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Longitudinal assessment of occupational determinants of chlorpyrifos exposure in adolescent pesticide workers in Egypt

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ABSTRACT

Chlorpyrifos (CPF) is an organophosphourus insecticide applied to cotton fields by adolescents employed by the Egyptian Ministry of Agriculture. Urinary 3,5,6-trichloro-2-pyridinol (TCPy) is a biomarker of CPF exposure that has substantial variability among these applicators. In order to identify predictors of CPF exposure, we conducted a longitudinal study of 43 adolescent pesticide applicators in Egypt from April 2010 to January 2011 in Egypt. Urinary TCPy was quantified at 25 time-points, prior to, during, and following application. We used log-linear regression and a best subset selection approach to identify the exposure determinants that were most predictive of cumulative TCPy and participants' highest TCPy values (peak exposure). Applicators had cumulative urinary TCPy levels ranging from 167 to 49,8208 μg/g creatinine. Total hours applying CPF (semi-partial $r^2 = 0.32$), and total hours in the field applying other pesticides (semi-partial $r^2 = 0.08$) were the strongest predictors of cumulative TCPy. Applicators had peak urinary TCPy levels ranging from 4 to 5715 μg/g creatinine. The amount of time applying pesticides prior to blood draw was the strongest predictor of peak TCPy (semi-partial $r^2 = 0.30$). We also observed evidence that wearing clean clothes to work was associated with lower longitudinal TCPy. Our results suggest there is an opportunity for targeted interventions, particularly related to hygiene or implementation of personal protective equipment usage to reduce CPF exposure among adolescent pesticide workers.

1. Introduction

Chlorpyrifos (CPF) is one of the most commonly applied organophosphorus pesticides (OPs) in the world (Foxenberg et al., 2011). Exposure to OPs, including CPF, is a public health concern because of the acute detrimental neurological impacts associated with acetylcholinesterase inhibition (Keifer and Firestone, 2007) and the potential of chronic exposures to produce deficits in neurobehavioral performance (Eskenazi et al., 2014; Khan et al., 2014; Rauh et al., 2011; Rohlman et al., 2016). OP exposure has also been associated with increased risk of developing certain cancers (Lerro et al., 2015) and poor respiratory function (Chakraborty et al., 2009; Fieten et al., 2009; Hoppin et al., 2002; Hoppin et al., 2006; Ohayo-Mitoko et al., 2000). These concerns are amplified when considering children and adolescents because they have a higher body surface area to volume ratio, which increases their dermal absorption of toxic compounds (Phillips et al., 1993). Furthermore, human and animal studies have demonstrated that younger individuals express lower levels of the OP detoxifying enzyme, paraoxonase (PON1) (Costa et al., 1999).

Previous studies in agricultural pesticide applicators in Egypt and Indonesia have reported that the primary route of exposure to CPF is through dermal absorption (Fenske et al., 2012; Kishi et al., 1995). An individual's internal dose of CPF is determined by the amount of CPF an individual is exposed to, which can be altered by workplace behaviors and hygiene, and their capability for metabolizing xenobiotics. In addition to the use of personal protective equipment (PPE) (Coble et al., 2011; Krenz et al., 2015), the method of pesticide mixing (Krenz et al., 2015), frequency and duration of spraying, pesticide formulation

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(granular vs. liquid) (Thomas et al., 2010a; Thomas et al., 2010b), contact with contaminated surfaces (Thomas et al., 2010b), and equipment malfunction (Alexander et al., 2006; Thomas et al., 2010b) can contribute to exposure. Personal hygiene (Curwin et al., 2002), laundering of clothing (Kishi et al., 1995), and eating while applying (Thomas et al., 2010b) have also been demonstrated to impact biomarkers of OP exposure.

Residential use of OPs (Fenske et al., 2002), diet (Beamer et al., 2012; Munoz-Quezada et al., 2012), parents who are employed in agriculture (Fenske et al., 2002; Lu et al., 2000; Rohitrattana et al., 2014), and living in close proximity to a farm (Fenske et al., 2002; Lu et al., 2000; Munoz-Quezada et al., 2012; Rohitrattana et al., 2014) are predictors of increased concentrations of OP metabolites in urine among children. We have previously reported that adolescents who apply pesticides, including CPF, seasonally in Egypt had higher urinary concentrations of TCPy and lower blood butyrylcholinesterase (BChE) activity when compared with non-applicators of similar age (Crane et al., 2013; Khan et al., 2014). Although occupation is the primary source of OP exposure among adults, (Munoz-Quezada et al., 2012) occupational exposure to OPs among adolescents has not been thoroughly evaluated. Determinants of exposure to OPs may differ between adolescents and adults. For instance, adolescent farmworkers in the US have been observed to engage in riskier work practices than their adult counterparts. These practices include engaging in higher pesticide exposure habits, such as less hand washing, wearing wet clothing, or wearing shorts instead of long pants (Kearney et al., 2015). In order to develop potential interventions to reduce CPF exposure among adolescents, determinants of exposure must be identified.

Our objective was to identify the determinants of CPF exposure among adolescent pesticide applicators by examining the relationship between urinary TCPy concentrations throughout a 10-month study period and workplace activities, hygiene habits, PPE use, and duration of applications. We expect that our results will aid in identifying methods to reduce exposure to CPF among adolescent applicators, a population that is particularly vulnerable to the deleterious effects of OPs.

