine.

4. Results

4.1. PBPK/PD modeling and derivation of BGVs

Fig. 2 presents the results for the PBPK/PD model for RBC ChE inhibition as a function of a single oral daily dose in a simulated population of 100,000 infants for the dose range of 0.005 mg/kg–5 mg/kg of CPF. The data for adults (not shown) are similar. In the dose response for an individual, the responses to any dose fall on a single curve. But in a population of individuals with variation of metabolism and physiology, the model predictions appear as a cloud. The horizontal line marks 10% RBC ChE inhibition. The range of 10% RBC ChE inhibition for the simulated population falls within a range of 0.08 mg/kg–3 mg/kg. This is consistent with earlier esti-mates (Juberg et al., 2011).

Fig. 3 presents the 95th, 50th, and 5th percentiles of the distributions of blood CPF and urinary TCPy concentrations for the 100,000 simulated infants, and Tables 3 and 4 present the blood CPF and urinary TCPy concentrations corresponding to these percentiles for adults and infants. As Table 3 indicates, there are approximately a 10-fold and 9-fold difference between the 5th (i.e., the biomarker concentration associated with 5% of the population having a RBC ChE inhibition of 10% or less) and 95th percentiles (i.e., the bio-marker concentration associated with 95% of the population having a RBC ChE inhibition of 10% or less) for blood CPF and urinary TCPy and about a 3-fold difference between the medians (i.e., the bio-marker concentration associated with 50% of the population having a RBC ChE inhibition of 10% or less) and 95th percentiles. Table 3 presents the predicted levels for the concentration of TCPy in urine (μg/L), the mass excretion rate of TCPy per hour adjusted for body weight (μg TCPy excreted per kg body weight per hour – μg/kg/h), and the μg of TCPy per gram of creatinine.

The age-related differences are generally smaller than the differences between individuals of a given age and vary with the bio-marker. BGV values for CPF in blood are a third lower for infants compared to adults (Tables 3 and 4). The age-related differences for the three BGVs for TCPy in urine also differ. These differences are influenced by the units used to normalize TCPy in urine. When reported on a concentration basis, the BGV is lower in infants as compared to adults, reflecting the greater body weight normalized urine production rate in infants. When reported on a creatinine basis, the BGV is higher in infants, reflecting the greater excretion rates of creatinine in adults (expressed on a bodyweight basis). When expressed as a body weight normalized total mass excreted basis, the values are nearly identical (Tables 3 and 4).
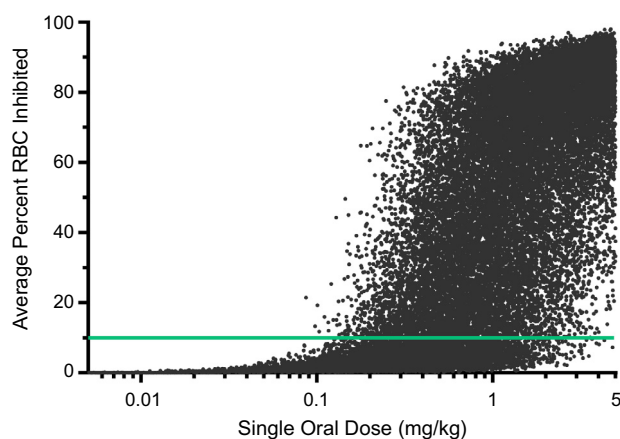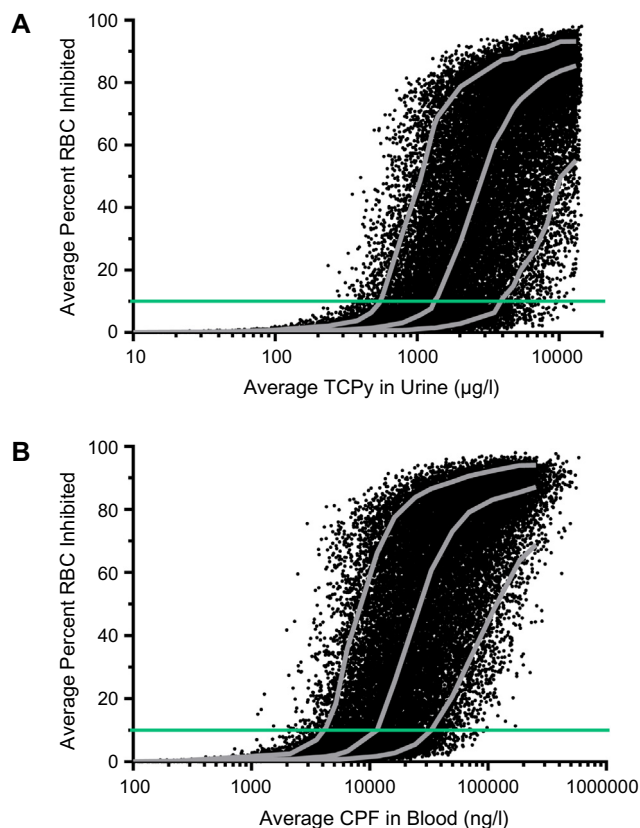

