Contributions of inhalation and dermal exposure to chlorpyrifos dose in Egyptian cotton field workers

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Objective: Chlorpyrifos exposures were assessed in 12 Egyptian cotton field workers.
Methods: 3,5,6-trichloro-2-pyridinol (TCPy) was measured in 24-hour urine samples to estimate absorbed dose. Workshift air samples were used to calculate chlorpyrifos inhalation dose.
Results: Patches on legs had the highest chlorpyrifos loading rates among body regions sampled. Geometric mean chlorpyrifos air concentrations were 5.1, 8.2, and 45.0 μg/m^3 for engineers, technicians, and applicators, respectively; peak TCPy urinary concentrations were 75–129, 78–261, and 487–1659 μg/l, respectively; geometric mean doses were 5.2–5.4, 8.6–9.7, and 50–57 μg/kg, respectively, considering TCPy excretion half-life values of 27 and 41 hours. All worker doses exceeded the acceptable operator exposure level of 1.5 μg/kg/day. An estimated 94–96% of the dose was attributed to dermal exposure, calculated as the difference between total dose and inhalation dose.
Discussion: Interventions to reduce dermal exposure are warranted in this population, particularly for the hands, feet, and legs.

Keywords: Pesticide, Application, Agriculture, Occupational, Worker, Dermal exposure, Inhalation exposure, Chlorpyrifos, Urinary metabolites, TCPy, Egypt

Introduction

Potential adverse health impacts of organophosphorus (OP) pesticide exposures are an ongoing public health concern for agricultural workers, particularly in low and middle income countries where the exposures are very high in some cases.[1–12] Knowledge of exposure pathways and routes of exposure is critical for the design of effective interventions for reducing pesticide exposures among applicators and other agricultural workers. Some studies have reported simultaneous inhalation and dermal exposure measurements but have not included biomonitoring to estimate total doses.[13,14] Others have focused on either inhalation exposure[15] or dermal exposure.[2,4,11] Still others have used biological monitoring (e.g. urinary metabolites) but have not provided dose estimates or quantitative information on routes of exposure.[16] Relatively few studies have been designed to estimate the relative contributions of inhalation and dermal exposure to internal dose. Durham *et al.*[17] were the first to provide definitive evidence that dermal exposure and subsequent skin absorption of pesticides are the major contributors to internal dose for pesticide applicators during orchard airblast spraying. A study of termiticide applicators using chlorpyrifos also demonstrated the predominance of the dermal exposure route.[18]

A study of chlorpyrifos (O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate) exposure among a small number of Egyptian cotton field workers demonstrated substantial deposition of pesticide on skin and clothing during applications, relatively low air concentrations, and 3,5,6-trichloro-2-pyridinol (TCPy) levels higher than most reports in the literature.[9] A more recent study[12] found that TCPy concentrations in urine samples collected in the summer of 2008 from ~40 workers over 2 weeks of chlorpyrifos applications in Egyptian cotton fields were in some cases even higher than those measured in the earlier Farahat *et al.* study.[9]

The first objective of this study was to determine the relative contributions of dermal and inhalation exposure to internal dose in Egyptian cotton production workers applying chlorpyrifos for pest control. We hypothesized that the primary route of exposure was dermal for three occupational categories: engineers, technicians, and applicators. A second, but equally important, objective was to document the

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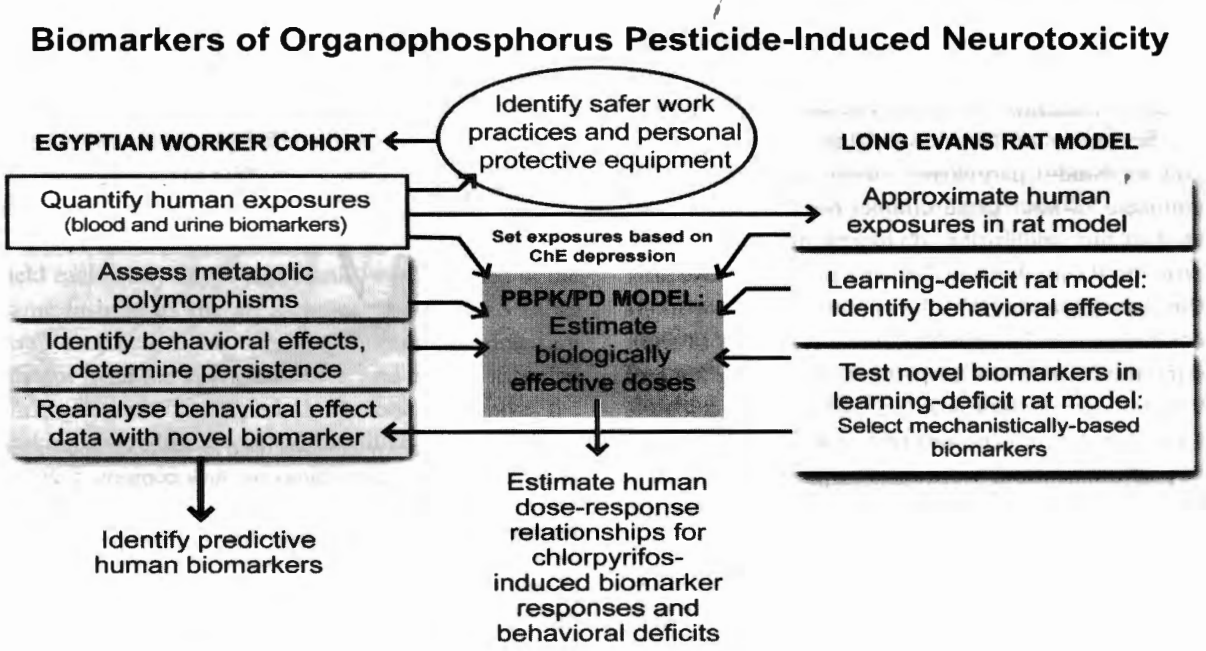

