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Longitudinal assessment of occupational exposures to the organophosphorous insecticides chlorpyrifos and profenofos in Egyptian cotton field workers

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ABSTRACT

Chlorpyrifos (CPF) and profenofos (PFF) are organophosphorus (OP) insecticides that are applied seasonally in Egypt to cotton fields. Urinary trichloro-2-pyridinol (TCPy), a specific CPF metabolite, and 4-bromo-2-chlorophenol (BCP), a specific PFF metabolite, are biomarkers of exposure, while inhibition of blood butyrylcholinesterase (BChE) and acetylcholinesterase (AChE) activities are effect biomarkers that may be associated with neurotoxicity. Urinary TCPy and BCP and blood BChE and AChE activities were measured in 37 adult Egyptian Ministry of Agriculture workers during and after 9–17 consecutive days of CPF application followed by an application of PFF (9–11 days), and a second CPF application (5 days) in 2008. During the OP applications, urinary TCPy and BCP levels were significantly higher than baseline levels, remained elevated following the application periods, and were associated with an exposure related inhibition of blood BChE and AChE. Analysis of blood AChE levels before and after the PFF application period suggests that individual workers with peak BCP levels greater than 1000 μg/g creatinine exhibited further inhibition of blood AChE with PFF application, demonstrating that PFF exposure had a negative impact on AChE activity in this highly exposed worker population. While large interindividual differences in exposure were observed throughout this longitudinal study (peak urinary BCP and peak TCPy levels for individuals ranging from 13.4 to 8052 and 16.4 to 30,107 μg/g creatinine, respectively), these urinary biomarkers were highly correlated within workers ($r = 0.75$, $p < 0.001$). This suggests that the relative exposures to CPF and PFF were highly correlated for a given worker. The variable exposures between job classification and work site suggest that job title and work location should not be used as the sole basis for categorizing OP exposures when assessing neurobehavioral and other health outcomes in Egyptian cotton field workers. Together, these findings will be important in educating the Egyptian insecticide application workers in order to encourage the development and implementation of work practices and personal protective equipment to reduce their exposure to CPF and PFF.

Introduction

Organophosphorus (OP) insecticides continue to be a significant public health concern due to their worldwide use, human exposures, and documented harmful effects. OPs have accounted for the majority of insecticide poisonings in the United States

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(Calvert et al., 2008; Lee et al., 2011) and worldwide (Aardema et al., 2008). Occupations such as insecticide manufacturers, agricultural field workers and crop dusters and can be exposed to significant amounts of OPs (Berkowitz et al., 2004; Farahat et al., 2010, 2011; Garabrant et al., 2009). Other occupations such as custodial workers, veterinary employees, and pet handlers may also be at risk of exposure to these OPs (Ames et al., 1989; Jaga and Dharmani, 2003).

OPs cause acute neurotoxicity by the inhibition of acetylcholinesterase (AChE), which disrupts cholinergic function in the nervous system, with brain AChE being the primary neurological endpoint of concern (Farahat et al., 2003; Jamal et al., 2002). Inhibition of AChE leads to a decrease in hydrolysis of acetylcholine in cholinergic synapses, resulting initially in overstimulation of nicotinic and muscarinic receptors followed by receptor down-regulation on post-synaptic membranes (Costa, 2006; Eaton et al., 2008; Koelle, 1981). It has been postulated that chronic OP neurotoxicity is due to different mechanisms, including CNS receptor deregulation, oxidative stress and inflammation (Banks and Lein, 2012; Costa, 2006; Jamal et al., 2002), and chronic low level exposure in non-poisoned subjects has been associated with impaired neurobehavioral performance (Farahat et al., 2003; Ray and Richards, 2001; Rohlman et al., 2011).

Chlorpyrifos is an organophosphorothionate insecticide that is used extensively throughout the world (Eaton et al., 2008). Chlorpyrifos undergoes cytochrome P450 (CYP)-mediated bioactivation to the potent B-esterase inhibitor, chlorpyrifos-oxon (CPF-O), in addition to being detoxified to trichloro-2-pyridinol (TCPy) (CAS 6515-38-4) (Foxenberg et al., 2007). TCPy is excreted in urine and has been used previously as a biomarker of exposure to chlorpyrifos in this worker population (Farahat et al., 2010, 2011; Fenske et al., 2012).

Profenofos (PFF) is an organophosphorothiolate insecticide that was developed for pests with resistance to chlorpyrifos and other OPs (Gotoh et al., 2001). In contrast to the majority of OP insecticides, which require bioactivation to their oxon metabolite, the parent form of PFF is a potent inhibitor of AChE (Das et al., 2006; Nillos et al., 2007). CYP-mediated metabolism of PFF results in oxidative bioactivation and detoxification reactions (Abass et al., 2007; Wing et al., 1983). PFF is metabolized to the detoxified metabolite 4-bromo-2-chlorophenol (BCP) (CAS 3964-56-5), which is excreted in urine and can be used as a sensitive and specific biomarker of exposure to PFF (Dadson et al., 2013).

OPs such as CPF and PFF inhibit cholinesterases (ChE) (Das et al., 2006; Sparks et al., 1999), thus, plasma BChE and red blood cell (RBC) AChE have been used as biomarkers of exposure and effect in both occupational and non-occupational OP exposure studies (Rohlman et al., 2011). Studies of occupational exposure to CPF demonstrate a concentration-dependent inverse relationship between urinary TCPy and the activity of both plasma BChE and RBC AChE (Farahat et al., 2011; Garabrant et al., 2009). BChE is more sensitive to inhibition by CPF than RBC AChE and thus, is considered a more sensitive biomarker (Farahat et al., 2011; Nolan et al., 1984). Inhibition of BChE is not known to cause detrimental health effects (Lotti, 1995; Zhao et al., 2006).

Approximately 40% of the Egyptian workforce is employed in agriculture, making it the largest industrial sector in Egypt (Abdel Rasoul et al., 2008). The Egyptian Ministry of Agriculture directs the application of multiple insecticides in the Nile delta to assure an optimal cotton crop. While relying primarily on CPF, the serial application of CPF, PFF and the pyrethroid insecticide alpha-cypermethrin (aCM) has been the recent practice. Previous studies have reported biomarkers of CPF and aCM in Egyptian agriculture workers (Farahat et al., 2011; Singleton et al., 2014). Farahat et al. (2011) investigated CPF exposure and effects in a cohort of Egyptian cotton field workers by determining the relationship between biomarkers of CPF exposure (urinary TCPy) and effect (blood AChE and BChE). However, there is little data available on human biomarkers of PFF (Dadson et al., 2013), and essentially nothing is known about combined exposures to PFF and CPF in humans.