Fig. 2. Average daily red blood cell (RBC) cholinesterase (ChE) inhibition from a single daily dose of chlorpyrifos from 0.005 mg/kg to 5 mg/kg in 100,000 simulated infants. Each data point is the average RBC ChE inhibition for the 24 h following a single oral dose. The horizontal line marks 10% RBC ChE inhibition.

Fig. 3. Average daily red blood cell (RBC) cholinesterase (ChE) inhibition from a single daily dose of chlorpyrifos (CPF) ranging from 0.005 mg/kg to 5 mg/kg in 100,000 simulated infants compared to (A) 24-h average concentration of TCPy in urine or (B) 24-h average blood CPF concentration. Gray lines delineate the 95th, 50th, and 5th percentiles of RBC inhibition across the population as a function of biomarker concentration. The horizontal line marks 10% RBC ChE inhibition.

Table 3
Concentrations of chlorpyrifos in blood and TCPy in urine associated with the fraction of the population of simulated adults with red blood cell (RBC) cholinesterase (ChE) inhibition of less than or equal to 10%.

Percent of population with ≤10% RBC ChE inhibition	Blood CPF (ng/L)	Urinary TCPy (µg/L)	Urinary TCPy (µg/kg × h)	Urinary TCPy (µg/g creatinine)
95	6100	2100	2.0	1700
50	19,000	5700	5.6	4600
5	64,000	19,000	18	15,000

Table 4
Concentrations of chlorpyrifos in blood and TCPy in urine associated with fraction of the population of simulated infants with red blood cell (RBC) cholinesterase (ChE) inhibition of less than or equal to 10%.

Percent of population with ≤10% RBC ChE inhibition	Blood CPF (ng/L)	Urinary TCPy (µg/L)	Urinary TCPy (µg/kg × h)	Urinary TCPy (µg/g creatinine)
95	4200	520	2.2	2900
50	12,000	1300	5.3	7100
5	33,000	3800	16	21,000

4.2. Comparison of the proposed BGV levels with available human biomonitoring data

The proposed BGV values are protective of public health and may be compared directly to human biomonitoring data to deter-mine a MOS. The MOS is defined here as the BGV divided by the bio-monitoring data (BGV/biomonitoring data). The target MOS used here is ⩾1, reflecting the fact that the associated uncertainty fac-tors (i.e., inter- and intra- species variability) have been accounted for in the derivation of the BGV. For clarity, this term is different than the term "Margin of Exposure", which is defined in this con-text as the ratio of the point of departure (such as a no observed adverse effect level) and the estimated exposure dose or concentra-tion. Since CPF and TCPy have short half-lives in the body and thus would be expected to yield high intraindividual variability (Meeker et al., 2005), the central tendency of biomonitoring levels from pop-ulation surveys are most indicative of longer term average expo-sures (Aylward et al., 2012; LaKind et al., 2008).

CPF can also be converted to TCPy in the environment. It is esti-mated that approximately 80% of urinary TCPy is the result of direct exposure to preformed environmental TCPy and not expo-sure to CPF (MacIntosh et al., 2001; Wilson et al., 2003). Two other pesticides chlorpyrifos-methyl and triclorpyr also are converted to TCPy in the environment (Eaton et al., 2008). Therefore, caution must be used when attempting to predict CPF exposure from mea-sured levels of TCPy for populations where the predominant source of exposure is dietary residues. To account for the other environ-mental sources of TCPy, MOSs are also presented assuming that 20% of the observed TCPy in urine results from exposure to CPF (Table 5).