Figure 1 Overall design for the biomarkers of organophosphorus pesticide-induced neurotoxicity project.[19] Doses in animal model studies are based on human exposure data from field studies. Multiple biomarkers are measured in both animal studies and human exposure studies. The quantification of human exposures and identification of safer work practices and personal protective equipment were the focus of this field study.

distribution of exposure across worker body regions. This information is important for developing risk communication messages and new safety measures designed to minimize exposure during applications.

Methods

Study design

This field study was undertaken as a component of a larger project focused on the development of novel biomarkers of OP pesticide neurotoxicity.[19] The overall study design is shown in Fig. 1. The left column depicts human research, the right column depicts animal research, and the middle column depicts physiologically based pharmacokinetic/pharmacodynamic modeling. Outcomes are shown in ellipses. This field study focused on quantifying human exposures through different routes in order to identify safer work practices and potential exposure reduction through use of personal protective equipment.

The field study was designed to use biomonitoring of the primary chlorpyrifos urinary metabolite, TCPy to provide an estimate of absorbed dose from a single

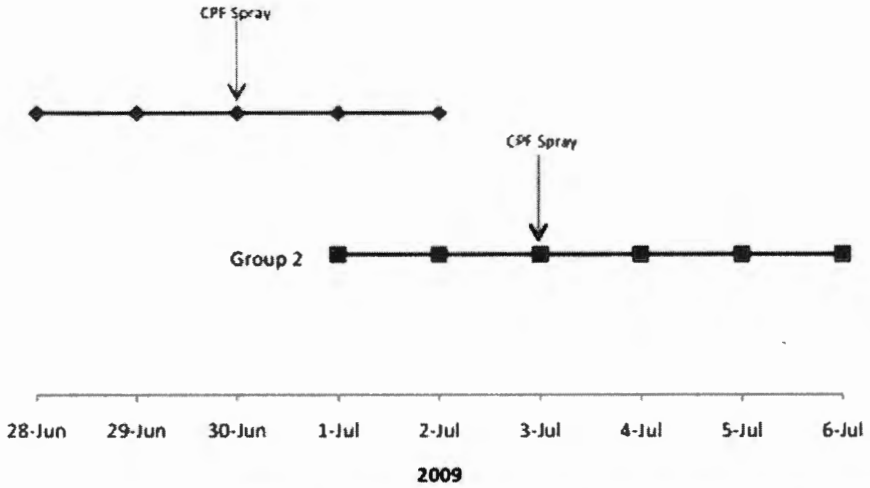

Figure 2 Study design. Two groups of Egyptian workers (six workers per group) provided 24-hour urine samples before, during, and after a single day of chlorpyrifos application on cotton fields. Diamonds and squares indicate the beginning and end of each 24-hour urine sample collection (approximately 13:00 each day). Spraying began in the late afternoon. Personal air sampling exposure was conducted during the application. Inhalation doses were calculated from the air samples and total doses were calculated from the urinary metabolite measurements. The difference between these dose estimates was considered to be the dose attributable to dermal exposure and absorption.

work shift. At the same time, inhalation exposure was measured throughout the 1-day work shift. The study design is presented in Fig. 2. The study took place at the beginning of the chlorpyrifos spray season for cotton. Study participants were asked to collect complete 24-hour urine samples for the 2 days prior to their first application of chlorpyrifos to the cotton crop, on the application day, and for 1 or 2 days after the application day. On the application day, workers conducted normal activities over a typical work shift. Air samples were collected in the breathing zone of workers during chlorpyrifos applications to calculate 1-day inhalation exposure and dose. After the single day of application, workers were assigned alternate duties for 1 day (group 1) or 2 days (group 2) that did not involve chlorpyrifos exposure. Workers wore dermal patches on the chest, back, forearms, upper and lower legs during chlorpyrifos applications to document dermal exposure patterns. Inhalation doses for the application day were calculated from the air samples, and the urinary metabolite data were used to estimate total dose attributable to the single application day. The internal dose attributable to dermal exposure and absorption was calculated as the difference between the urinary metabolite-based dose estimate and the inhalation dose estimate.