Since human occupational exposures to insecticides often involve multiple agents, it is useful to characterize the comprehensive exposure to multiples OPs to better assess human risk over the duration of the insecticide application period. The present study is the first to report a longitudinal assessment of serial occupational exposure to the OPs, CPF and PFF. The objectives of the present study are to: (1) Conduct a comprehensive characterization of exposure to CPF and PFF in Egyptian cotton field workers; (2) determine the relationship between urinary BCP and blood ChE activity during PFF exposure in these workers; (3) Determine if individuals who are highly exposed to CPF are also highly exposed to PFF; (4) Determine if and to what extent urinary TCPy and BCP levels return to baseline after the application of CPF and PFF has ceased.

Materials and methods

Study setting, population, and insecticide application

A detailed description of this study population has been reported elsewhere (Dadson et al., 2013; Farahat et al., 2011; Singleton et al., 2014). In brief, the current study takes place in Menoufia, a governorate of Egypt, which is situated in the Nile River Delta north of Cairo. Egypt's Ministry of Agriculture directs the use and application of insecticides in cotton fields and employs agricultural workers. Ministry of Agriculture employees are assigned to one of the following job categories as described by the Ministry of Agriculture: applicators who apply insecticides with backpack sprayers; Technicians who walk each row with the applicator to direct the path of application; and Engineers who periodically walk the field but mostly direct the application process from the edge of the field. Mixing and loading of pesticides in backpack sprayers, where there was opportunity for exposure, was informally observed to be completed primarily by applicators, and occasionally by technicians and engineers. The workers are based out of regional field stations that serve as a place to receive training and direction, and a storage area for insecticides and application equipment. During the summer of 2008, exposure and effect biomarkers (for PFF and CPF) were comprehensively assessed prior to, during, and following application of CPF and PFF at 3 field stations (Q1–Q3). Demographic characteristics of the workers at each field station are summarized in Table 1. Table 2 summarizes the pesticide application schedule for each of the 3 field stations.

Urine samples

During the summer of 2008, spot urine samples were collected daily from the workers at the beginning of each work day. Samples were placed on wet ice in a cooler and transported to Menoufia University (Shebin El-Kom, Egypt) where they were stored at −20 °C until shipped on dry ice to the University at Buffalo (Buffalo, NY) for analyses.

BCP and TCPy analysis

Urine specimens were analyzed for the presence of BCP, the detoxified metabolite of PFF, using previously described methods (Dadson et al., 2013). In brief, a 1 ml aliquot of each urine sample was thawed and mixed prior to the addition of internal standard 2,4,5 trichlorophenol. Samples were then hydrolyzed to free any conjugated BCP (Gotoh et al., 2001) at 80 °C for 1 h with 100 μL of 12 N HCl, and extracted with 1 ml of toluene. The toluene

Table 1
Demographic characteristics of 2008 cohort of Ministry of Agriculture workers.

Characteristic	Applicators ($n=14$)	Technicians ($n=12$)	Engineers ($n=12$)	ANOVA p-value
Age (years)	25.1 ± 11.4*	48.8 ± 3.8	46.3 ± 3.2	<0.0001
Height (cm)	169.5 ± 6.5	169.8 ± 5.0	172.8 ± 2.8	0.227
Weight (kg)	73.5 ± 14.6	79.5 ± 9.7	81.6 ± 12.6	0.247
Body mass index (kg/m^2)	25.4 ± 3.7	27.5 ± 2.4	27.3 ± 4.1	0.263

* $p<0.0001$ Compared with the two other job categories, determined by one-way ANOVA with Tukey's post hoc analysis (from Farahat et al., 2011).

Table 2
Daily insecticide application schedule for chlorpyrifos, α-cypermethrin, and profenofos during the summer of 2008.

Field station[a]	Chlorpyrifos application		α-cypermethrin application		Profenofos application		Chlorpyrifos application	
	Start	End	Start	End	Start	End	Start	End
1	1-Jul	17-Jul	19-Jul	28-Jul	30-Jul	9-Aug	12-Aug	16-Aug
2	6-Jul	14-Jul	19-Jul	26-Jul	30-Jul	7-Aug	12-Aug	16-Aug
3	7-Jul	14-Jul	19-Jul	24-Jul	30-Jul	7-Aug	12-Aug	16-Aug

[a] Region where cotton fields were sprayed daily by Ministry of Agriculture workers; Blood was drawn on June 25, July 2, July 10, July 24, August 9, August 23.

extract was then derivatized with BSTFA at 70 °C for 1 h and analyzed by gas chromatography–micro electron capture detection (GC/μECD). Urine specimens were analyzed for TCPy (the detoxified metabolite of CPF) by negative-ion chemical ionization gas chromatography–mass spectrometry, using 13C-15N-3,5,6-TCPy as an internal standard, as described previously (Crane et al., 2013; Farahat et al., 2010, 2011; Khan et al., 2014). Briefly, samples for TCPy analyses were hydrolyzed with hydrochloric acid, extracted with toluene and derivatized using MTBSTFA. Creatinine concentrations were measured for all workers using the Jaffe reaction (Fabiny and Ertingshausen, 1971). Urinary BCP and TCPy concentrations are expressed as μg/g creatinine.

Blood collection and analysis of BChE and AChE activity

A single pre-exposure blood sample was collected on June 25th, prior to the start of the official government-regulated insecticide application season (Table 2) to establish the baseline ChE activity for each worker. Additional blood samples were collected on July 2nd and July 10th (during the CPF application); prior to the PFF application on July 24th, and a post-PFF exposure blood sample was collected when PFF spraying had ended at all three field stations on August 9th. The final blood sample was collected at the end of the insecticide application season on August 23rd. The blood sample collection procedure has been described in detail previously (Crane et al., 2013; Farahat et al., 2011). In brief, blood samples were collected by venipuncture into 10 ml lavender top (EDTA) Vacutainer tubes and analyzed in triplicate for AChE and BuChE activity using an EQM Test-Mate kit (EQM Research Inc., Cincinnati, OH, USA). The intraclass correlation coefficient for BChE with this method is 0.987 and for AChE is 0.898.

