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Characterization of α-cypermethrin exposure in Egyptian agricultural workers

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ABSTRACT

Pyrethroids are neurotoxic insecticides that exert their effects by prolonging the open time of sodium channels, which increases the duration of neuronal excitation. α-cypermethrin (αCM) is derived from the 8-stereoisomers that together make up the pyrethroid cypermethrin, which is one of the most common pyrethroids being used in agriculture throughout the world. The objective of this study was to characterize the occupational exposure to αCM in a cohort of Egyptian agriculture workers ($n = 37$) before, during and after 6–10 consecutive days of application of αCM to cotton fields. Daily spot urine specimens were collected and analyzed by GC-MS NCI for the αCM metabolites 3-phenoxybenzoic acid (3-PBA) and cis-3-(2′,2′-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (cis-DCCA). Prior to αCM application, median urinary levels of 3-PBA (4.59 nmol/g creatinine) were greater than cis-DCCA (0.33 nmole/g creatinine) demonstrating low background exposures to pyrethroids. During the application period for αCM, median urinary levels of both biomarkers increased (13.44 nmol 3-PBA/g creatinine and 7.76 nmol cis-DCCA/g creatinine) and ranged from 2.3–93.96 nmol 3-PBA/g creatinine and 0.09–90.94 nmol cis-DCCA/g creatinine, demonstrating that workers had a wide range of exposures to αCM. The data also demonstrate that pesticide applicators had greater exposures to αCM than workers who play a supporting role in the seasonal application of pesticides on the cotton crop. Urinary cis-DCCA and 3-PBA concentrations were elevated at 7–11 days after the cessation of αCM application, compared to baseline levels. This study is the first to use these biomarkers to quantify occupational exposures specifically to αCM. This urinary biomarker data will be useful for estimating daily internal dose, comparing exposures across job categories within the Egyptian pesticide application teams, and for modeling human exposures to αCM.

Introduction

Pyrethroids are the most commonly utilized residential insecticides partly due to the generally held belief that they pose minimal risk to human health. In addition, there are numerous worldwide applications for pyrethroids in agriculture, horticulture, public health (hospitals and commercial aircraft) and textiles (Berger-Preiss et al., 2002; Wei et al., 2012; Worthing and Hance, 1991). They have insecticidal activity in their parent form and do not require metabolic activation to exert their neurotoxic effects, which are mediated by increased open time of voltage-gated sodium channels (Soderlund, 2012; Soderlund et al., 2002). The initial symptoms of acute occupational pyrethroid intoxication include parasthesia consisting of burning and itching sensations on the skin or dizziness that develops approximately 4–6 h after exposure, although dermal symptoms can manifest after minutes of application. Systemic symptoms (dizziness, headache, nausea, anorexia and fatigue) can occur up to 48 h after acute exposure (He et al., 1988, 1989; Vijverberg and Vandenbercken, 1990).

Pyrethroids are classified as Type I or Type II pyrethroids. Type I pyrethroids are esters of primary or secondary alcohols, whereas Type II pyrethroids are esters of secondary alcohols with a cyano group at the α-carbon of the alcohol component. The acid and

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alcohol moieties both contain chiral centers, leading to the possibility of several stereoisomers for each pyrethroid, which may exhibit isomer-specific insecticidal activity (Knaak et al., 2012; Leicht et al., 1996).

The type II pyrethroid, αCM, is derived from the 8-stereoisomers that comprise the pyrethroid cypermethrin, which is one of the most common pyrethroids in agricultural and residential use. αCM is a racemate of two cypermethrin stereoisomers: (S)-α-cyano-3-phenoxybenzyl-(1R)-cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate, and (R)-α-cyano-3-phenoxybenzyl-(1S)-cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate (Knaak et al., 2012), which are considered the two most stable cis-isomers (Leicht et al., 1996; Liu et al., 2005). The major detoxification pathway of αCM is through hydrolysis by esterases and hydroxylation by cytochrome P450s (Abernathy and Casida, 1973; Ross et al., 2006; Scollon et al., 2009). *In vitro* studies have shown that alcohol and aldehyde dehydrogenases can also contribute to the metabolism of pyrethroids (Choi et al., 2002). Dosing studies with αCM and cypermethrin in 6 human volunteers indicate an elimination half-life range of 8–22 h for a single dermal exposure (Eadsforth et al., 1988; Woollen et al., 1992).