2. Materials and methods

2.1. Study population and setting

We conducted a longitudinal study of Egyptian adolescents from April 2010 to January 2011 that included 57 applicators and 38 non-applicators. The analyses reported herein are restricted to 43 applicators that had information on body surface area. Of the applicators included in these analyses nine were 18 years old and one was 19 years old, the rest were under age 18. Details regarding the study setting and pesticide application process of this cohort have been described previously (Khan et al., 2014). Briefly, applicators employed seasonally by the Ministry of Agriculture to spray pesticides were recruited from two field stations in the region. Duties of the applicators included mixing pesticides and filling backpack sprayers, which were then used to apply pesticides to cotton fields in the Nile delta. Although CPF was the primary pesticide applied, participants also reported applying other agents, including bacillus thuringiensis, chlorfluazuron, penconazole, propamocarb hydrochloride, profenofos, atrazine, alpha cypermethrin, diflubenzuron, lambda cyhalothrin, and spinosad.

At enrollment participants completed a self-administered questionnaire with research staff available to provide clarification if needed to obtain information regarding participants' age; education; medical history; usual PPE use, including waterproof gloves; and pesticide use in their homes and gardens. Thirty-four subsequent assessments were conducted throughout the study at one of two field stations where participants donated spot urine samples for TCPy analyses; completed a brief follow-up questionnaire that queried recent symptoms, personal hygiene, and work behaviors, including hours worked and pesticides applied. Quantifications of urinary TCPy were available for baseline and 24 subsequent study sessions. Not all participants attended each session. Written consent was obtained from all participants and their legal guardian. The study was approved by the Oregon Health & Science University Institutional Review Board, and by the Medical Ethics committee of the Faculty of Medicine, Menoufia University.

The typical work schedule was from 8 am–12 pm and 3 pm–7 pm six days per week. Spot urine specimens were collected at field stations during the lunch break where they were placed on wet ice and transported to Menoufia University where they were stored at −20 °C until they were shipped on dry ice to the University at Buffalo for analysis.

2.2. Laboratory measurements

Negative-ion gas chromatography-mass spectrometry was used to quantify urinary TCPy. Samples were hydrolyzed with hydrochloric acid, extracted with toluene, and derivatized using N-(*tert*-butyldimethylsilyl)-N-methyltrifluoro-acetamide (Sigma Aldrich, USA). A 1 mL aliquot of each urine specimen was thawed and mixed prior to the addition of 100 ng of internal standard (13C-15N-3,5,6-TCPy). TCPy values were corrected for creatinine and are expressed as μg TCPy/g creatinine. Urinary creatinine was quantified using the Jaffe reaction. The within-run precision for TCPy analyses was excellent, as demonstrated by a $< 2\%$ coefficient of variation and an intraclass correlation coefficient between analytical replicates of 0.997 (Farahat et al., 2011). Participants provided an average of 19 samples over the course of the study. The limit of detection for the method was 0.5 ng/ml urine. The isotope-labeled analogue of 3,5,6-TCPy (13C-15N-3,5,6-TCPy) was used as the internal standard to account for matrix effects. The same degree of ion suppression or enhancement (if there is any resulted from the matrix) will be observed for the target native TCPy and its isotopically labeled analogue. The ratio of the two signals should not be affected, allowing for correct quantification in different matrices.

2.3. Statistical analyses

Cumulative urinary TCPy excretion was estimated by calculating the area under the curve. Each participant's excretion curve was graphed and integrated using the trapezoid rule to calculate the total TCPy excreted over the course of the study via Stata's pharmacokinetic function (Stata Corp. 2009. Stata Statistical Software: Release 11, StataCorp LP: College Station, TX, USA). Log-linear regression models were used to identify predictors of cumulative TCPy excretion (cumulative exposure model) as well as predictors of the highest TCPy concentration an individual experienced (peak exposure model). Concentrations of TCPy were natural log-transformed in these models. Beta coefficients from these models were transformed using the formula {[(exp(beta)) − 1]*100} to be presented as percent change in geometric mean urinary TCPy per unit increase of the characteristic.

Predictors considered for inclusion in the cumulative exposure model were age; body surface area (BSA), which was calculated using the DuBois formula ($BSA = 0.00718 \times \text{height cm}^{0.725} \times \text{weight kg}^{0.425}$)(Verbraecken et al., 2006); total hours applying CPF; total hours applying pesticides other than CPF; and applying pesticides at home. We also considered what clothing participants reported usually wearing while working in the field during the baseline questionnaire. These clothing options were pants, shorts to the knee, length of shirtsleeves, hat or head covering, closed shoes or open sandals, socks, bare feet, and bandana or neck scarf over the face. The response options were always, often, sometimes, seldom, or never, which were parameterized as ordinal values.

We assessed differences in the peak TCPy a participant excreted by workplace and hygiene behaviors using the Kruskal Wallis test. We restricted peak TCPy analyses to applicators who worked the day of their peak TCPy excretion. Potential predictors considered for inclusion in the peak exposure log-linear regression model, were, age, BSA, days