Urinary TCPy has been detected in the general US population in the CDC National Health and Nutrition Examination Survey (NHANES) biomonitoring program (CDC, 2009) (Table 5). With no legal residential uses since 2001, diet is the presumed route of exposure and a substantial proportion of the measured TCPy is due to ingestion of preformed environmental TCPy (Barr et al., 2005). As shown in Table 5, urinary TCPy is ubiquitously detected in children and adults. Using the relevant BGV and assuming that all the TCPy was from the parent CPF, the MOSs for US children, teens, and adults are 677, 583, and 1105, respectively. Using the BGVs derived for infants, the MOSs for children are 168 and 839, assuming 100% and 20% contribution of CPF, respectively. Collected several years later, urinary TCPy results from a biomon-itoring study of California families were slightly lower than the CDC US general population data (Trunnelle et al., 2014). Urinary TCPy levels for the children were just above the LOD (limit of detection) with a resulting MOS of 1235 (100% from CPF) and 6176 (20% from CPF). Even using the most conservative assump-tions (i.e., 100% TCPy was derived from CPF and using the infant BGV value for children), the MOS of 306 indicates no RBC ChE inhi-bition would be expected in this population. Other studies of preg-nant women in which MOSs have been calculated indicate similar results (Castorina et al., 2010; Whyatt et al., 2009; Yan et al., 2009).

Recent studies have collected blood in pregnant women and infant cord blood to measure parent CPF (Huen et al., 2012; Whyatt et al., 2005; Yan et al., 2009). However, for blood CPF (Table 6), CDC or similar national programs have not published data representative of the general population. Notably, the geo-metric mean blood CPF level in the New York City cohort declined with the withdrawal of residential uses and by 2003 was not detected in the New Jersey cohort (Eaton et al., 2008; Whyatt et al., 2005; Yan et al., 2009). The MOSs are all well above 1 (when the geometric mean was <LOD, the LOD was used to determine the MOS). The California study reported by Huen et al. (2012) used a different analytical technique and the LOD of 21 ng/L was higher than the geometric mean values/LOD for the other cohorts. As a result using the LOD as the exposure estimate, the estimated MOS for this study is lower than the other studies, and may not actually reflect a higher risk.

Table 5
Summary of the margin of safety (MOS) of TCPy in urine for selected US biomonitoring studies under assumptions of 100% of TCPy in urine is from direct exposure to chlorpyrifos (CPF) or that only 20% of TCPy in urine is a result of direct exposure to CPF.

References	Population	Group	Time period	N	LOD[a] (μg/L)	TCPy in urine 50th percentile (μg/L)	MOS Assuming 100% CPF	MOS Assuming 20% CPF
Castorina et al. (2010)	California pregnant women	Prenatal, 13th week	1999–2001	538	0.3	2.1	1000[c]	5000[c]
CDC (2009)	US population	Ages 6–11	2001–2002	573	0.4	3.1	168[b]/677[c]	839[b]/3387[c]
		Ages 12–19	2001–2002	823	0.4	3.6	583[c]	2917[c]
		Ages 20–59	2001–2002	1113	0.4	1.9	1105[c]	5526[c]
Whyatt et al. (2009)	New York City pregnant women	Prenatal, 34th week	2001–2004	95	0.3	0.5	4200[c]	21,000[c]
		Maternal, day after delivery	2001–2004	73	0.3	<LOD	>7000[c]	>35,000[c]
Yan et al. (2009)	New Jersey pregnant women	Maternal, day of delivery	2003–2004	34	0.4	<LOD	>5250[c]	>26,250[c]
Trunnelle et al. (2014)	California children	Ages 2–8	2007–2009	83	0.6–1.2	1.7	306[b]/1235[c]	1529[b]/6176[c]
	California parents	Ages 18–57	2007–2009	90	0.6–1.2	1.2	1750[c]	8750[c]

[a] LOD (limit of detection) value used to calculate the MOS when the 50th% <LOD.
[b] The BGV derived for infants of 520 μg/L was used to determine the MOS.
[c] The BGV derived for adults of 2100 μg/L was used to determine the MOS.

Table 6
Summary of the margin of safety (MOS) for selected US biomonitoring studies of chlorpyrifos (CPF) in blood.