The assessment of human exposure to insecticides such as pyrethroids is often based on quantification of metabolites excreted in urine (Barr et al., 2010; Eadsforth et al., 1988; Elflein et al., 2003; Fortin et al., 2008; Hardt and Angerer, 2003; Kolmodin-Hedman et al., 1982; Leng et al., 1996, 1997; Wang et al., 2007; Woollen et al., 1992; Xia et al., 2008; Zhang et al., 1991). The major urinary metabolites of αCM in humans are 3-phenoxybenzoic acid (3-PBA) and cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (cis-DCCA), which are conjugated prior to being excreted in the urine (Eadsforth et al., 1988; Leng and Gries, 2005; Woollen et al., 1992) (Fig. 1). 3-PBA is a metabolite common to a large number of pyrethroid insecticides, while cis-DCCA is a more specific metabolite and useful urinary biomarker of exposure for αCM, permethrin and cyfluthrin (Barr et al., 2010; Hardt and Angerer, 2003; Heudorf and Angerer, 2001; Wang et al., 2007). Thus, urinary concentrations of 3-PBA may serve as a general biomarker of pyrethroid exposure, while cis-DCCA represents a more specific biomarker for human exposure to αCM.

Currently, there are no published studies specifically assessing the occupational exposure to αCM. One pyrethroid study investigated occupational exposure in Chinese cotton workers spraying deltamethrin, fenvalerate and a deltamethrin methamidophos mixture (Zhang et al., 1991). Hardt and Angerer (2003) evaluated occupational exposure in individual workers after applying a mixture of up to 7 synthetic pyrethroids, including αCM. Another study described occupational exposure to permethrin and fenvalerate (Kolmodin-Hedman et al., 1982), and a number of other studies have documented general pyrethroid exposure in non-occupational settings utilizing 3-PBA as a general biomarker of pyrethroid exposure (Barr et al., 2010; Fortin et al., 2008; Heudorf and Angerer, 2001; Schettgen et al., 2002).

The primary objective of the present study was to investigate occupational exposure to αCM by quantitating the daily urinary levels of cis-DCCA and 3-PBA before, during, and after the application of αCM in a cohort of Egyptian agriculture workers who were spraying αCM on cotton fields daily for up to 10 consecutive days. A biomonitoring study on a subset of this Egyptian agriculture worker population determined that 94–96% of the dose was due to dermal exposure (Fenske et al., 2012). While chlorpyrifos exposure has previously been characterized in these individuals (Farahat et al., 2011), this is the first study to describe a longitudinal assessment of exposure to αCM.

Materials and methods

Chemicals

1,1,1,3,3,3,-Hexafluoroisopropanol (HFIP), N,N-diisopropylcarbodiimide (DIC), 3-Phenoxybenzoic acid (3-PBA 98%) and internal standard, 2-Phenoxybenzoic acid (2-PBA 98%) were purchased from Sigma–Aldrich Corp (St. Louis, MO, USA). cis- and trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (cis-DCCA 5.6% and trans-DCCA 92.7%) were purchased from Chemservice (PA, USA).

Population and sampling

A detailed description of the study setting was previously published (Farahat et al., 2011), and a previous chlorpyrifos biomonitoring study on a subset of this Egyptian agriculture worker population estimated that 94–96% of the dose was due to dermal exposure (Fenske et al., 2012). Briefly, the present study was conducted in Menoufia, one of 29 governorates in Egypt, which is located in the Nile River Delta north of Cairo. Daily urine samples were collected from a cohort of 37 Ministry of Agriculture workers which were divided into three job categories: (1) applicators who spray the insecticide on the cotton crop with a backpack mistblower sprayer; (2) technicians who walk the fields with the applicator in order to point out particular areas which need attention; and (3) engineers who direct the work mainly from the edge of the field. These workers were assigned to 3 regions (designated as field stations) where αCM was sprayed daily in 3–5 h work shifts for up to 10 consecutive days in the summer of 2008. The workers provided daily spot urine samples before, during, and after the insecticide application cycle. The urine samples used for analysis were collected just prior to start of the work day, on 24 h intervals at approximately 3 pm. One technician (field station 2) was included in the demographic analysis, but did not provide any urine specimens during the pyrethroid application and was therefore excluded from the current study. 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 being shipped to the State University of New York at Buffalo (Buffalo, NY, USA) on dry ice for analysis. Creatinine concentrations were measured using the Jaffe reaction (Fabiny and Ertingshausen, 1971); urinary cis-DCCA and 3-PBA concentrations are expressed as micrograms or nanomoles per gram creatinine. All protocols and questionnaires were approved by Institutional Review Boards of Menoufia University and Oregon Health & Science University, the institute administering the parent grant that funded the field studies.

