PBPK/PD Model Simulations of the Effects of Changes in Enzyme Levels on Plasma Esterase Inhibition and Urinary TCPy Elimination Following Chlorpyrifos Exposures

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Physiologically based pharmacokinetic (PBPK) modeling is a quantitative tool that can help describe and interpret the exposures associated with specific biomarker response (i.e. urinary metabolite levels) and to estimate the potential health implications from those exposures. To this end, the US EPA requested that a PBPK/pharmacodynamic (PD) model that has been developed to describe the pharmacokinetics of the organophosphorus insecticide chlorpyrifos (CPF) be interrogated to simulate a number of scenarios, including an estimation of biomarkers of exposure in individuals with differencing enzyme activity levels (Part I) and to estimate exposure levels that might result in urinary metabolite levels from biomonitoring studies (Part II). CPF is primarily metabolized to the major metabolite trichlorpyridinol (TCPy) and CPF-oxon which is responsible for blood and tissue cholinesterase (ChE) inhibition. The model simulation scenarios included: I) scenarios in which metabolic variability within the human population might alter the pharmacokinetics/pharmacodynamics of chlorpyrifos and II) exposure levels in children and applicators. The PBPK model was published in 2002 (Timchalk et al., 2002a) and has been updated and improved as more data have become available (Poet et al. 2003a; Poet et al. 2003b; Poet et al. 2004; Slikker et al. 2005; Timchalk et al. 2002b; Timchalk et al. 2003; Timchalk et al. 2005; Timchalk et al. 2007).

It is important to acknowledge that these model simulations have not been subjected to peer review and a number of assumptions were made in conducting these simulations. Hence, care should be taken in the application and interpretation of these results.

Part I. Changes in enzyme activities during pregnancy

Metabolic activities are often altered during pregnancy (Anderson 2006; Anger and Piquette-Miller 2008; Bologa *et al.* 1991; Carpintero *et al.* 1996; Czekaj *et al.* 2000; Czekaj *et al.* 2005; Dickmann *et al.* 2008; Ejiri *et al.* 2005; Ferre *et al.* 2006; Hines 2007; Homma *et al.* 2000; Howard and Sugden 1993; Tsutsumi *et al.* 2001). Organophosphorous pesticides, like chlorpyrifos (CPF) are highly metabolized and their toxic mode of action is linked to a bioactivated metabolite. Chlorpyrifos is both detoxified and bioactivated by CYP450 enzymes, primarily in the liver, and the active oxon metabolite (CPF-oxon) is detoxified by esterases, primarily in the liver and blood. Therefore, changes in both CYP450s and PON1 esterases over the course of pregnancy may alter the consequences of exposures. In addition, butyrylcholinesterases (BuChE) also change over the course of pregnancy, and BuChE inhibition by CPF-oxon is an endpoint of concern.

The changes in enzyme activates are generally highest in the third trimester, so estimates of common enzyme activity levels were determined and fractionally applied to a PBPK/PD model to estimate the effect of these enzyme activities on plasma and brain ChE inhibition. To estimate relative enzyme activity levels in late gestation, reports in the open literature were examined. Most of the CYPP450 activity estimates for pregnancy are based on data from either epileptic or AIDS patients, who must maintain pharmacologically active levels of therapeutic drugs throughout pregnancy. When human data were not available orthologous rat enzyme data were used.

Hepatic P450s

Overall, cytochrome P450 activity does not change significantly in pregnancy, at least in rats (Czekaj *et al.* 2000), but individual P450 activities can change dramatically. The most important P450s for CPF metabolism in humans are 1A2, 2B6, 2C19, and 3A4. To estimate late pregnancy enzyme changes, the activities of these four enzymes were changed in 25% increments based on literature data for related compounds. Bologa et al (1991) found that production of a marker substrate for 1A2 activity dropped to 41% of non-"pregnant" levels by the third trimester in epileptic women, so 1A2 activity toward CPF was estimated to decrease 50%. In rats, 2B activity was shown to be 25% lower in late gestation (Czekaj *et al.* 2000). While 1A2 and 2B activities decrease in pregnancy, 2C and 3A activities increase. In rats, 2C activity was shown to increase 1.5 to 2- fold (Dickmann *et al.* 2008) and, in pregnant AIDS patients, endogenous cortisol metabolism (marker for 3A4 activity) increased by more than 2-fold (Homma *et al.* 2000).