Statistical analysis

Selected demographic characteristics between job categories (applicators, technicians, and engineers) were compared using one-way analysis of variance (ANOVA) and Tukey's post hoc analysis with significance set at $p<0.05$ (Table 1). Urinary metabolite levels among the applicators, technicians, and engineers were each compared by time point using the non-parametric Kruskal–Wallis 1-way ANOVA followed by Mann–Whitney U post hoc analysis (alpha = 0.05) (Fig. 2). The correlation of urinary TCPy and BCP excretion was assessed by Pearson product-moment correlation coefficient (Fig. 3). ChE activities were compared to baseline using the paired t-test (Table 3) and across job categories by using 1-way ANOVA with Dunnett's post hoc analysis (Figs. 4 and 5).

Results

Study population and application schedule

Table 1 summarizes demographic data for the cohort of Egyptian Ministry of Agriculture workers that applied insecticides to cotton fields during the summer of 2008. Applicators were younger than the technicians and engineers, while all other characteristics were similar between the three job classifications. Table 2 shows the

Table 3
Average BChE and AChE activity from Egyptian agricultural workers prior to (June 25), during (July 24, Aug 9) and post (August 23) OP application.

	Field station 1			Field station 2			Field station 3		
	Applicators	Technicians	Engineers	Applicators	Technicians	Engineers	Applicators	Technicians	Engineers
	($N=5$)	($N=5$)	($N=4$)	($N=4$)	($N=2$)	($N=3$)	($N=5$)	($N=5$)	($N=5$)
	Mean ± standard deviation								
Plasma BChE (U/ml)									
25-Jun	0.07 ± 0.06	1.55 ± 0.47	0.99 ± 0.39	1.18 ± 0.61	1.49 ± 0	1.55 ± 0.31	1.45 ± 0.62	0.83 ± 0.46	1.79 ± 0.53
24-Jul	0 ± 0.00*	0.81 ± 0.19	0.53 ± 0.33*	0.02 ± 0.04*	1.24 ± 0	1.18 ± 0.19	0.59 ± 0.38*	0.85 ± 0.62	1.56 ± 0.76
9-Aug	0 ± 0.01	0.57 ± 0.37*	0.52 ± 0.34	0.10 ± 0.09*	1.29 ± 0.04	1.25 ± 0.46	0.59 ± 0.31*	0.88 ± 0.41	1.62 ± 0.68
23-Aug	0.24 ± 0.41	0.79 ± 0.35	0.79 ± 0.44	0.10 ± 0.13	1.04 ± 0	1.38 ± 0.15	0.88 ± 0.59	1.05 ± 0.56	1.66 ± 0.67
RBC AChE (U/g Hgb)									
25-Jun	23.7 ± 4.0	25.5 ± 3.7	24.4 ± 2.7	23.2 ± 2.0	27.1 ± 3.0	25.7 ± 4.1	27.7 ± 2.3	24.7 ± 1.5	27.2 ± 3.6
24-Jul	7.1 ± 3.9	25.4 ± 4.1	27.0 ± 2.3	20.8 ± 2.6	26.7 ± 0	24.7 ± 3.6	26.4 ± 3.2	25.8 ± 2.1	29.9 ± 2.7
9-Aug	4.3 ± 4.0*	25.5 ± 5.8	23.8 ± 3.1	16.5 ± 7.1	29.3 ± 2.9	29.5 ± 4.4	28.8 ± 2.8	26.8 ± 2.1	29.0 ± 3.4
23-Aug	4.3 ± 2.9*	24.2 ± 5.5	27.2 ± 4.0	24.0 ± 3.1	29.5 ± 0	24.9 ± 4.5	28.7 ± 2.7	26.6 ± 1.8	30.8 ± 1.6

* $p<0.05$. Depressed when compared with the baseline (25 June) activities for each group by job category and field station, determined by paired t-test.

2008 insecticide application schedule consisted of daily application of CPF followed by αCM, PFF, and a second CPF application for each of the 3 field stations or regions.

Urinary TCPy concentrations (CPF exposure biomarker)

Fig. 1 illustrates a comprehensive, longitudinal assessment of daily urinary TCPy values for the cotton field workers in the three field stations prior to, during, and after the insecticide application season. This is a more comprehensive assessment than reported previously (Farahat et al., 2011) and clearly demonstrates that each field station represents a distinct exposure scenario. At the start of the first CPF application in field station 1(June 28), TCPy levels in applicators increased compared to their respective baseline values and remained elevated from baseline until July 26 (Fig. 1). Following the cessation of the first CPF application (on July 17), average urinary TCPy levels decreased for applicators, technicians and engineers in field station 1 until August 8th when values increased with the start of the second CPF application. Engineers and technicians in field station 1 demonstrated urinary TCPy levels greater than their respective baselines levels after the start of the CPF application and remained above baseline throughout the longitudinal study. Field station 2 workers demonstrated an elevation in urinary TCPy levels throughout the first CPF spray and average levels for applicators, technicians and engineers did not return to baseline throughout the study. Workers in field station 3 did not experience peak TCPy levels of the same magnitude as the other 2 field stations; however, the mean urinary TCPy of applicators were elevated from baseline throughout most of the longitudinal study. At the start of the second CPF application on August 12, urinary TCPy levels steadily increased for all job categories in all 3 field stations, and (with the exception of field station 3 engineers) were still elevated compared to baseline on the final day of the study (August 18).

Urinary BCP concentrations (PFF exposure biomarker)

The mean urinary BCP concentrations were compared for each job category within each field station (Fig. 2). A log scale was used to illustrate the urinary BCP concentration prior to, during, and after the PFF spraying period for the three field stations. Prior to the start of PFF application on July 30th, all workers, regardless of work-station or job category, had detectable levels of BCP. In field station 1, the peak urinary BCP levels during the PFF spray period (August 3), were significantly higher for applicators (1234.1 μg/g creatinine) than technicians (36.9 μg/g creatinine) and engineers (49.5 μg/g creatinine). Field station 1 workers had the highest levels of urinary BCP when compared to workers from the other two field stations. In field stations 2 and 3, the applicators were also the most highly exposed work group (Fig. 2). At the end of the PFF application (August 7–9), urinary BCP levels gradually decreased across all three-job categories. When comparing the PFF post-spraying period (August 10–August 16) to the PFF pre-spraying period (July 22–July 28), the urinary BCP levels remained elevated for 6–10 days after the cessation of the PFF application for applicators, technicians and engineers in all three field stations.