Sample preparation and analysis

The sample extraction method was adapted from previously described methods (Fortin et al., 2008; Leng and Gries, 2005). First, urine samples were spiked with 2-PBA internal standard at a final concentration of 20 ng/ml of sample. The samples were then treated with hydrochloric acid and hydrolyzed at 100 °C for 2 h to form free conjugated metabolites. The urine samples were then subjected to a liquid-liquid extraction with methyl *tert*-butyl ether, the organic phase was removed, concentrated under nitrogen, resuspended in acetonitrile, derivitized with 1,1,1,3,3,3-Hexafluoroisopropanol (HFIP) and N,N-diisopropylcarbodiimide, and the HFIP-esters were transferred to a microinjector vial. Sample analysis was performed using an Agilent GC 6890 gas chromatograph, a Hewlett Packard 6890 Series autoinjector, a Phenomenex Zebron ZB-5HT Inferno column (30 m × 250 μm, film thickness 0.10 μm) coupled to an Agilent 5973 inert Mass Selective Detector (MSD). The oven temperature program was held at 60 °C for 1 min and ramped to 300 °C
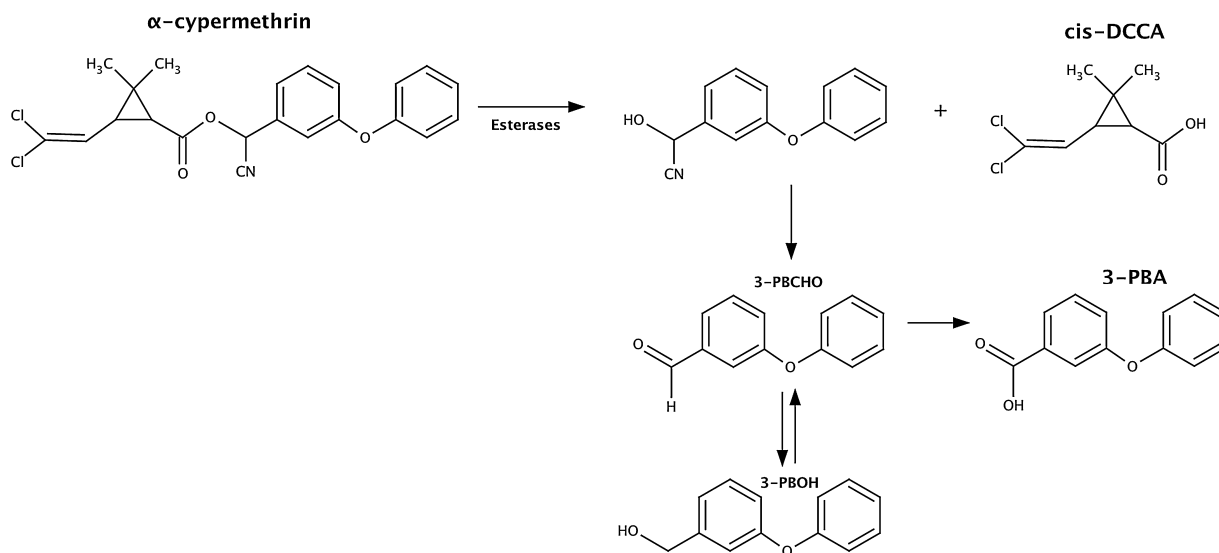
Fig. 1. α-Cypermethrin metabolic scheme and urinary metabolites cis-DCCA and 3-PBA.

at 10 °C per minute and then held at 300 °C for 5 min. The interface heater was held at 250 °C for the duration of the program. Helium was used as the carrier gas (99.999%) with a flow rate of 1.2 ml/min in constant flow mode. 1 μl of sample was injected in splitless mode. The MSD was operated in Selective Ion Mode (SIM) using negative chemical ionization. The GC retention times (in min) for cis-DCCA, 3-PBA, and 2-PBA were 7.372, 12.094, and 11.886 respectively. The MS SIM mode target ion 322 (m/z) was used to detect cis-DCCA; and ion 364 (m/z) was used for the detection of both 2-PBA and 3-PBA.

Quality control

The analytical limit of detection (LOD) for cis-DCCA and 3-PBA, was 0.054 and 0.22 ng/ml, respectively (US EPA MDL). A spiked QC sample was routinely run with the analytical samples and the metabolite concentration was determined from a standard curve for the peak area for the selective ion. The cis-DCCA standard curve was linear from 0.056 ng/ml to 28 ng/ml with a correlation coefficient of 0.999, while the 3-PBA linearity was tested from 0.1 to 100 ng/ml with a correlation coefficient of 1.000. Spiked samples (n = 6) consistently gave metabolite recoveries above 93% and the within series relative standard deviations were 3.4% and 2.1% for cis-DCCA and 3-PBA, respectively.