The relative activities of P450s 1A2, 2B6, 2C19, and 3A4 toward metabolism to either TCPy or the CPF-oxon were recently presented by Griffith, Ramaprasad, and Faustman (RASS teleconference, 5/14/2008). These data were used, along with the fractional increases in activities from the above examples, to adjust total P450 activity (V_{max} for TCPy or CPF-oxon production) to 82 and 129% of default optimized activity, respectively (**Figure 1**).

Serum Paraoxonase

Recently, Ferre et al (2006) showed that the hydroxylation of paraoxon (PON1 activity marker) in serum decreases from a nonpregnant background of 146 U/L to 111 U/L in late gestation, indicating 76% of normal activity in late gestation "pregnant" women. Carpintero, et al (1997), however, found that phenyl acetate metabolism increased from 23.6 to 33.5 µkat/g in the third trimester. The relative activity of serum PON1 was therefore adjusted to 75% of the PBPK model optimized activity (V_{max}) and 125% of default activity. Also, The US EPA provided a 5^{th} percentile PON1 activity estimate for newborns of only ~8% of adult activity based on on-going calculations (which will be reviewed by the FIFRA SAP in July, 2008), so an extreme PON1 activity change of approximately this percentage of PON1 activity was also simulated.

Acetyl and Butyryl Cholinesterases (ChE)

The activities of the target ChE are also altered in pregnancy. In rat heart, total ChE activity was shown to be 64% of adult activity on postnatal day-1 (PND-1) and 125% of adult on PND-30 (Slavikova and Tucek, 1986). The same study showed that acetylcholinesterase (AChE) was 31% of adult activity on PND-1 and 138% on PND-30. In rat diaphragm, BuChE and AChE were higher than adult levels for the first 2-weeks after birth (Sketilj et al., 1981). Although several studies have looked at histochemical staining of various brain regions and shown that the forms and amounts of BuChE vary over the course of development, information needed to estimate the level of change was not available. Nominal changes of -25 and +25% was investigated.

Carboxylesterase Activity

Was not investigated.

Simulations

The LOEL for plasma ChE inhibition in humans is 100 µg/kg/day for 9 days. The simulations were run for a single dose at the LOEL and at 10 and 3 µg/kg. These doses were chosen so an effect could be seen. Adult (default), child (~5 yrs old, 20 kg) and "pregnant" female scenarios were investigated using the percentage changes described above and outlined in (**Table 1**).

The output parameters of interest were maximal blood BuChE inhibition and urinary TCPy over 24 hr. Blood BuChE inhibition can be measured and is the most sensitive parameter to CPF exposures and urinary TCPy is a commonly used biomarker of exposure.

Additional Assumptions and Limitations

For these modeling exercises, the "pregnant" female was modeled as an assumed genderless human based on previous modeling efforts (Timchalk et al., 2002). There are a number of physiological and biochemical changes known to occur beyond the enzyme changes investigated in these simulations that were not addressed. For example, Lowe et al., (2008), have found that chlorpyrifos portioning into blood is altered along with the increased in plasma lipid levels that occur in pregnant women. These increases in blood partitioning would be expected to change tissue distribution of chlorpyrifos and chlorpyrifos-oxon, which are both lipophilic. The 5 year old child was likewise modeled as an assumed genderless adult, but with tissue and default enzyme activity levels scaled to a 20 kg body weight using standard scaling methods.

Part II: Biomonitoring

Biomonitoring Data

Biomonitoring for CPF exposures is most often carried out using urinary TCPy levels. The biomonitoring data that were chosen to investigate using the PBPK/PD model to describe exposures in children was from Lu et al (2008). In this study, the longitudinal exposure of 23, 3-11 year old children in the greater Seattle, WA area was assessed by measuring TCPy in spot urine samples. While a seasonal variation in urinary TCPy was observed, the annual average was 5.1 µg/L urine, the 10^{th} percentile was 0.2 and the 90^{th} percentile was 11.3 µg/L. To estimate total daily urinary TCPy levels in these children, urinary output was estimated using the data of Mattsson and Lindstrom (1995), and the children's body weight was estimated using CDC growth charts (http://www.cdc.gov/nchs/about/major/nhanes/growthcharts/clinical_charts.htm) (**Table 2**).