Correlation between TCPy and BCP concentrations

Peak urinary TCPy and BCP concentrations were compared for each worker during the CPF or the PFF application period, respectively, to determine if there was a correlation between the peak levels of the 2 biomarkers of exposure (Fig. 3). Statistical analysis of the scatter plot in Fig. 3 found a significant positive linear correlation between worker peak urinary concentrations of TCPy and BCP

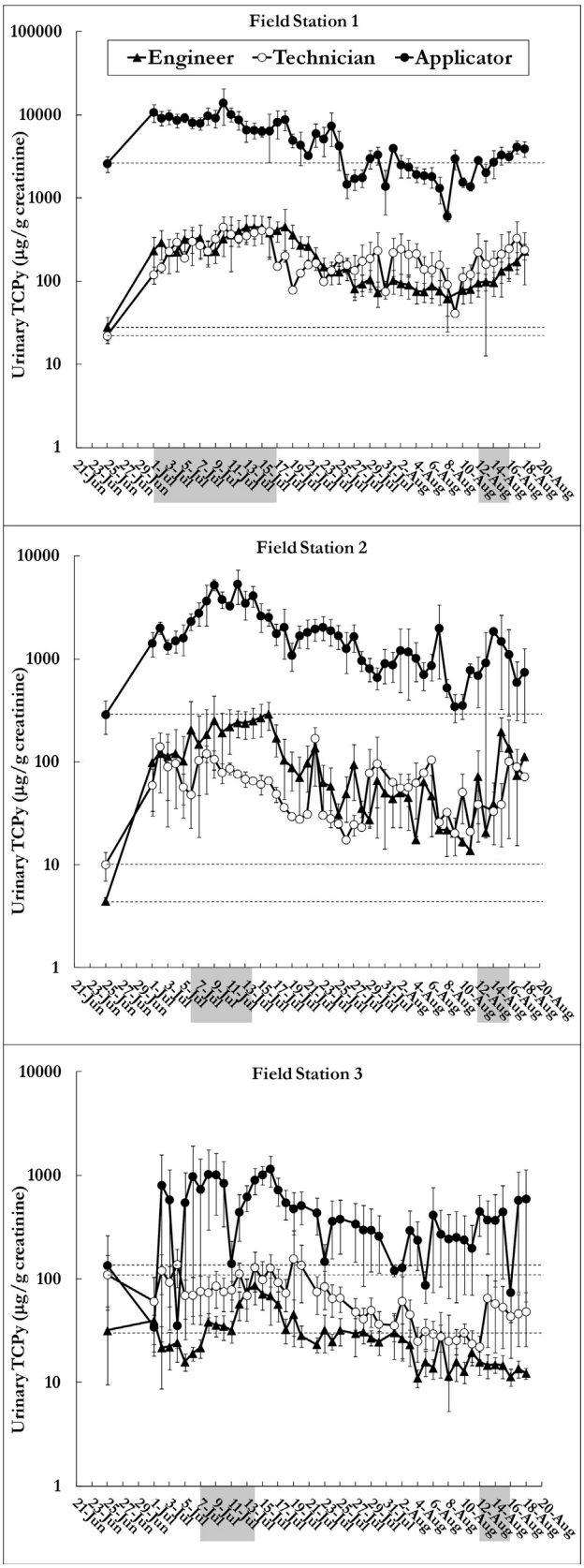

Fig. 1. Longitudinal assessment of urinary TCPy levels (mean ± SE) for applicators, technicians, and engineers in 2008. $n = 2–5$ workers for each job category in a given field station. Gray shading identifies the dates of the CPF application.