Pesticide handling activities

The study took place from June 28 through 6 July 2009. Field activities took place at cotton farms in the Menoufia Governorate, Egypt. Laboratory activities took place at Menoufia University Hospital, Shebin El-Kom, Egypt. Potential study participants were recruited at two field stations prior to the beginning of the spray season. Participants were selected based on planned spraying activities and willingness to carry out the study procedures. The purpose of the study was explained and informed consent was obtained. Discussions with workers and the consent forms were in Arabic. The Institutional Review Boards of Menoufia University and Oregon Health & Science University approved the study procedures and associated documents.

Twelve workers from two field stations were enrolled in the study, six from each station. In each case, the six workers made up a pesticide application team that included two applicators who dispensed pesticides with motorized backpack mistblowers, two technicians who walked each row to direct the path of the applicator, and two engineers who mainly directed the application process from the edge of the fields, periodically entering the fields to assist the other workers. Mixing and loading activities were shared among the technicians and engineers depending on availability. The backpack mistblower apparatus is illustrated in Fig. 3. Exposures can occur

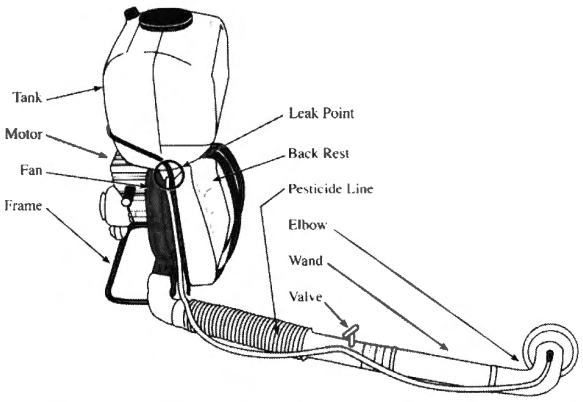

Figure 3 Backpack mistblower used for pesticide application in Egyptian cotton production. The tank contains ~ 20 l of water with Pestban. The motor powers a fan and pressurizes the tank. The pesticide solution flows through a plastic tube from the tank to the tip of the mistblower wand, where it is injected into the air stream produced by the fan. A valve on the wand controls the air speed. A primary point where leakage can occur is at the junction of the tank and plastic tubing.

when the tank is loaded (overflow), if the tank lid is not secure (leakage of pressurized spray solution), and at the pesticide line–tank connection (leak point). All 12 workers completed full work shifts.

The OP pesticide formulation Pestban™ was used during the study. Pestban is an emulsifiable concentrate that contains 48% chlorpyrifos as the active ingredient. One liter of Pestban was mixed with water in a 40-l barrel. A plastic scoop was then used to transfer this mixture from the barrel to the 20-l tank of the backpack mistblower. The mixture typically overflowed onto the backpack mistblower tank during loading. About half the time, a piece of plastic sheeting was draped over the tank to protect it from the overflow.

According to the Egyptian Ministry of Agriculture, 80 l of the Pestban solution should be applied on each feddan (60×70 m^2; equivalent to 0.42 hectares or 1.04 acres) of cotton plants with a motorized backpack mistblower. The applicators are trained to walk 40 steps/minute, and it takes about 20 minutes for four mistblower applicators to full cover a feddan. The application rate was therefore estimated to be 0.02 l of diluted Pestban per square meter. The following information was recorded on standardized field data sheets: (1) types and amounts of pesticides mixed and loaded in the mistblower during the day; (2) amount of time spent in the fields; (3) types of work clothing worn; (4) use of any personal protective equipment; and (5) worker body weight.

Air sampling

Air sampling procedures were based on NIOSH Method 5600 for OP pesticides.[20] OSHA versatile sampler (OVS) tubes (SKC, Inc., Covington, GA, USA) attached to personal air sampling pumps were used for air sampling. The OVS tubes were placed in the workers' breathing zone. SKC pumps were

calibrated to run at 2 l/minute with a Defender™ calibration instrument (Bios International Corporation, Butler, NJ, USA) before and after each study period. The air sampling pumps were placed in belt packs worn by the workers. The OVS tubes were replaced with fresh tubes mid-way through the sampling period.

Blank and spiked OVS tubes were taken into the field each study day. Field blanks were handled in the same fashion as the air samples. Spiked OVS tubes were attached to air sampling pumps at a location distant from the application site, and air was sampled throughout the study period. For one field spike, the spiking procedure breached the filter in front of the XAD-2 resin, and the resin fell out of the OVS tube part way through sampling. This sample was considered invalid and was therefore not analyzed.