Adult biomonitoring data of urinary TCPy from pesticide applicators were also simulated. Post application, the geometric mean TCPy concentration was 15 µg/ml and the 99^{th} percentile was 170 µg/ml. These concentrations were scaled to 24 hr total urinary output assuming a steady state exposure as outlined in **Table 2**.

Simulations

For children, the dietary exposures to CPF were simulated assuming 3 meals/day with an equal amount of CPF in each meal. Simulations were carried out for 60 days to assure steady state and to minimize the contribution of the first few, pre-steady state days on urinary TCPy output. Thus, 24 hr urinary TCPy was estimated by taking the total, 60 day urinary TCPy and dividing it by 60 days. Blood esterase inhibition was reported as the minimum esterase activity from any given simulation. As in Part I, children were modeled with parameters matching the adult defaults, but model parameters scaled to lower body weights (Table 2). The adults were assumed to be 70 kg.

Results and Discussion

Part I

At the lowest simulated exposures (3 µg/kg) plasma inhibition was nominal for all scenarios. The maximal change simulated involved a child with 10% of the default PON1 activity, an estimate of the 5th percentile in newborns; at this dose the maximal plasma BuChE inhibition in these individuals was less than 4% of control.

At 10 µg/kg very slight differences in maximal plasma BuChE inhibition were observed for most scenarios, and only the worst case scenario of a child with 10% of the default PON1 activity resulted in a substantial different in inhibition (87.6 % of control). At 100 µg/kg, the LOEL, differences between the scenarios become more apparent. The scenario of 10% of the default PON1 activity, however, is the only scenario where a substantial decrement in maximal plasma BuChE inhibition is predicted (**Figure 2**).

In all adult, "pregnant" women simulations, differences in maximal plasma BuChE inhibition were nominal (**Figure 3**). For these simulations, select enzyme kinetics were changed. Cholinesterase levels in tissues other than blood and brain were not altered, the activity, or concentrations of these enzymes in other tissues may represent sinks to bind up CPF-oxon, which may result in some differences from these simulations. For these simulations, however, only the enzyme activity levels deemed most important were simulated.

Regardless of exposure scenario, the urinary elimination of TCPy over the first 24 hr was unchanged, indicating that biomonitoring of urinary TCPy is not sensitive enough to estimate PON1, BuChE, AChE, or P450 activities in individuals. Urinary elimination of TCPy is not complete by 24 hr post-exposure, so total urinary elimination, or urinary elimination after steady state has been reached for multiple dosing (See Part II) will be different than these examples for elimination in the first 24 hr.

Adjusting the total activity of BuChE and AChE in plasma and brain by 25%, did not result in significant changes in maximal plasma BuChE inhibition in any scenario, indicating the model is not sensitive to these parameters. Changes in the amount of tissue ChE were not simulated.

As suggested previously, maximal plasma BuChE inhibition seems to be the most sensitive to PON1 activity. PON1 activity has been reported to be low at birth (~30% of

adult: Holland et al., 2006) and plateau at 6-15 months of age (Cole et al., 2003), it is unlikely that this very low PON1 activity will be observed in an older individual.

Part II

To demonstrate the type of model output obtained, a simulated adult exposure to 0.5 µg/kg/day (broken up into three equal doses of 0.1667 µg/kg separated by 4 hr throughout a day) is shown in **Figure 4**. Blood CPF levels will reach a theoretical maximum of 0.024 nmol/L and plasma ChE inhibition will be ~98 percent of unexposed control.

The exposure level in the meals of the children from the study of Lu et al (2008) were estimated by assuming the urinary TCPy concentrations outlined in **Table 2** and determining the concentration (per kg body weight) divided into three meals that will result in that daily concentration after 60 days in a 20 kg (approx 5 year old) child. The model estimates a daily chlorpyrifos intake of 0.27 µg/kg/day in a 20 kg child will result in the average urinary TCPy concentrations from the study of Lu et al. (2008). See **Table 3** for estimated CPF doses, urinary TCPy and plasma ChE activities from these simulations.