Estimated exposure

Assuming steady-state conditions, it is possible to estimate the daily internal dose of αCM in each worker utilizing the urinary concentration of the more specific αCM metabolite cis-DCCA (per gram creatinine) and the following equation (adapted from (Eadsforth et al., 1988; Farahat et al., 2011; Fenske et al., 2012):

$$Dd = (DCCAc \times Cd \times IEF \times MWratio)/BW \qquad (1)$$

where Dd = estimated daily dose (μg/kg/day); DCCAc = creatinine-adjusted cis-DCCA concentration (μg/g creatinine) for each worker; Cd = daily creatinine clearance of 1.7 g creatinine/day (Farahat et al., 2011); IEF = incomplete excretion factor of 1.0/0.46 based on a human αCM dosing study (Eadsforth et al., 1988); MW ratio = molecular weight ratio of αCM and cis-DCCA (416.3/209.07 = 1.99; and BW = body weight (kg). Weights of the workers (ranging from 65 to 88 kg) were utilized to estimate daily exposure (internal dose).

Statistical analysis

Data analysis was performed with IBM SPSS statistics version 19. Urinary metabolite levels before, during, and after the αCM application period and the metabolite levels among the applicators, technicians, and engineers were each compared using the non-parametric Kruskal–Wallis 1-way ANOVA followed by Mann–Whitney U *post hoc* analysis (alpha = 0.05). The correlation of urinary cis-DCCA and 3-PBA excretion was assessed by Pearson product-moment correlation coefficient. In situations where concentrations were below the analytical limit of detection (LOD), a value equal to the LOD divided by the square root of 2 was used (Barr et al., 2010).

Results

Study population

Table 1 summarizes demographic data for the 2008 cohort of Egyptian Ministry of Agriculture workers that applied αCM during the summer of 2008. Farahat et al. (2011) previously reported the exposure of those workers to chlorpyrifos. Table 2 shows the 2008 pesticide application schedule consisted of daily application of chlorpyrifos followed by α-cypermethrin for 3 field stations or regions.

Urinary cis-DCCA and 3-PBA concentrations

Fig. 2 shows median urinary cis-DCCA and 3-PBA concentrations (normalized to creatinine) stratified by job category for each of the three field stations (Q1, Q2, Q3). The error bars represent the inter-quartile range. July 17 was chosen for baseline analysis of urinary metabolite concentrations because this date was prior to the start of the government regulated αCM application for the three field stations. In the event that a urine sample was not available from a worker on July 17, the sample that was collected one or two days prior to that date was used for baseline analysis of that particular worker. The 3 field stations (Q1–Q3) demonstrate 3 distinct exposure scenarios, including different length of spray period (Table 2) and peak urinary metabolite levels during the application (Fig. 2 and Table 3). Of the 3 stations, Q1 had the longest duration of daily αCM application and Q2 had the highest median peak excreted levels of urinary metabolites cis-DCCA and 3-PBA. Only 1 technician from Q2 provided samples during the

Table 1
Demographic characteristics of 2008 cohort of Ministry of Agriculture workers (From Farahat et al., 2011).

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

αCM spray and this particular worker had very low urine levels of both cis-DCCA (<1 μg/g creatinine) and 3-PBA (<2 μg/g creatinine) at all time points analyzed throughout the study. At the baseline, there was no significant difference in the median metabolite levels among the 3 job categories. When the application of αCM began, the median urinary cis-DCCA and 3-PBA levels increased for all three