All pumps ran properly for group 1 throughout the June 30 application, but three pumps used for group 2 stopped operating during the July 3 application: one pump was replaced with a backup pump after 22 minutes of application; two other pumps shut off during the last few minutes of the work shift. In each case, the total work time was recorded and we considered the air concentration measured to be representative of the total work time. All tubes were capped and transported on ice from the field to the laboratory at Menoufia University where they were stored at −20°C. They were later shipped to the University of Washington (UW) on dry ice for analysis.

Dermal patch samples

A modification of the traditional patch technique[21] was used to characterize potential dermal exposures. This method has often been used to quantify total dermal exposure and dose, but such an approach requires extrapolation from patches to body surfaces, development of clothing penetration factors, measurement of hand, foot and head exposures, and determination of chemical-specific skin absorption rates. Instead, we used the method to determine differential pesticide loading across major body regions as the basis for exposure reduction strategies.

Dermal patches (7 × 7 cm) were constructed in the laboratory from filter paper backed by aluminum foil. Duct tape secured the aluminum foil to the filter paper on all sides, leaving approximately 25 cm^2 of exposed filter paper. Patches were attached to clothing with safety pins. If the body region was not covered by clothing (e.g. the forearms), then patches were attached directly to skin with surgical tape. Eight patches were attached to each worker in the following locations: mid-chest; mid-upper back; left and right forearms; front of left and right thighs; front of left and right lower legs. Patches were worn for the duration of the work period, then removed and covered with aluminum foil and stored in a cooler until transport to the Menoufia University Hospital laboratory.

A leather punch was used to remove a 2.38 cm diameter circle from the center of each patch. Right and left patches for the forearms, upper legs, and lower legs were combined for each worker, resulting in five patch samples per worker. Patch samples were stored at −20°C until shipment on dry ice to the UW Environmental Health Laboratory for analysis. Patch loading was calculated by dividing chlorpyrifos mass by patch surface area. Loading rate was calculated by dividing chlorpyrifos loading values by the exposure time period for each worker.

Urine sample collection

Workers were asked to collect all of their urine for 24 hours (Fig. 2). For group 1, sampling began on June 28 and ended on July 2. For group 2, sampling began on July 1 and ended on July 6. Collection began and ended at 13:00 each day. Total urine volume was recorded for each worker for each day. The total 24-hour void was mixed and aliquots were drawn and stored at −20°C prior to analysis.

Laboratory analysis

The front and back portions of the OVS tubes were analyzed separately, and no significant breakthrough occurred. OVS tube resin and patch samples were extracted with acetonitrile and analyzed for chlorpyrifos residues using LC-MS-MS (Agilent 6410) and a deuterated chlorpyrifos internal standard (Cambridge Isotope Labs) by the UW Environmental Health Laboratory.[22] Extraction efficiencies were 90.5% (SD, 5.1%) for air samples and 108% (SD, 8.0%) for patches. Limits of detection were 1 and 5 ng per sample for OVS tubes and patches, respectively.

Chlorpyrifos can be converted to its oxygen analog as a part of the air sampling process.[22] Both chlorpyrifos and chlorpyrifos-oxon were measured in these samples. Chlorpyrifos-oxon represented on average 1.7% of total chlorpyrifos. The oxygen analog was considered an artifact of sampling, so total chlorpyrifos mass per sample was calculated as the sum of chlorpyrifos and chlorpyrifos-oxon, with the oxon mass converted to chlorpyrifos mass through the molecular weight ratio (350.6/334.5 g/mol, chlorpyrifos/chlorpyrifos-oxon).

Urine samples were analyzed at the University at Buffalo for TCPy by negative-ion chemical ionization gas chromatography–mass spectrometry, with 13C–15N–3,5,6-TCPy as an internal standard, as described previously.[9] Creatinine concentrations were measured using the Jaffe reaction.[23]

Inhalation dose calculation

Chlorpyrifos mass from each sample was divided by sample volume to produce chlorpyrifos air concentrations. Each worker had two air samples, so

time-weighted averages were calculated. Inhalation dose was calculated as follows:

$$D_i = [C \times MV \times T \times A]/BW \quad (1)$$

where D_i is the inhaled dose for study period (μg/kg/day), C is the time-weighted average air concentration (μg/m^3), MV is the minute respiratory volume (l/minute), T is the time of exposure for day (minute/day), A is the fraction absorbed (default=1.0) and BW is the body weight (kg).