All of these simulations assume that urinary TCPy comes from CPF exposures, but it is known that TCPy is both a metabolite of other pesticides and is in the environment itself (Barr et al., 2005; Bradman et al., 2005; Timchalk et al., 2007).

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The research that has gone into developing and implementing this model has been funded by a number of sources, including:

The Dow Chemical Company, Midland, MI 48674

- Adult rat and human PBPK/PD model (completed 2002)
- Human gestational PBPK/PD model (ongoing)
- Model modifications to simulate inhalation exposure (ongoing)

Centers for Disease Control and Prevention (CDC), R01 OH003629

- Complex mixture modeling of organophosphate insecticides (completed)
- Mixture modeling: pesticide drug interactions (ongoing)

Centers for Disease Control and Prevention (CDC), R01 OH008173

- Non-invasive biomonitoring of pesticides (ongoing)

U. S. Environmental Protection Agency's STAR program through grant R828608

- Development of a physiologically based pharmacokinetic/pharmacodynamic model to quantitate biomarkers of exposure for organophosphate insecticides. (completed)

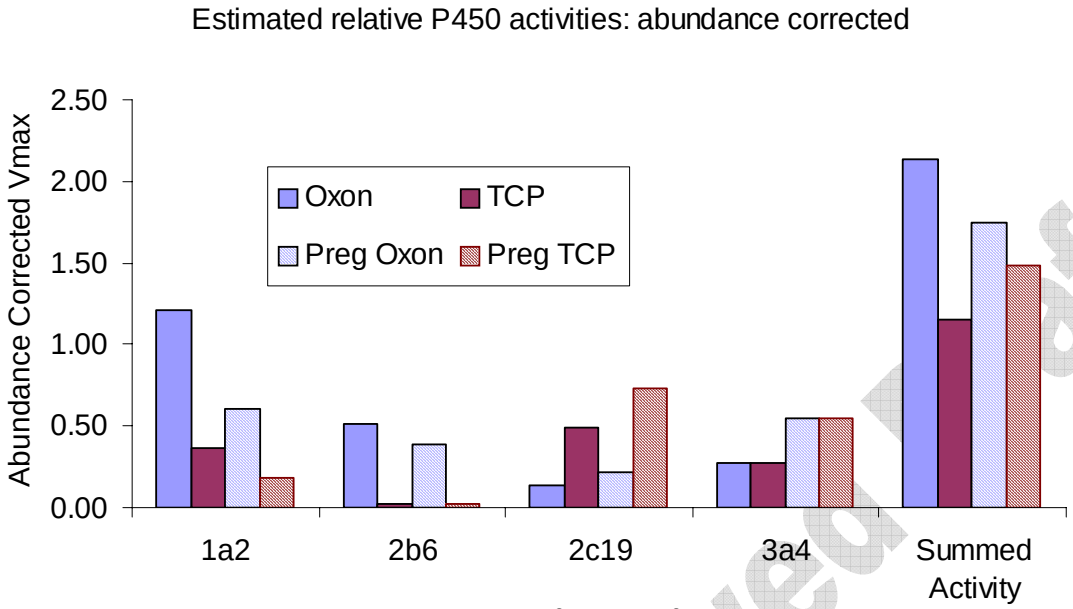


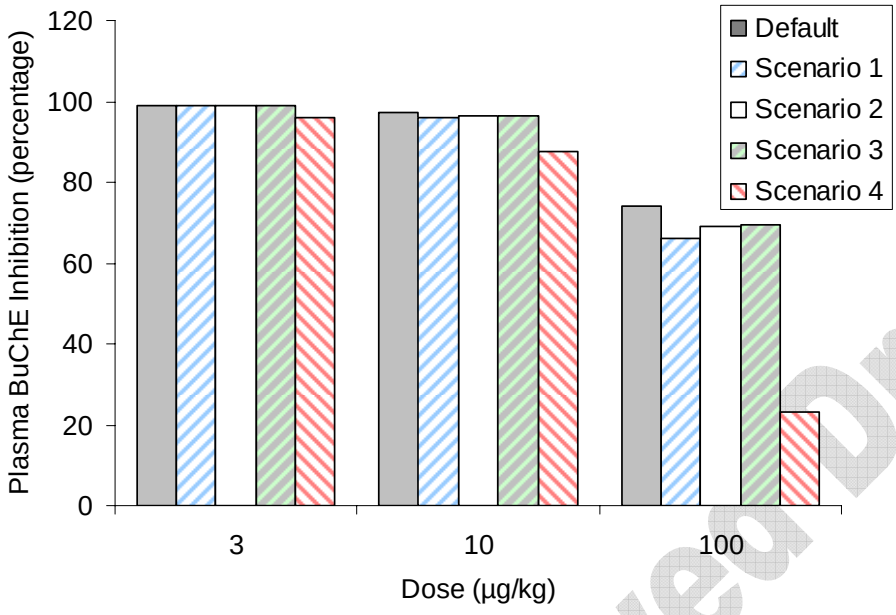
Figure 1. The estimated relative activity of each of the 4 major CPF metabolizing enzymes toward total metabolism, corrected by abundance of each enzyme in the average human liver. Based on this estimate of summed activity of CYP450s leading to oxon (blue) or TCPy (red), P450 Vmax Pregnancy activities were increased or decreased in 25% increments based on literature marker substrate activities in late gestation.. This estimate suggests that oxon Vmax will be decreased ~25% whereas TCPy Vmax may be increased ~25%.