job categories, with the applicators having significantly higher levels during the spray period as compared to the engineers (Q1–Q3) and technician (Q1 and Q3) ($p < 0.001$). In all three-field stations, the median urinary metabolite levels during the application period followed the rank order: Applicator > Engineer > Technician. For αCM, urinary metabolite concentrations decreased over the 7-day
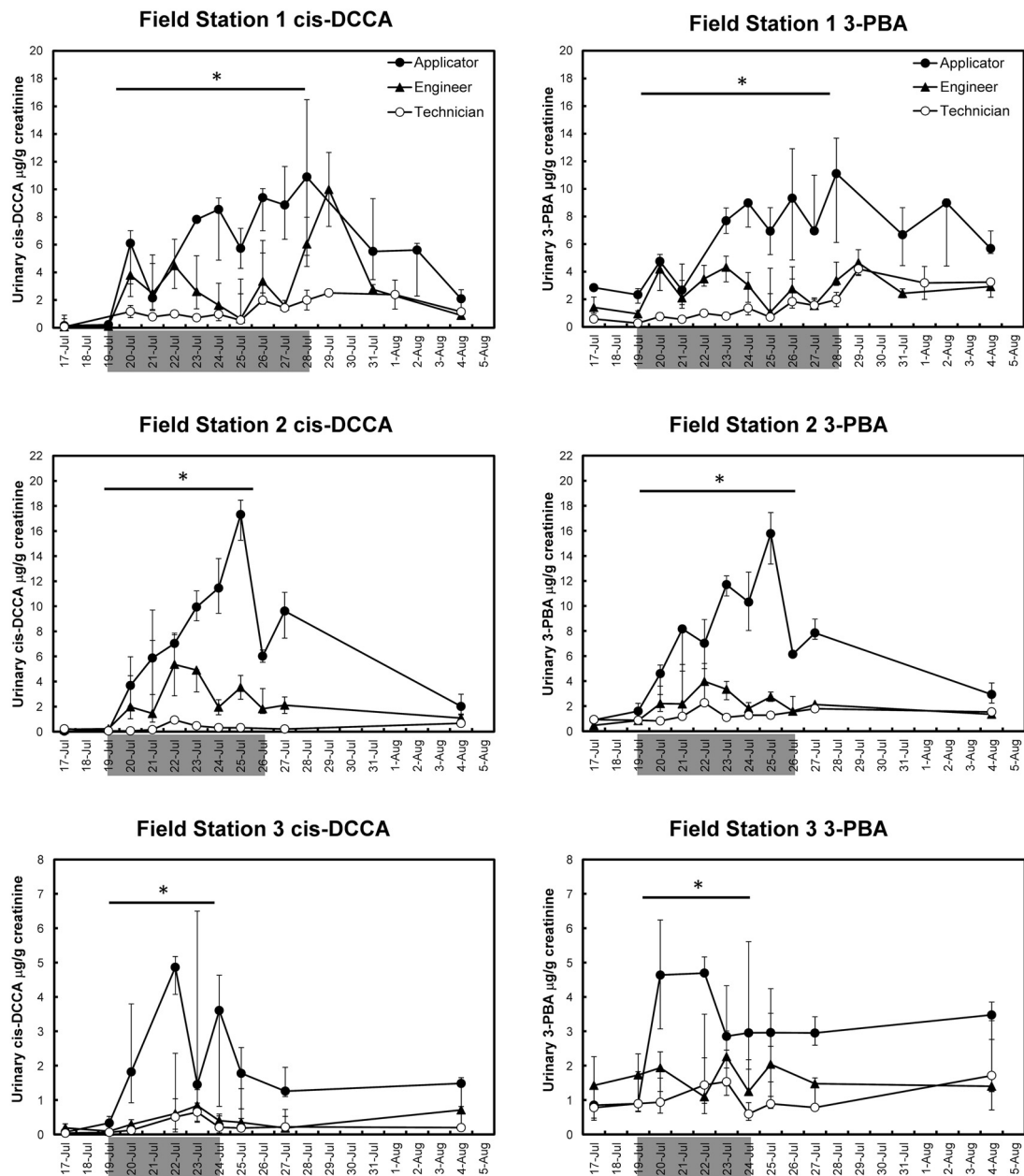

Fig. 2. Urinary concentrations of cis-DCCA and 3-PBA in applicators, technicians, and engineers from field stations 1, 2, and 3 in 2008. Shaded areas represent the period of α-cypermethrin application. Data are presented as median ± interquartile range ($n = 2–5$ workers for each job category in a field station, with the exception of field station 2, where $n = 1$ technician) * = $p < 0.001$ for the difference between job categories.

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

Field station[a]	Chlorpyrifos application		α-cypermethrin application	
	Start	End	Start	End
Q1 A(n = 5) T(n = 5) E(n = 4)	1 July	17 July	19 July	28 July
Q2 A(n = 4) T(n = 2) E(n = 3)	6 July	14 July	19 July	26 July
Q3 A(n = 5) T(n = 5) E(n = 5)	7 July	14 July	19 July	24 July

[a] Region where cotton fields were sprayed daily by Ministry of Agriculture workers; A = applicators, T = technicians, E = applicators

post-application period. cis-DCCA and 3-PBA concentrations were generally still elevated compared to baseline levels on August 4th (7–11 days after the cessation of αCM application).

Peak metabolite levels compared to baseline

For field station 1 (Q1), all workers across all job categories had detectable levels of both cis-DCCA and 3-PBA in their baseline urine sample. After the start of the αCM application (July 19th), peak median urinary cis-DCCA concentrations for Q1 showed a ~70-fold increase for applicators, ~86-fold increase for engineers, and ~35-fold increase for technicians, when compared to respective median baseline values. Q1 urinary metabolite 3-PBA levels were detectable at baseline, and after the start of αCM application peak levels showed a ~4-fold increase for applicators and a 3-fold increase for engineers, and technicians, as compared to respective median baseline values.

In field station 2, applicators baseline cis-DCCA levels were <LOD, and during the αCM application period peak median cis-DCCA levels increased to 17.32 µg/g creatinine. Engineers peak median cis-DCCA levels during the αCM application period

Table 4
Estimated daily internal dose of α-cypermethrin in applicators prior to (baseline), during (peak median), and post α-cypermethrin application.