Minute respiratory volume values specific to pesticide handlers and workers associated with pesticide applications were used:[24] light activity (16.7 l/minute) for engineers and technicians; moderate activity (26.7 l/minute) for applicators. An absorption fraction of 1.0 was used. This value was recommended recently by the California Environmental Protection Agency after review of existing literature on inhalation absorption efficiency for hazardous chemicals, including pesticides.[25]

Calculation of absorbed chlorpyrifos dose

Total TCPy mass excreted was converted to a chlorpyrifos equivalent, adjusted for incomplete excretion,[26] and divided by worker body weight, producing an absorbed dose attributable to a 1-day exposure event for each worker.

$$D_{ed} = [\text{TCPy} \times MW_{CPE}/MW_{TCPy} \times 1/IE]/BW \quad (2)$$

where D_{ed} is the chlorpyrifos dose attributable to 1-day exposure event (μg/kg), TCPy is the total mass of TCPy excreted (μg), MW_{CPF} is the chlorpyrifos molecular weight (=360.6 g/mol), MW_{TCPy} is the TCPy molecular weight (=198.4 g/mol), IE is the incomplete TCPy excretion fraction of 0.7,[26] and BW is the body weight for each worker (kg).

The background level of TCPy in urine can be affected by pesticide use at home or at another job site. Some workers had high pre-exposure levels relative to their post-exposure levels. We used the pre-exposure sample collected the day before the study as the best indicator of background. We calculated the amount of TCPy that was in subsequent samples using the two TCPy excretion half-life values available in the published literature: 27 hours (Ref. 26) and 41 hours.[27] We also calculated urinary elimination of TCPy due to the 1-day exposure event that continued beyond our urine collection periods using these same half-life values. We calculated TCPy mass excreted for four additional days for group 1 and three additional days for group 2. The daily TCPy excreted on the day of application and the five subsequent days, corrected for background TCPy, was then summed to produce a total TCPy mass excreted due solely to the exposure event.

Data analysis

Data appeared to have log-normal distributions, so air concentrations, patch loading rates, and biomonitoring data were log transformed for analysis (SPSS Statistics software, version 17). Descriptive statistics were reported as geometric means (GM) and geometric standard deviations (GSD). A one-way analysis of variance (ANOVA) was used to detect differences in time-weighted average chlorpyrifos air concentrations and biomonitoring dose estimates across job categories. The Levene statistic was used to test for homogeneity of variances. Variances for both air concentration and biomonitoring data were not equal, so Dunnet's T3 test was used for *post-hoc* tests.

The five body locations from which patch samples were collected were grouped into three body regions, so they could be prioritized for potential interventions: (1) arms; (2) legs (average of upper and lower leg); and (3) torso (average of front and back torso). Each job category was evaluated separately with a one-way ANOVA. These data had equal variances, so Tukey's honestly significant difference *post-hoc* tests were used to determine differences among the body regions.

A general linear model repeated measures analysis was used to detect differences in TCPy concentrations across job categories over a 3-day period: pre-spray day, spray day, and first post-spray day. The second post-spray day was excluded from the analysis because data were not available for group 2. Dunnet's T3 *post-hoc* test for multiple comparisons was used to determine differences between job categories. The non-parametric Kruskal–Wallis test was used to determine if the percent dermal dose differed across job categories.

Results

Application began at 17:30 for group 1 and at 17:00 for group 2. Work shifts ranged from 184 to 208 minutes for group 1, and from 214 to 226 minutes for group 2. Workers did not wear protective clothing. Some wore long-sleeve shirts, while others

Table 1 Time-weighted average chlorpyrifos air concentrations (μg/m^3) for three job categories of Egyptian workers during a single day of pesticide application to cotton fields

Job	*n*	Geometric mean*	Geometric standard deviation*
Engineer	4	5.1†,‡	1.3
Technician	4	8.2†,‡	1.1
Applicator	4	45‡	1.4

Notes: *Data were treated as log-normally distributed.
†Engineer and technician air concentrations were not significantly different (P=0.087).
‡Applicator air concentrations were significantly higher than engineer concentrations (P<0.001) and technician concentrations (P=0.003).

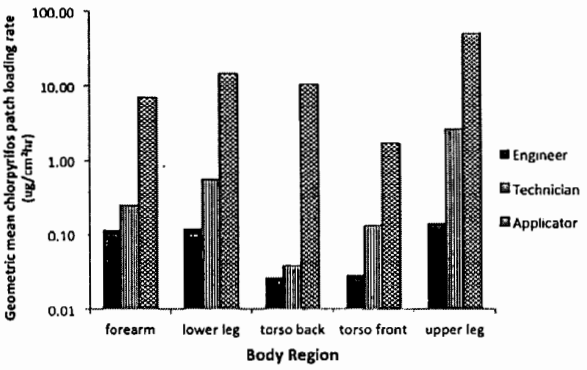

Figure 4 Geometric mean chlorpyrifos loading rates across body regions based on patch measurements (μg/cm²/hour) for three job categories of Egyptian workers for a single day of chlorpyrifos application in cotton fields.