TABLE 1. Simulation of effects of enzyme inhibition on human dose metrics

Scenario		Default	1	2	3	4
P450				% of Default		
	Oxon	100%	100%	75	75	75
	TCPy	100%	100%	125	125	125
PON1						
	Liver	100%	*75*	*75*	*75*	*10*
	Blood	100%	*75*	*75*	*75*	*10*
Cholinesterase						
	Brain AChE	100%	*75*	*75*	*125*	*75*
	Brain BuChE	100%	*75*	*75*	*125*	*75*
	Blood BuChE	100%	*75*	*75*	*125*	*75*
Adult Simulation Results*						
3 µg/kg Dosing						
Blood BuChE Activity (%)		***99.05***	***98.74***	***98.87***	***98.91***	
24 hr Urinary TCPy (µmol)		***0.153***	***0.153***	***0.153***	***0.153***	
10 µg/kg Dosing						
Blood BuChE Activity (%)		***96.87***	***95.84***	***96.25***	***96.40***	
24 hr Urinary TCPy (µmol)		***0.511***	***0.511***	***0.510***	***0.510***	
100 µg/kg Dosing						
Blood BuChE Activity (%)		***72.17***	***64.52***	***67.47***	***68.41***	
24 hr Urinary TCPy (µmol)		***5.103***	***5.106***	***5.103***	***5.103***	
"Child" (20 kg) Simulation Results						
3 µg/kg Dosing						
Blood BuChE Activity (%)		***99.13***	***98.79***	***98.91***	***98.95***	***96.16***
24 hr Urinary TCPy (µmol)		***0.0438***	***0.0438***	***0.0438***	***0.0438***	***0.0438***
10 µg/kg Dosing						
Blood BuChE Activity (%)		***97.12***	***96.02***	***96.41***	***96.53***	***87.55***
24 hr Urinary TCPy (µmol)		***0.146***	***0.146***	***0.146***	***0.146***	***0.146***
100 µg/kg Dosing						
Blood BuChE Activity (%)		***74.30***	***68.85***	***69.67***	***68.85***	***23.14***
24 hr Urinary TCPy (µmol)		***1.460***	***1.460***	***1.460***	***1.460***	***1.460***