Median values	Applicators		
Field station	Baseline	Application	Recovery
Q1	July 17	July 28	August 4
cis-DCCA (µg/g creatinine)	0.15	10.89	2.09
Alpha-cypermethrin (µg/kg/day)	0.01	0.97	0.19
Q2	July 17	July 25	August 4
cis-DCCA (µg/g creatinine)	<LOD	17.32	2.01
Alpha-cypermethrin (µg/kg/day)	–	1.53	0.18
Q3	July 17	July 22	August 4
cis-DCCA (µg/g creatinine)	<LOD	4.86	1.48
Alpha-cypermethrin(µg/kg/day)	–	0.43	0.13

increased ~30-fold when compared to baseline values. Peak 3-PBA levels in this field increased ~18-fold for applicators and ~8-fold for engineers.

Field station 3 had the shortest application period (6 days) as well as the lowest peak levels of cis-DCCA and 3-PBA when compared to the other two field stations. During the αCM application, peak cis-DCCA levels increased from <LOD to 4.86 µg/g creatinine for applicators, and ~4-fold for both engineers and technicians. Peak 3-PBA levels increased ~6-fold for applicators, ~2-fold for engineers, and ~2-fold for technicians, as compared to their respective median baseline values.

Correlation between cis-DCCA and 3-PBA

cis-DCCA and 3-PBA concentrations were compared in each urine sample during the αCM application period to determine if there was a correlation between the excreted levels of the 2 metabolites. Statistical analysis of the scatter plot in Fig. 3 found a significant positive linear correlation between daily urinary concentrations of 3-PBA and cis-DCCA (Pearson correlation (95% confidence interval) r = 0.873 (0.815–0.935), n = 270 urine samples, p < 0.001). The slope of the regression line = 0.76.

Estimated daily dose of αCM

Table 4 summarizes daily αCM dose estimates calculated from peak median urinary cis-DCCA concentrations for applicators
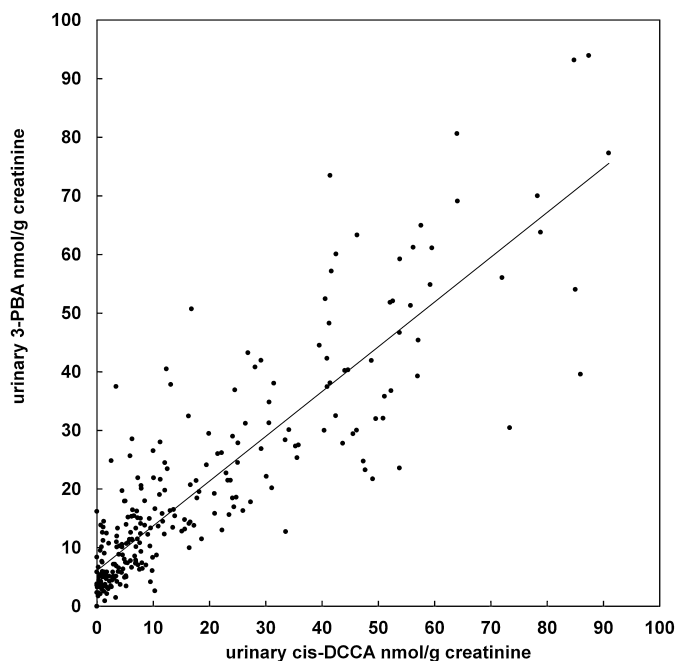
Table 3
Median urinary cis-DCCA and 3-PBA levels prior to (baseline), during (peak), and post application of α-cypermethrin.

	Field station	Baseline µg/g creatinine		Peak µg/g creatinine		Post µg/g creatinine	
		cis-DCCA	3-PBA	cis-DCCA	3-PBA	cis-DCCA	3-PBA
Applicator	1	0.15	2.85	10.89	11.11	2.09	5.68
	2	<LOD	0.90	17.32	15.78	2.01	2.93
	3	<LOD	0.85	4.86	4.69	1.48	3.48
Engineer	1	0.07	1.41	6.05	4.32	0.90	2.92
	2	0.18	0.47	5.36	3.97	1.09	1.35
	3	0.19	1.43	0.84	2.26	0.71	1.40
Technician	1	0.06	0.58	2.00	1.99	1.15	3.25
	2	<LOD	<LOD	0.93	2.26	0.67	1.53
	3	0.01	0.78	0.65	1.44	0.19	1.71
	Field Station	Baseline nmol/g creatinine		Peak nmol/g creatinine		Post nmol/g creatinine	
		cis-DCCA	3-PBA	cis-DCCA	3-PBA	cis-DCCA	3-PBA
Applicator	1	0.74	13.31	52.10	51.84	9.99	26.52
	2	<LOD	4.19	82.84	73.68	9.64	13.66
	3	<LOD	3.96	23.26	21.91	7.09	16.22
Engineer	1	0.32	6.60	28.94	20.18	4.28	13.63
	2	0.87	2.17	25.64	18.55	5.20	6.29
	3	0.90	6.66	4.00	10.55	3.40	6.54
Technician	1	0.31	2.69	9.55	9.27	5.52	15.18
	2	<LOD	<LOD	4.44	10.55	3.18	7.16
	3	0.03	3.62	3.10	6.70	0.93	7.99

Fig. 3. Relationship between cis-DCCA and 3-PBA after the start of α-cypermethrin application; 270 samples (Pearson's r (95% CI)= 0.873 (0.815–0.935, $p < 0.001$).