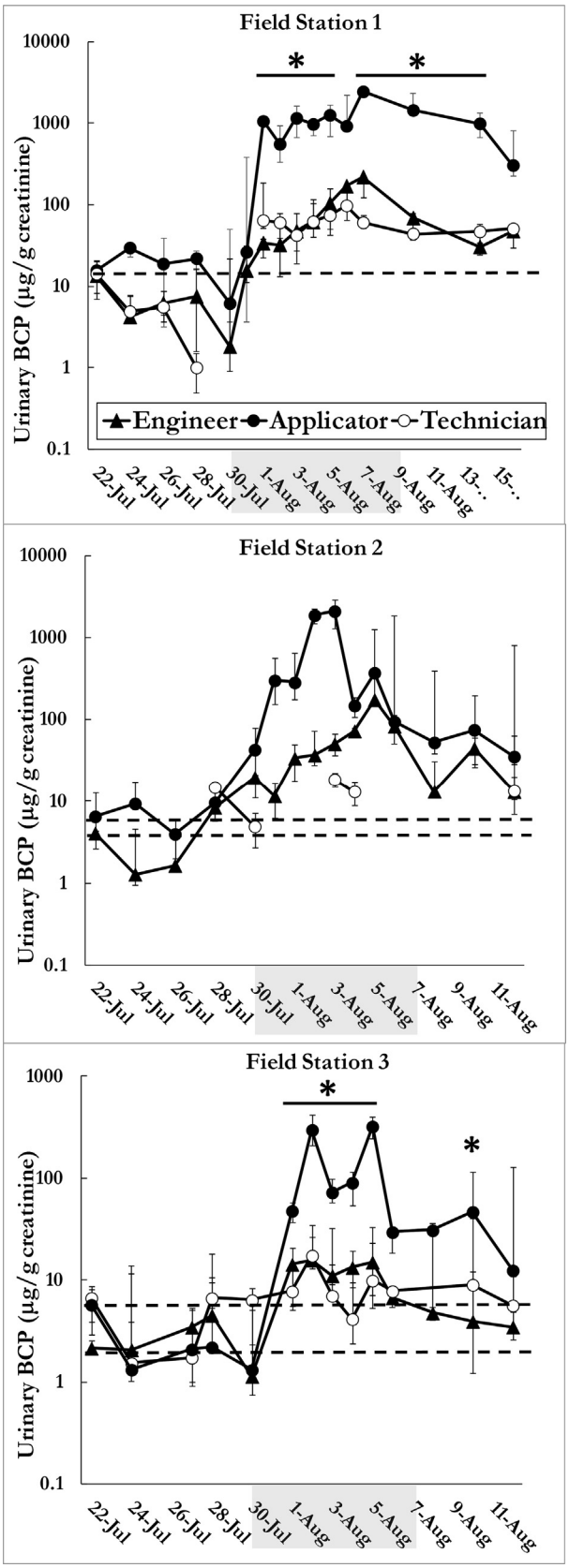

Fig. 2. Longitudinal assessment of urinary BCP levels (median ± interquartile range) for applicators, engineers and technicians before, during and after the profenofos application (gray shaded region) to the cotton fields. $n = 2–5$ workers for each job category in a given field station. * $p < 0.05$ for applicators compared to the other job categories; Kruskal–Wallis one-way ANOVA with Mann–Whitney U post hoc analysis.

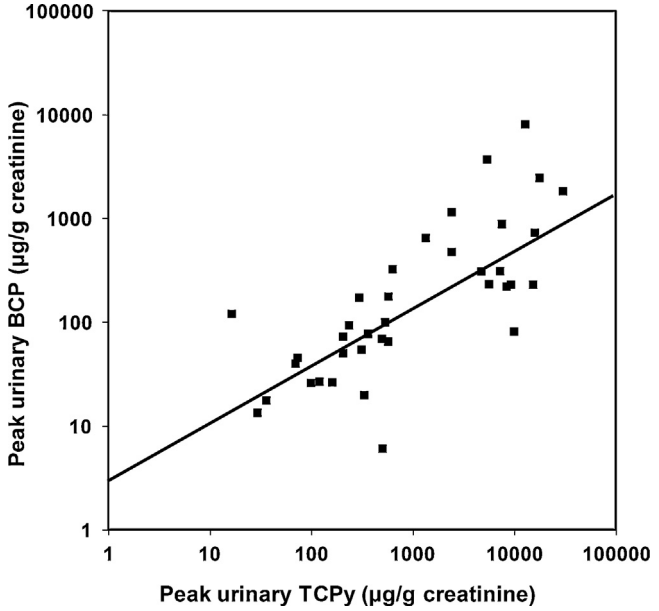

Fig. 3. The correlation between peak urinary levels of BCP and TCPy for applicators, technicians and engineers during the summer of 2008 (Pearson correlation (95% confidence interval) $r = 0.75$ (0.52–0.98), $n = 37$ workers, $p < 0.001$).

(Pearson correlation (95% confidence interval) $r = 0.75$ (0.52–0.98), $n = 37$ workers, $p < 0.001$).

Cholinesterase activity

The plasma BChE and RBC AChE activity measured in blood samples during the summer of 2008 insecticide application cycle are shown in Figs. 4 and 5, and Table 3. The activity for June 25, July 2, 10 and 24 has been previously reported (Farahat et al., 2011). Individual baseline measurements (June 25) of BChE activity ranged widely (0–2.48 U/ml blood) depending on job category and field station. Interestingly, the mean baseline BChE activity of field station 1 applicators was close to zero (0.07 U/ml blood), and remained suppressed throughout the study until August 9th when activity increased slightly (although not statistically significant) to 0.23 U/ml blood (Fig. 4). On July 24th, eight to ten days after the first designated CPF application period had ended, BChE activity was suppressed relative to baseline measurements in most workers, regardless of field station or job category (Fig. 4; Table 3). The agriculture workers were stratified by field station and job category resulting in small sample sizes, limiting the power of statistical analyses. However, a statistically significant decrease was observed in the BChE activity of applicators in all three field stations compared to the other job categories for two or more time points (Fig. 4). Baseline RBC AChE activities varied for all workers across all 3 field stations and between job categories, with individual values ranging from 20.9 to 30.7 U/g hemoglobin. After cessation of the first CPF application (July 24th), applicators from field station 1 were the only group to show a statistically significant decrease in AChE activity ($p < 0.05$) (Table 3). On August 9th, just after the end of the PFF application, average applicator AChE activities in Field stations 1 and 2 were depressed when compared to the pre-PFF activity level on July 24th (Fig. 5; Table 3). Field station 3 AChE activity was not significantly depressed compared to baseline, and remained in the average range of 24.7–30.8 U/g HGB for engineer, technicians, and applicators throughout the study (Fig. 5). Field station 1 AChE activity was significantly depressed in applicators when compared to the other job categories in the middle of the first CPF application (July 10) and remained depressed for the rest of the study (Fig. 5). In