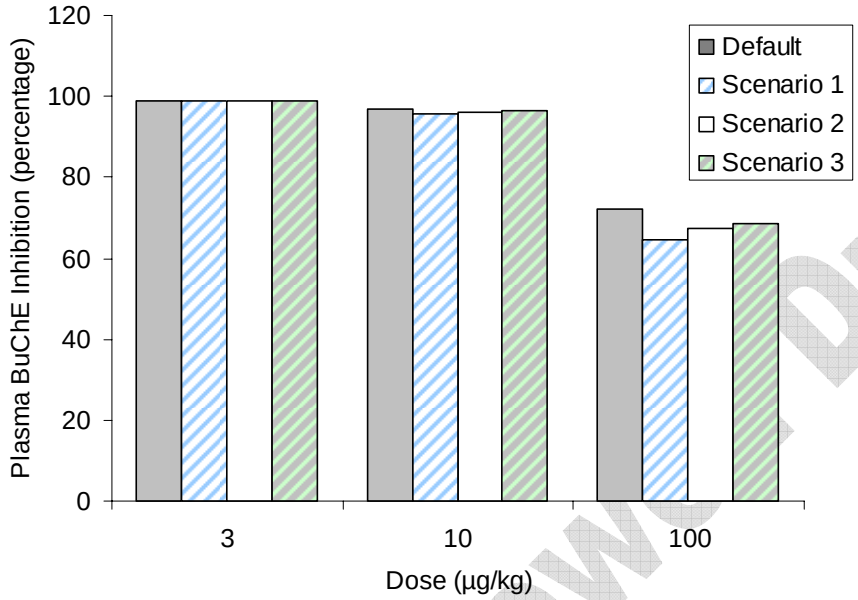
* Note that scenarios 2 and 3 in the adult represent “pregnant” women

Figure 2. 20 kg "child" Simulations



See Table 1 for description of Scenarios.

Figure 3. 70 kg "pregnant woman"



See Table 1 for description of Scenarios.

Table 2. Estimated total urinary TCPy/day in children and adult applicators

LU et al 2008					
Mean TCPy (annual)		5.1	µg/L		
	10th percentile	0.2	µg/L		
	90th percentile	11.3	µg/L		
Estimated Body weights (CDC 2000 growth charts)					
3 yr old		13	kg		
5 yr old		20	kg		
11 yr old		35	kg		
Estimated urinary output (Mattsson and Lindstrom 1995)					
Median		0.9	ml/hr/kg		
	× 24 hr	21.6	ml/kg		
	13 kg × 21.6 ml/kg	281	ml/day		
	20 kg × 21.6 ml/kg	432	ml/day		
	35 kg × 21.6 ml/kg	756	ml/day		
TCPy					
13 kg				÷ 198.6 g/mol	
Mean	0.281 L × 5.1 µg/L	1.43	µg/day	0.0072	µmol/day
10th percentile	0.281 L × 0.2 µg/L	0.06		0.00028	
90th percentile	0.281 L × 11.3 µg/L	3.17		0.0160	
20 kg					
Mean	0.432 L × 5.1 µg/L	2.20	µg/day	0.0111	µmol/day
10th percentile	0.432 L × 0.2 µg/L	0.09		0.00044	
90th percentile	0.432 L × 11.3 µg/L	4.88		0.0247	
35kg					
Mean	0.756 L × 5.1 µg/L	3.86	µg/day	0.0195	µmol/day
10th percentile	0.756 L × 0.2 µg/L	0.15		0.00076	
90th percentile	0.756 L × 11.3 µg/L	8.54		0.0431	
Urinary TCPy in adult applicators					
Geometric Mean	15	µg/L			
99th percentile	170	µg/L			
Estimated urinary output (Hyun et al., 2006)					
	20	ml/24 hr/kg			
	× 70 kg	1.4	L/day		
				÷ 198.6 g/mol	
	× 15 µg/L	21	µg TCPy/day	0.106	µmol/day
	× 170 µg/L	238	µg TCPy/day	1.20	µmol/day

Table 3. Estimated Exposure Doses (oral)

Child (20 kg)		
Estimated Dose[1] (µg/kg/day)	**Urinary TCPy[**] (µmol/day)**	**Estimated Plasma Cholinesterase (percent)**
0.03	0.0004	100
0.3	0.0112	98.9
0.5	0.0207	98.0
0.6	0.025	97.6
Adult (70 kg)		
Estimated Dose[2] (µg/kg/day)	**Urinary TCPy[**] (µmol/day)**	**Estimated Plasma Cholinesterase (percent)**
0.5	0.073	97.9
0.6	0.107	97.3
8.0	1.208	71.9

[1] For children, the oral dose was modeled as 3 meals/day.
[2] For adults, the oral dose was modeled as 1 meal/day.
[**]The 0.5 µg/kg/day was chosen as a reference dose to compare between children and adults. The other estimated doses were designed to approximate urinary TCPy levels from children (Lu et al., 2008) or to approximate TCPy urinary levels in pesticide applicators (Lowit, A. personal communication) (See **Table 2**).