(Eq. (1)). Applicators in field station 2 had the highest peak median cis-DCCA levels 17.32 μg/g creatinine, which corresponds to an estimated αCM dose of 1.52 μg/kg bw/day (Table 4).

Discussion

cis-DCCA and 3-PBA concentrations after daily αCM exposure

The Type II pyrethroid αCM is metabolized by hydrolytic cleavage of the ester bond to form the metabolite cis-DCCA, while the alcohol moiety is further metabolized to 3-PBA likely by oxidative enzymes such as cytochrome P450s and aldehyde dehydrogenase (Choi et al., 2002; Godin et al., 2007; Scollon et al., 2009). Urinary levels of cis-DCCA and 3-PBA were measured in the Egyptian cotton field worker to estimate their exposure to αCM over the course of the spray schedule (pre-, during, and post-application). The results show great variability from worker to worker, even within the same job category. The 3 field stations (Q1–Q3) demonstrate 3 distinct exposure scenarios, including different length of spray period and peak metabolite levels during the application. Accordingly, not all applicators were highly exposed, while several individual engineers and technicians were highly exposed.

To our knowledge this is first study to conduct a longitudinal assessment of occupational human exposure specifically to αCM. This cohort is unique because the workers were applying a single pesticide (αCM) for up to 10 consecutive days. Similar to the previously reported occupational exposure to chlorpyrifos exposure in this cohort (Farahat et al., 2011), applicators had the highest levels of urinary metabolites and thus greater exposure to the parent compound, while technicians and engineers had lower exposures.

Correlation between cis-DCCA and 3-PBA

The αCM exposure characterization included analysis of the more specific urinary metabolite cis-DCCA, and the less specific, 3-PBA, which is a more general biomarker of exposure to many

pyrethroids exposure (Barr et al., 2010; Fortin et al., 2008; Heudorf and Angerer, 2001; Schettgen et al., 2002). This approach allows for a more robust characterization of the exposure to αCM, the only pyrethroid applied by the occupational cohort. The strong positive linear correlation between the urinary levels of the two metabolites (nmoles/g creatinine) suggests that αCM was the only pyrethroid that the workers were exposed to during this application. However, pre-spray baseline levels of 3-PBA were consistently higher than pre-spray cis-DCCA levels, suggesting low level environmental exposure to one or more other pyrethroids from which 3-PBA can be derived as a urinary metabolite. The slope of the regression line in Fig. 3 is 0.76, which indicates that the amount of 3-PBA excreted during the aCM application was 76% of that for cis-DCCA and suggests that the 3-PBA molecule is further metabolized to additional metabolites that were not analyzed in this study. Hardt and Angerer (2003) examined the relationship between 3-PBA and total DCCA (the sum of cis and trans) excreted by pest control operators after a single application of permethrin and found that the amount of 3-PBA was approximately 54% the amount of total DCCA, which is in line with our findings in the present study.

Comparison with other pyrethroid exposure studies

Although there are no other published studies on exposure specifically to αCM alone, our data can be compared to previous studies of both non-occupational and occupational exposures to several pyrethroids. In comparing the urinary concentrations of the non-specific pyrethroid metabolite, 3-PBA, the Egyptian agriculture applicator's peak urinary levels (mean 15.0 and median 15.8 μg/g creatinine) are about 50 times greater than the levels found in the 2010 US NHANES study of the general U.S. population in 2001–2002 (geometric mean 0.324 μg/g creatinine) (Barr et al., 2010). Hardt and Angerer (2003) assessed background excretion of urinary pyrethroid metabolites including (combined cis- and trans-) DCCA from 45 urine samples in 25 women and men in Germany who had no occupational exposure to pesticides. These studies suggest substantial non-occupational exposure to pyrethroids in the general population. The mean DCCA urinary concentration found in these samples was 0.8 μg/g creatinine. The peak cis-DCCA concentrations in the Egyptian workers (mean 16.4, median 17.4 μg/g creatinine) were more than 20 fold higher than the levels in this German population.