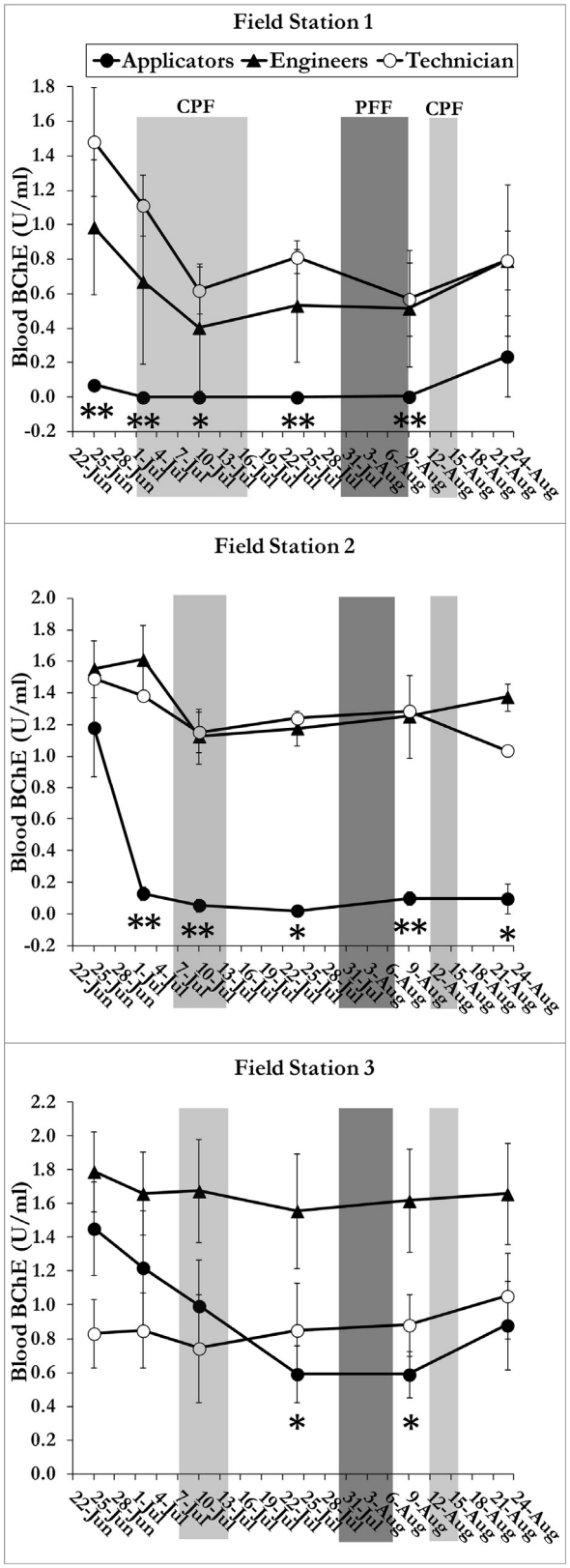

Fig. 4. Longitudinal assessment of plasma butyrylcholinesterase activity (mean ± SE) in applicators, engineers and technicians before during and after the insecticide application. $n = 2–5$ workers for each job category in a given field station. CPF = chlorpyrifos (light shading); PFF = profenofos (dark shading). ** Applicators depressed as compared to the other 2 job categories; * applicators depressed as compared to technicians only (field station 1); or engineers only (field stations 2 and 3); one-way ANOVA with Dunnett's post hoc analysis ($p < 0.05$).

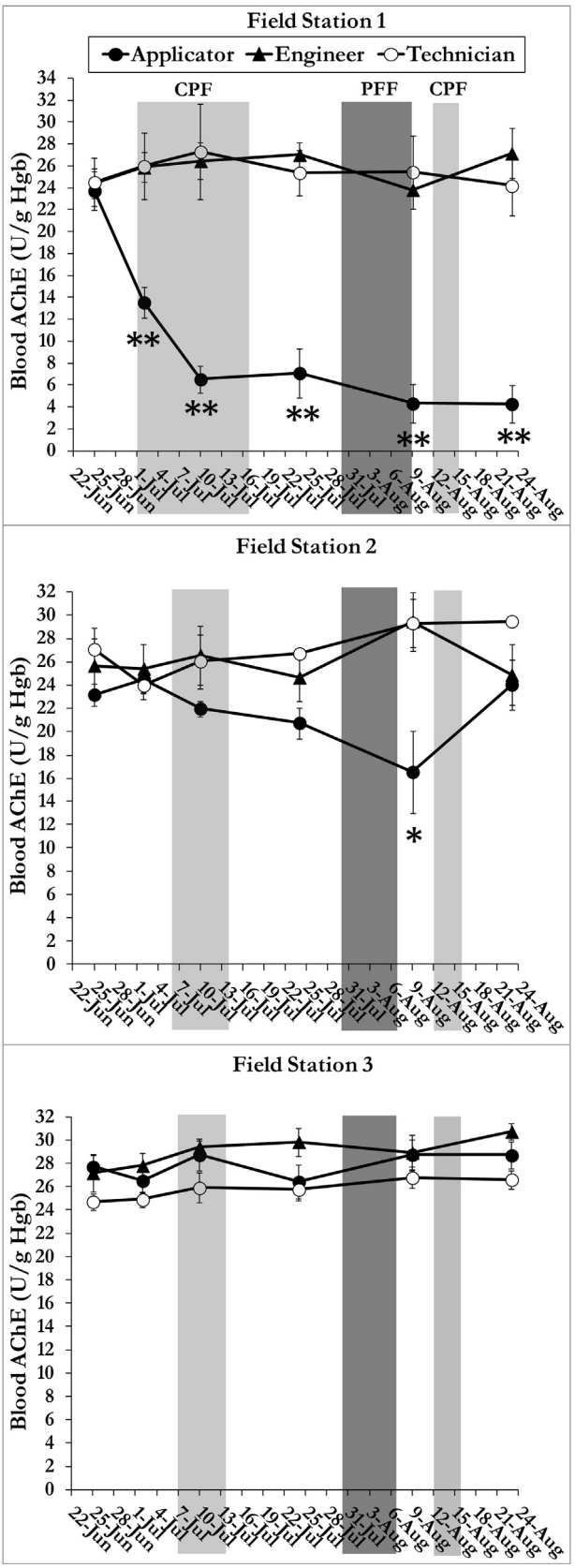

Fig. 5. Longitudinal assessment of erythrocyte acetylcholinesterase activity (mean ± SE) in applicators, engineers and technicians before during and after the insecticide application. $n = 2–5$ workers for each job category in a given field station. CPF = chlorpyrifos (lightshading); PFF = profenofos (dark shading). ** Applicators depressed as compared to the other two job categories; * applicators depressed as compared to engineers; one-way ANOVA with Dunnett's post hoc analysis ($p < 0.05$).

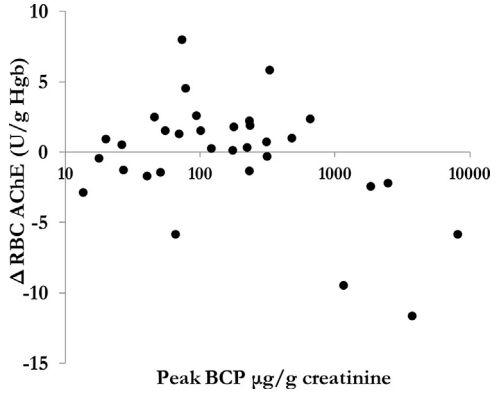

Fig. 6. The relationship between peak urinary BCP levels and the change in red blood cell acetylcholinesterase for each subject (n = 32 workers) before (July 24) and after (August 9) the PFF application.

field station 2, applicators AChE activity was significantly depressed compared to the other workers on August 9th, just after the end of the PFF application.