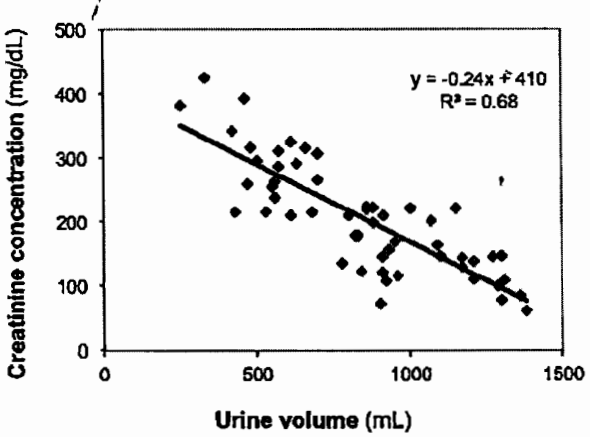

Figure 5 The relationship between urine volume and creatinine concentrations in 24-hour urine samples from Egyptian cotton production workers. The inverse relationship between creatinine concentration and urine volume suggests that most 24-hour urine samples were complete.

wore short-sleeve shirts or T-shirts. All of the workers wore long pants. Some of the workers were barefoot. None wore respiratory, eye, face, or hand protection.

Inhalation exposure

Time-weighted average (TWA) air concentrations for the three job categories are presented in Table 1. GM concentrations for engineers (5.1 μg/m^3) and technicians (8.2 μg/m^3) were not significantly different, but the mean concentration for applicators (45.0 μg/m^3) was significantly higher than those of engineers ($P<0.001$) and technicians ($P=0.003$).

Dermal exposure

GM chlorpyrifos loading rates (μg/cm²/hour) on patches are presented in Fig. 4. Overall, applicators had much higher loading rates than either technicians or engineers for each of the five body locations. Table 2 presents the analysis of loading rates across three body regions for each of the job categories. In the calculation of torso values, only the front torso values were used for the applicators, since two applicators had extremely high loading rates on their backs. Each job category showed a significant difference for body region ($P=0.04$ for engineers; $P=0.001$ for technicians; $P=0.001$ for applicators), with legs as the most highly exposed region for all job categories. *Post-hoc* multiple comparisons across body regions indicated that loading rates on the legs were significantly greater than those on the torso for engineers (GM of 0.15 versus 0.03 μg/cm²/hour). Loading rates on the legs were significantly greater than loading rates on either the torso or the arms for technicians (GM of 1.6 versus 0.33 versus 0.25 μg/cm²/hour). Loading rates on the legs were also significantly greater than loading rates on either the torso or the arms for applicators (GM of 30.1 versus 1.7 versus 7.0 μg/cm²/hour). For all job categories, no significant differences were found between torso and arm loading rates.

Urinary metabolite monitoring

The study design was based on 24-hour urine sample collection on multiple days for each worker. Workers received instructions regarding the importance of complete samples and indicated that they were providing complete samples at the time of collection. A review of the number of bottles returned indicated protocol compliance, but some samples had very low

Table 2 Chlorpyrifos loading rates (μg/cm²/hour) by job category for three body regions

	Body region				*Post-hoc* tests[¶]	
Job	Legs[†] GM (GSD)	Torso[‡] GM (GSD)	Arms GM (GSD)	ANOVA[§] *P* value	Body region	*P* value*
Engineer	0.149 (2.7)	0.029 (2.8)	0.116 (2.4)	0.039	Leg>torso	0.044*
					Leg=arm	0.087
Technician	1.62 (1.6)	0.332 (2.3)	0.246 (2.5)	0.001	Leg>torso	0.001*
					Leg>arm	0.017*
Applicator	30.1 (4.2)	1.73 (2.6)	6.96 (2.0)	0.001	Leg>torso	0.001*
					Leg>arm	0.037*

Notes: GM, geometric means; GSD, geometric standard deviations; ANOVA, analysis of variance.
*Significant *P* value <0.05.
[†]Legs: average of each subject's upper and lower leg patch loading rates.
[‡]Torso: average of each subject's front and back patch loading rates, except for applicators which is the front (chest) loading rate only because of extremely high rates for two applicators on the back patches, likely due to leaking tanks.
[§]One-way ANOVA conducted for each job category separately, since the goal was to identify job-specific interventions.
[¶]Tukey's honestly significant difference test was used as variances were homogeneous for each job category.

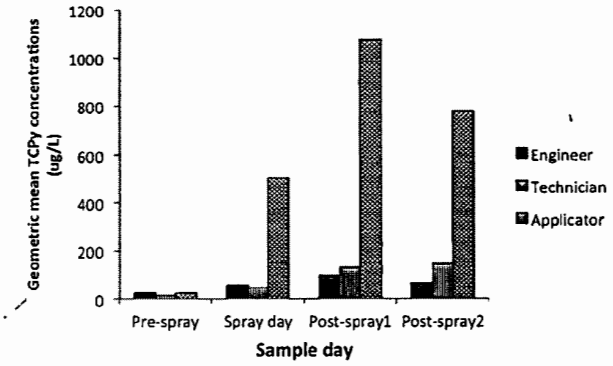

Figure 6 Geometric mean TCPy concentrations (μg/l) in urine of Egyptian cotton production workers for the day prior to spraying chlorpyrifos, the spray day, and for post-spray days (one post-spray day for group 1; two post-spray days for group 2). Collection of 24-hour urine samples took place each day at 13:00. Applications on the spray days occurred between 17:00 and 21:00.

urine volumes. We therefore examined the relationship between urine volume and creatinine concentration in these samples.