Biological monitoring was carried out in a population of German agriculture workers involved in applications of various pyrethroid insecticides, including αCM ($n = 19$ workers, 24 urine samples) (Hardt and Angerer, 2003). The mean cis-DCCA and 3-PBA concentrations found in these workers were 0.6 and 1.8 μg/g creatinine, respectively. These concentrations are also several fold lower than the peak (during spray) levels observed in the Egyptian applicators.

Comparison to chlorpyrifos exposures in this cohort

During the summer of 2008, the Egyptian workers applied the organophosphorus pesticide, chlorpyrifos, to the cotton fields prior to the αCM application. Farahat et al. (2011) estimated chlorpyrifos exposures in these workers using urinary TCPy as a biomarker for chlorpyrifos. During the chlorpyrifos application, Farahat et al. (2011) reported peak urinary TCPy levels on July 10 in field station 1 (mean 14012, median 10505 μg/g creatinine). This peak median urinary TCPy level corresponds to estimated internal chlorpyrifos dose of 578.4 μg/kg/day. The peak median αCM doses estimated in the present study (1.52 μg/kg BW/day) are several orders of magnitude lower than the estimated chlorpyrifos doses in this cohort. The route of exposure for chlorpyrifos in this occupational setting is primarily dermal (Fenske et al., 2012) and because the application method for both insecticides is the same (backpack mistblower

sprayers), it is plausible that the primary route of exposure for aCM in this study is also dermal. Human dosing studies have shown that there are differences between the elimination half-life and the dermal adsorption for CPF and aCM. Griffin et al. (1999) and Meuling et al. (2005) both reported average CPF elimination half-lives of 41 h and ~1% adsorption, based upon the recovery of the applied dose as urinary metabolites and the amount of chlorpyrifos recovered from the skin subtracted from the amount applied to the skin. Two human dosing studies with aCM and cypermethrin calculated an elimination half-life range of 8–22 h after the application of a single dermal dose, and ~0.1% of the applied dose was recovered as urinary metabolite DCCA (Eadsforth et al., 1988; Woollen et al., 1992). The variance between the half-life and the absorption of CPF and aCM can explain, in part, why the levels of the aCM urinary metabolites cis-DCCA and 3-PBA did not reach the exceedingly high levels as seen for the CPF urinary metabolite TCPy.

Several *in vitro* studies have shown that metabolism of pyrethroids is mediated by carboxylesterases (Crow et al., 2007; Nishi et al., 2006; Ross et al., 2006). Crow et al., 2007 demonstrated that the activity of human carboxylesterase 1 and 2 (hCE1 and hCE2) is inhibited after treatment with chlorpyrifos-oxon, the active metabolite of chlorpyrifos, and metabolism assays indicate that hydrolysis of trans-permethrin is inhibited by chlorpyrifos-oxon in human liver fractions (Choi et al., 2004). The Egyptian agriculture workers were spraying chlorpyrifos for up to 17 days just prior to the application of αCM on the cotton fields. Thus, there is potential for interactions in the metabolism of chlorpyrifos and αCM *in vivo*. Prior exposure to chlorpyrifos could inhibit the carboxylesterases ability to detoxify αCM, thereby altering the pharmacokinetic and toxic potential of αCM. Future studies designed to assess the interactive effects of these two classes of insecticides will be critical in examining the impact of combined and sequential exposures on human health.

Urinary cis-DCCA and 3-PBA concentrations were generally still elevated compared to baseline levels 7–11 days after the cessation of application (on August 4). This result was unexpected based upon the reported mean urinary half-life of 13 h for aCM after a single dermal exposure (Woollen et al., 1992). This finding may be explained by poor washing habits after handling the insecticide, continued exposure from wearing αCM drenched clothing, or by workers re-entering the cotton fields and contacting αCM residue on the cotton plants or in the soil. Another possible route of continued exposure is from skin or deep compartments such as fat tissue. αCM is highly lipophilic and the internalized compound may get into skin layers where it is not readily removed by typical washing procedures (Eadsforth et al., 1988), and fat compartments where it can be slowly released over time, metabolized, and excreted as urinary cis-DCCA and 3-PBA. Another explanation for the accumulation of urinary cis-DCCA and 3-PBA (Fig. 2) is the possibility that the pharmacokinetics of aCM metabolism and excretion in man are different from repeated and sustained exposure when compared to the single dermal dose that was used to estimate the half life range of 8–22 h (Woollen et al., 1992).

Conclusion

This study is the first longitudinal assessment of occupational exposures specifically to the pyrethroid insecticide αCM. The results may be utilized for future risk assessment efforts of αCM. These findings will also be useful to inform the Egyptian agriculture workers of the extent of their exposures so that they may take adequate preventative measures to reduce exposure by implementing the use of personal protective equipment and proper working practices.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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