Relationship between urinary BCP and cholinesterase activity

While Fig. 5 suggests that PFF application is producing a further inhibition in blood AChE activity, the results in Fig. 4 suggest that PFF application in not altering BChE activity. In an attempt to assess the impact of PFF exposure on blood AChE activity, the relative change in blood AChE activity from the pre- (July 24) to the post- (August 9) PFF application was plotted against the peak urinary BCP for individual workers from all 3 field stations (Fig. 6). 13 of 32 participants showed a decrease in blood AChE activity on August 9th relative to that prior to PFF exposure on July 24th. Several workers had peak urinary BCP levels greater than 1000 μg/g creatinine, and all five of these participants had a decrease in blood AChE activity following the PFF exposure period.

Temporal changes in blood AChE levels over the entire study period are shown in Fig. 7 for individual workers with peak urinary BCP levels greater than 1000 μg/g creatinine. These five individual workers had baseline blood AChE levels (June 25) ranging from 20.7 to 30.7 U/g Hgb. Field station 2 workers (Q2–27 and Q2–28), blood AChE levels were approximately equal to their respective baseline (June 25) levels on July 24 (just prior to the start of the PFF application), and demonstrated the greatest decrease in blood AChE (11.6 and 9.4 U/g Hgb, respectively) after the PFF application (August 9). The observed inhibition in blood AChE activity following PFF application (August 9) was followed by a recovery to near baseline levels for these 2 workers at the end of the study (August 23). Field station 1 workers (Q1–6, Q1–7, and Q1–8) average blood AChE levels were depressed following CPF exposure on July 24th (7.1 U/g Hgb) compared to their baseline levels (25.2 U/g Hgb) (Fig. 7). Following the cessation of PFF application on August 9th, average AChE activity for these 3 workers was further depressed to 3.7 U/g Hgb. No apparent recovery of blood AChE activity was observed in these 3 workers at the end of the study (August 23).

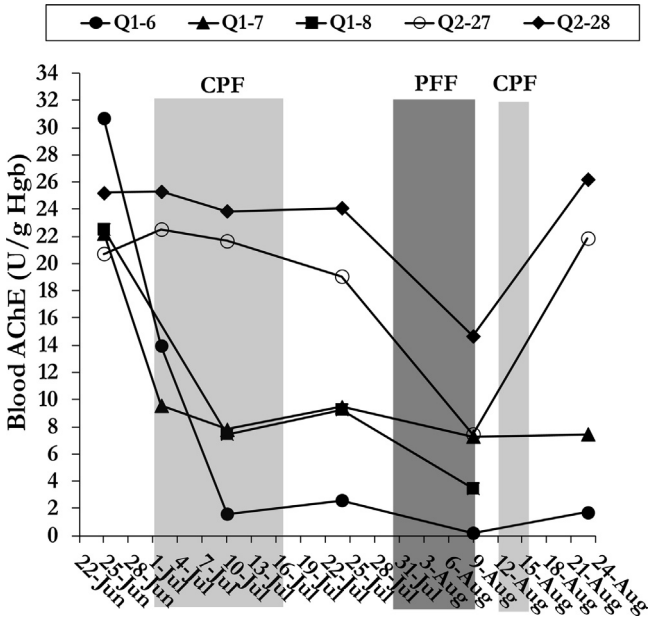

Fig. 7. Longitudinal assessment of red blood cell acetylcholinesterase in individual workers having peak urinary BCP levels greater than 1000 μg/g creatinine. CPF = chlorpyrifos (light shading); PFF = profenofos (dark shading). Q1 = field station 1; Q2 = field station 2.

Discussion

The present study characterized longitudinal changes in biomarkers of exposure and effect for the OPs, CPF and PFF over the insecticide application season. Adult Egyptian workers were hired by the Egyptian Ministry of Agriculture to apply insecticides to the 'national' cotton crop, which is planted and harvested by independent family farms. Teams of insecticide applicators, technicians, and engineers work out of small regional field stations, and coordinate the daily application of insecticides on cotton fields near each field station. Urinary TCPy and BCP levels were measured throughout the summer in the Egyptian agriculture workers as biomarkers of exposure for CPF and PFF, respectively. Plasma BChE and RBC AChE were measured as OP biomarkers of effect. The results demonstrate a wide range of exposures between workers within the same job category, between job category, and between field station. Detectable amounts of urinary TCPy prior to the start of CPF application (June 25), and BCP prior to the start of PFF application (July 22), are likely due to the periodic use of insecticides at home or elsewhere, and background environmental exposures from food, water and soil in the Nile delta. Mixing procedures that are carried out in the days leading up to the start of application are another potential source of CPF and PFF exposure. The length of the CPF and PFF spray period (Table 2) and the magnitude of urinary biomarkers levels (Figs. 1 and 2) varies between field stations, indicating that the three field stations represent three distinct exposure scenarios. It also suggests that work practices differed between field stations 2 and 3, specifically that engineers were more involved in pesticide mixing and/or walking the fields than technicians in field station 2, and less involved in these practices in field station 3.

Biomarkers of exposure

Measurable urinary TCPy levels were detected in all cotton field workers from all three field stations throughout the season, suggesting chronic exposure to CPF throughout the longitudinal study (Fig. 1). A biomonitoring study on a similar Nile delta agriculture worker population determined that ~95% of the OP dose is via the dermal route (Fenske et al., 2012) and likely occurs when workers enter previously treated fields and are exposed to insecticides because of skin contact with treated plants. Throughout the period prior to, during, and following CPF exposure, the applicators had higher levels of urinary TCPy than the technicians and engineers. These levels increase with the CPF application periods and in many cases do not return to baseline levels.

This is the first longitudinal study conducted to assess the occupational exposure of agriculture workers to PFF. Urinary BCP

serves as a sensitive and specific biomarker of exposure to PFF (Dadson et al., 2013). These workers exhibit a wide range of urinary BCP levels, with applicators consistently showing the highest levels relative to engineers and technicians. This is consistent with their respective job descriptions. Workers in each field station had increased urinary BCP levels associated with the period of PFF application (Fig. 2). The lack of personal protective equipment (PPE) among these workers allows for direct contact of the skin with spray residue containing OPs. As with urinary TCPy, significantly greater urinary BCP concentrations were found in workers at field station 1. This likely reflects the fact that workers in this field station were applying insecticides to larger cotton fields than workers in field stations 2 and 3. As the application time periods were largely set at a national level, workers in field station 1 had extra days of CPF and PFF application and longer work-days, and thus greater opportunity for exposure to CPF and PFF (Table 2).