Creatinine is a waste product from muscle metabolism of creatine, eliminated through the kidney by glomerular filtration.[28] Creatinine excretion rate is independent of urinary flow rate, so creatinine concentration should be inversely related to urine flow rate, or urine volume in this case, where all samples were of 24-hour duration. We found a strong inverse correlation between creatinine concentration and urine volume (R^2=0.68), suggesting that most 24-hour urine samples were complete (Fig. 5). The World Health Organization has proposed a creatinine concentration range of 300–3000 mg/l as appropriate for biological samples.[29] No samples were below 300 mg/l, but nine samples exceeded 3000 mg/l. These high concentrations are indicative of possible dehydration that can occur under the very hot conditions during summer in the Nile delta. World Health Organization does not recommend using highly dilute or highly concentrated samples when adjusting xenobiotic concentrations by creatinine concentrations. In this study, however, we have not used creatinine concentration as an adjustment factor, since we are interested in total TCPy mass in each 24-hour urine sample. We therefore included all 54 samples in our analysis.

Peak TCPy urinary concentrations during and following exposure ranged from 75 to 129 μg/l for engineers, 78–261 μg/l for technicians, and 487–1659 μg/l for applicators. GM TCPy concentrations by day for the three job categories are presented in Fig. 6. Mauchly's test for the repeated measures analysis for 24-hour TCPy excretion indicated that the assumption of sphericity was not violated (P=0.55). Day had a significant effect (P<0.001) on 24-hour urine TCPy concentrations and change in concentration was different for each job category (P=0.001 for the day–job category interaction term). Job category alone was also significant (P=0.004). Applicator concentrations were significantly different from those of engineers (P=0.03) and technicians (P=0.03), but engineer and technician concentrations did not differ significantly.

Dose estimates

Dose estimates are presented in Table 3. GM total chlorpyrifos doses based on TCPy metabolite measurements in urine were 5.2, 8.6, and 49.8 μg/kg for engineers, technicians, and applicators, respectively, using a 27-hour TCPy excretion half-life, and 5.4, 9.7, and 56.7, respectively, using a 41-hour TCPy excretion half-life. Chlorpyrifos inhalation doses averaged 0.20, 0.30, and 3.0 μg/kg for engineers, technicians, and applicators, respectively. Applicator doses were significantly different from engineers and technicians (P=0.001 and 0.05, respectively), but engineers and technicians were not different from one another. Chlorpyrifos doses attributable to dermal exposure and subsequent absorption through skin were estimated to be 94–96% of total dose for these workers regardless of the TCPy excretion half-life used for calculations. The estimated percent dermal doses did not differ across job categories (P=0.47).

Discussion

Dermal contact and absorption was the dominant exposure route for these workers. Distribution of dermal exposure over body regions was consistent across job categories, except for the backs of two applicators. The very high dermal exposure values for these workers were likely due to leakage or spillage while using backpack mistblowers (Fig. 3). Consistently high loading rates on legs compared to the front torso for all job categories are persuasive evidence that contact with treated foliage is a major source of dermal exposure for these workers.

An earlier study by our group measured chlorpyrifos loading rates on patches among a similar team of Egyptian cotton production workers.[9] The highest loading rates recorded in that study (e.g. 422 μg/cm^2/hour on applicator upper leg; 197 μg/cm^2/hour on technician forearm) did not occur in this study. In the current study, applicators were more careful in directing spray away from both themselves and from the technicians who accompanied them in the fields. Additionally, more body contact with foliage likely occurred in the Farahat *et al.* study,[9] since data were collected in mid-August when cotton was more than waist-high, whereas the current study occurred at the end of June and early July when cotton was closer to knee height.