References	Population	Group	Time period	N	LOD[a] ng/L	CPF in blood GM[b] ng/L	MOS
Huen et al. (2012)	California mother/infant pairs	Maternal, at delivery	1999–2001	234	21	<LOD	>290[d]
		Cord blood	1999–2001	256	21	<LOD	>200[c]
Whyatt et al. (2005) and Eaton et al. (2008)	New York City mother/infant pairs	Maternal, at delivery	1999–2002	326	0.5	1.7	3588[d]
		Cord blood	1999	109	0.5	3.7	1135[c]
		Cord blood	2000	104	0.5	2.0	2100[c]
		Cord blood	2001	67	0.5	<LOD	>8400[c]
		Cord blood	2002	9	0.5	1.1	3818[c]
Yan et al. (2009)	New Jersey mother/infant pairs	Maternal, at delivery	2003–2004	138	1	<LOD	>6100[d]
		Cord blood	2003–2004	148	1	<LOD	>4200[c]

Yan et al. (2009) publication has an unit error. The CPF blood should be ng/L (Personal communication D. Barr).
[a] LOD (limit of detection) value used to calculate the MOS when the geometric mean <LOD.
[b] GM = geometric mean.
[c] The BGV derived for infants of 4200 ng/L was used to determine the MOS.
[d] The BGV derived for adults of 6100 ng/L was used to determine the MOS.

5. Discussion

5.1. BGVs and their implications for CPF biomonitoring

The use of human biomonitoring data where concentrations of chemicals and/or their metabolites are measured in blood or urine from a population or specific sub-populations is increasingly being used as a preferred approach to assessing human exposure (Sexton et al., 2004). Biomonitoring data reflect integrated exposure from all routes and pathways (Hays et al., 2008). The concept of the BE was designed to put these data into a human health risk context (Hays et al., 2007). The BGVs presented here provide a robust method that allows interpretation of biomarkers for CPF exposure to the endpoint of interest, RBC ChE inhibition.

We propose to set the BGVs using the blood CPF and urinary TCPy values that are associated with 95% of the population having a RBC ChE inhibition rate less than or equal to 10%. Observed biomonitoring levels below the BGVs are unlikely to be associated with RBC ChE inhibition, and since RBC ChE inhibition is a conservative marker of ChE inhibition in the central and peripheral nervous systems, the BGVs are expected to be protective against actual adverse effects for adults and infants.

The BGVs were developed using the assumption that diet was the primary source of exposure and that high daily doses would occur as isolated events (Juberg et al., 2011; Price et al., 2011). Occupational exposures that can occur by multiple routes and may be repeated over multiple days were not part of the modeling exercise. However, the relationship between TCPy in urine and the extent of RBC ChE inhibition observed in Egyptian workers (who applied CPF to crops) are remarkably close to the same relationship predicted here using the PBPK/PD model (Farahat et al., 2011). Using TCPy concentrations from urine collected over 9–17 days and data on RBC ChE levels, Farahat et al. (2011) calculated "inflection points" where RBC ChE inhibition was detected for various groups of workers. The estimated inflection points in three groups of workers ranged from urinary TCPy levels of 2000 μg/g creatinine to 3200 μg/g creatinine. Using the PBPK/PD model, the TCPy levels in urine that are predicted to cause a small amount of RBC ChE inhibition (10%) in 50–95% of adults was estimated to be 4600 μg/g creatinine to 1700 μg/g creatinine (Table 3). The close agreement of the model findings with the Farahat et al. (2011) study suggests that the relationship of TCPy excretion to RBC ChE inhibition is likely to be independent of the route and duration of exposure. This is an area where additional research could be performed.

6. Conclusion

PBPK/PD modeling provides a useful tool for investigating the relationship between observable biomonitoring measurements and potential adverse effects. In the case of CPF, the model is able to generate BGVs that can be used to evaluate biomarkers for CPF in blood and TCPy in urine. The PBPK/PD model was constructed and validated using human data. In addition, the results of the model compared favorably to an Egyptian worker study where ChE inhibition was observed. A comparison of the BGV levels to available biomonitoring data suggests MOSs of greater than 150 for current US general population exposures. Such values indicate that current exposure levels are well below a level that would pose a potential risk to either adults or infants. If it is assumed 20% of urinary TCPy is derived from CPF exposure, and the Huen et al., 2012 study is disregarded because of the high blood CPF LOD, then all MOSs are greater than 800.

Conflict of interest

Michael Bartels, Paul Price, Scott Arnold and Carol Burns are employees of The Dow Chemical Company. Manoj Aggarwal, Joseph Velovitch, Daland Juberg and Alistair Morriss are employees of Dow AgroSciences LLC. Dow AgroSciences LLC is a wholly owned subsidiary of The Dow Chemical Company and manufactures chlorpyrifos. Sean Hays and Torka Poet report personal fees from Dow AgroSciences, during the conduct of the study.

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