In general, the urinary BCP levels remained above the pre-spray period for approximately 8 days after the end of the PFF spray period (Fig. 2). This may be explained by several factors, including continued exposure to PFF, slow metabolism or inter-individual variability in metabolism and excretion of the urinary biomarker of exposure. The lack of information regarding the half-life of PFF in humans precludes further interpretation of this observation. Similar trends in exposure are observed in the same population spraying CPF, where the urinary metabolite TCPy remained elevated in urine from baseline (pre-spray) for 7 or more days after the end of the CPF spraying period (Fig. 1) (Farahat et al., 2011). Additional factors that may account for these similar findings include: (1) workers may not regularly wash their insecticide-contaminated clothing, which could result in prolonged dermal absorption even after the end of spraying; (2) workers from all three job categories may use insecticides in some capacity outside of their ministry jobs (Farahat et al., 2011); and (3) daily dermal exposure to these OP insecticides may result in a subcutaneous deposit of insecticide in deep compartments (such as fat), slowing the rate of metabolism and excretion of metabolites (Wester and Maibach, 1985).

Peak BCP vs. peak TCPy

Peak TCPy and peak BCP concentrations were compared for each worker during the CPF and PFF application period, respectively, to determine whether there is a correlation between the peak urinary levels of the two biomarkers of exposure. Statistical analysis of the scatter plot (Fig. 3) indicates that the peak levels of these two metabolites were positively correlated, suggesting that workers who were highly exposed to CPF were also highly exposed to PFF. This common magnitude of exposure may be explained by the individual's job category, field station location and, additionally, by their work and hygiene practices. Subjects who consistently work in bare feet, mix and load insecticides with bare hands, and wear insecticide soaked clothing consistently maintain these habits and, thus, would be expected to consistently receive greater exposure to the insecticides. Together these observations support the need to implement better work practices and efforts to protect the workers from these high exposures.

Cholinesterase activity

Average BChE activity (0.07 U/ml) at baseline (June 25) was significantly depressed in field station 1 applicators when compared with technicians and engineers. This low level of BChE activity prior to the start of OP application in these applicators may indicate chronic toxicity, but may also occur due to the use of insecticides outside of their Ministry of Agriculture jobs. At the start of the first CPF application, the average BChE activity of applicators fell below detectable levels and remained so until the final day of the study (August 23), corresponding to eight days after cessation of the second CPF application. Field station 1 workers did not show any significant differences in their baseline AChE activities, but following the start of the first CPF application, average AChE levels were severely depressed (down to 18% of baseline activity in applicators) and remained depressed throughout the longitudinal study. At the end of the PFF application (August 9), in field stations 1 and 2, average AChE and BChE activities did not significantly recover when compared to the PFF pre-spray activities (July 24) and were significantly depressed in applicators when compared to technicians and engineers. This is not surprising given the high levels of average urinary BCP in applicators.

PFF was sprayed approximately 2 weeks following the end of a CPF spraying period followed by a second CPF application. The combination of PFF and CPF exposure has greater potential to inhibit ChE activity. A study by Lakew and Mekonnen (1998) of 81 pest control workers with exposures to both OPs reported that worker mean blood BChE and AChE activity was significantly lower (~55% and ~77% from baseline, respectively) than pre-exposure levels. Future additional studies are needed to assess the impact of exposures to both of these insecticides on metabolism kinetics and cholinesterase inhibition.

Relationship between urinary BCP and cholinesterase activity

This is the first study to attempt to identify a relationship between urinary BCP and ChE activity. While these analyses are limited to associations with urinary BCP levels, it is acknowledged that workers were previously exposed to CPF which may contribute to the observed results since the magnitude of CPF exposure was highly correlated with that of PFF (Fig. 3). Blood for ChE activity was collected on August 9th, which corresponds to the end of the PFF application period. A previous longitudinal study with CPF found that when assessing relative magnitude of CPF exposure, cumulative TCPy and peak TCPy were highly correlated for each worker and considered to be equal dosimetric options (Crane et al., 2013). Analysis of blood AChE levels before (July 24) and after (August 9) the PFF application period suggests that individual workers with peak BCP levels greater than 1000 μg/g creatinine exhibited further inhibition of blood AChE with PFF application (Figs. 6 and 7), demonstrating that PFF exposure had a negative impact on AChE activity in this highly exposed worker population. Individual workers Q2–27 and Q2–28 had the greatest depression in blood AChE over the course of the PFF spray period (August 9), and their pre-PFF (July 24) AChE activity was not significantly different from their baseline (June 25) levels (Fig. 7). These results indicate that their respective blood AChE depression was due to either PFF exposure alone, or possibly a consequence of the combined serial exposures to both CPF and PFF. In addition to having the greatest magnitude of blood AChE depression before and after the PFF exposure period, subjects Q2–27 and Q2–28 demonstrated a recovery to near baseline levels at the final blood collection of the study (August 23). The observed recovery was surprising, and while these results are extremely limited, the observations in these 2 heavily exposed workers suggest that PFF may have a less than expected prolonged effect on its interaction with blood AChE. Given the long half-life of blood AChE in humans (~50 days), the fast recovery of AChE activity seen in these individuals is presumably due to regeneration of the inhibited enzyme, in the absence of de novo synthesis.

Conclusion

Urinary TCPy and BCP are sensitive and specific biomarkers of exposure to the OPs, CPF and PFF, respectively. While large interindividual differences in exposure were observed throughout

this longitudinal study, these urinary biomarkers were highly correlated within workers, suggesting that relative exposures to CPF and PFF were highly correlated for a given worker. The variable exposures between job classification and work site suggest that job title should not be used as the sole basis for categorizing OP exposures when assessing neurobehavioral and other health outcomes in Egyptian cotton field workers. Individual workers that were highly exposed to PFF demonstrated the greatest magnitude of blood AChE depression following the PFF application, however, the observation of a relatively rapid recovery of AChE following PFF exposure will need to be confirmed by future studies. Together, these findings will be important in educating the Egyptian insecticide application workers in order to encourage the development and implementation of work practices and personal protective equipment to reduce their exposure to CPF and PFF.

Conflicts of interest statement

The authors declare that there are no conflicts of interest.

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