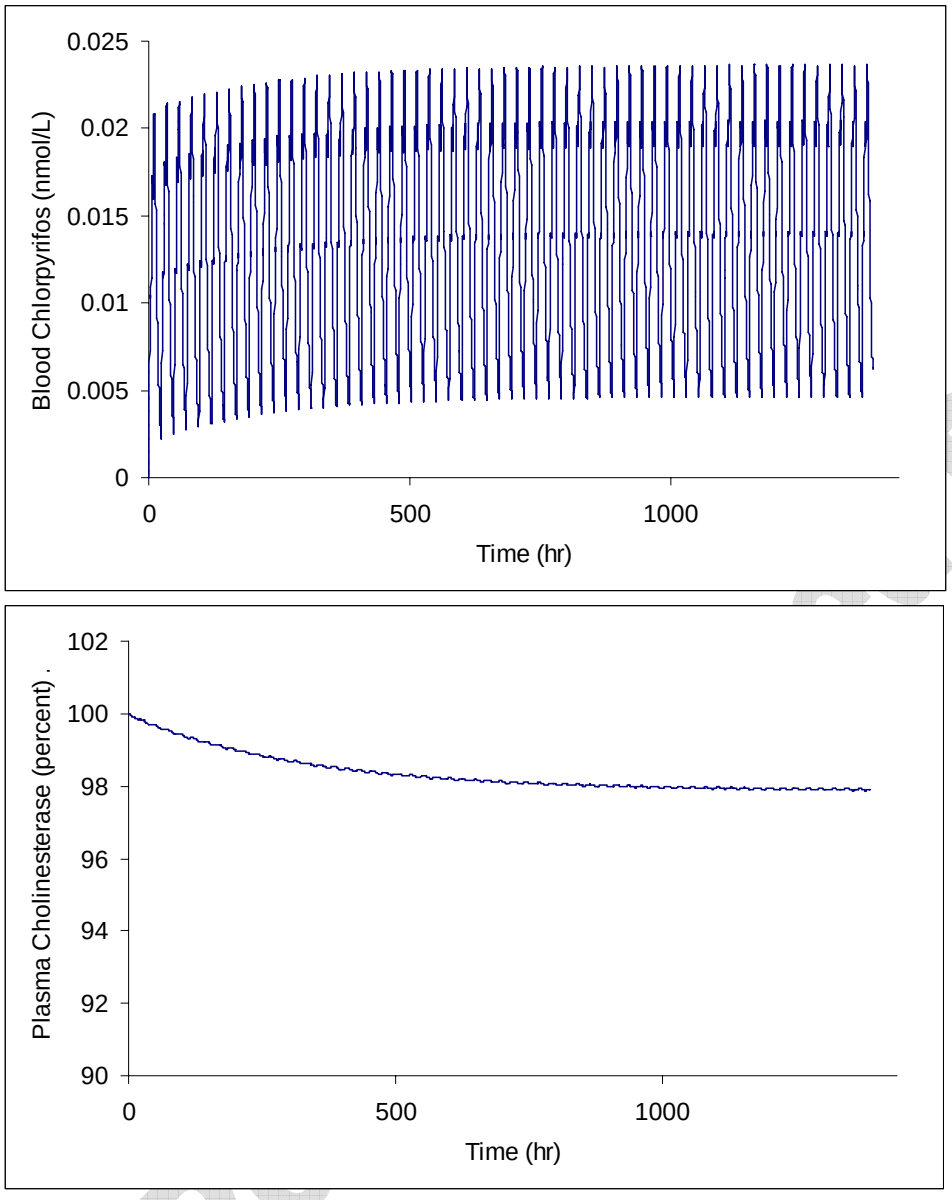


Figure 4. Example simulated exposures of a 70 kg individual to 3 daily meals containing 0.1667 µg/kg (0.5 mg/kg/day). Top, blood chlorpyrifos concentrations, bottom, plasma cholinesterase inhibition.

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