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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