It was clear from observations in our previous study and in the current study that substantial

Table 3 Chlorpyrifos dose estimates and relative percent contributions of the inhalation and dermal exposure routes to total dose in Egyptian workers during a single day of pesticide application to cotton fields*

		27-hour TCPy excretion half-life				41-hour TCPy excretion half-life			
Job category	Estimated inhaled dose (μg/kg)	Total chlorpyrifos dose (μg/kg)	Calculated dermal dose† (μg/kg)	Percent inhalation	Percent dermal	Total chlorpyrifos dose (μg/kg)	Calculated dermal dose† (μg/kg)	Percent inhalation	Percent dermal
Engineer									
GM‡	0.20	5.16§,**	4.96	3.9	96.1	5.35§,**	5.15	3.7	96.3
Range	0.11–0.28	3.2–7.5				3.6–5.0			
Technician									
GM	0.35	8.60§,¶	8.25	4.1	95.9	9.67§,¶	9.32	3.6	96.4
Range	0.31–0.39	3.9–19.0				4.7–20.2			
Applicator									
GM	3.0	49.80¶,**	46.80	6.0	94.0	56.70¶,**	53.70	5.3	94.7
Range	2.1–4.1	32.4–85.2				37.1–95.8			

Notes: *Total dose was based on urinary metabolite data; inhalation dose was based on air sampling; dermal dose was calculated as the difference between total dose and inhalation dose.
†Dermal dose is the difference between total dose GM and inhalation dose GM.
‡GM, geometric mean.
§Engineer and technician doses were not significantly different (P=0.68).
¶Technician and applicator doses were significantly different (P=0.05).
**Applicator and engineer doses were significantly different (P=0.001).

exposure occurred to the hands and feet, since none of the workers wore gloves or protective footwear. We did not directly measure exposure to these regions due to time and resource constraints, but considered that reduction in exposure to these body regions would be beneficial to these workers.

Internal dose estimates

Calculation of internal dose using urinary metabolites of pesticides requires adequate human pharmacokinetic data. Chlorpyrifos is one of the few pesticides for which multiple intentional dosing studies in humans are available. Most studies that have estimated doses from urinary metabolites have used spot urine samples and adjustment to daily dose using creatinine concentrations.[9,12,30–32] Collection of 24-hour urine samples in this study obviated the need for this type of adjustment. There appeared to be good compliance with the study protocol for complete 24-hour urine collection. The inverse relationship between creatinine concentration and urine volume suggests that most 24-hour urine samples were complete (Fig. 5). To the extent that any samples were not complete, our calculated total doses and dermal doses are underestimates of the true doses, and the contribution of the dermal route to total dose would be even larger.

We used TCPy excretion half-life values available in the published literature to adjust for pre-exposure TCPy levels and to extrapolate TCPy excretion beyond the sample collection periods. The doses based on these half-life values differed to a small degree, but the percents of total chlorpyrifos dose attributable to dermal exposure and absorption were equivalent.

Calculation of total dose included adjustment for incomplete excretion of the TCPy moiety of chlorpyrifos. If this adjustment is not warranted, then our dose calculations would be lower. The adjustment was based on a human dosing study of six subjects in which only 70% of an oral dose (0.5 mg/kg) was excreted as TCPy in urine.[26]

A recent evaluation of biomonitoring data from Dow manufacturing workers calculated internal doses from TCPy urine concentrations without this adjustment, since the authors considered the missing TCPy to be due to incomplete absorption of chlorpyrifos in the gastrointestinal tract.[33] However, a recent determination of absorbed doses from chlorpyrifos applicators in the farm family exposure study assumed incomplete excretion of TCPy and adjusted dose estimates based on the Nolan study, as we did in this analysis.[34]

A study by Feldmann and Maibach[35] determined percent excretion for intravenous doses of five OP pesticides, although chlorpyrifos was not among them. They found that percent of dose excreted ranged from 38 to 90%, with a median of 70%, demonstrating that metabolites in urine do not represent the entire absorbed dose for OP pesticides. We conclude from the available evidence that an adjustment for incomplete excretion of chlorpyrifos as TCPy is appropriate and likely provides more accurate dose estimates.

The dose estimates reported here were substantially higher than the acceptable operator exposure level (AOEL) calculated for chlorpyrifos. The US Environmental Protection Agency reported a no observable adverse effect level of 150 μg/kg/day for chlorpyrifos, based on a dermal exposure study in rats that was adjusted for 3% absorption.[36] The AOEL is calculated by adjusting the animal no observable adverse effect level by two 10-fold assessment factors,[37] resulting in a chlorpyrifos AOEL of 1.5 μg/kg/day. Estimated doses for all 12 workers exceeded the AOEL. GM doses for engineers, technicians, and applicators were about 3.5 times, 6 times, and 35 times greater than the AOEL, respectively.

Inhalation dose estimates

The method used to estimate inhalation dose, expressed in equation (1), is consistent with definitions and procedures developed by the International Programme on Chemical Safety.[38] The use of a 1.0 (100%) absorption factor may result in an overestimation of dose, but this value is recommended in the absence of a reliable alternative.

The use of the standard NIOSH air sampling method for OP pesticides[20] appears to be problematic for agricultural pesticide use. The recommended OVS tubes are not able to capture inhalable particles, thereby underestimating air concentrations. OVS tubes can also convert parent compounds to their oxygen analogs; if the oxygen analogs are not part of the laboratory analysis, then air concentrations will be underestimated. In a recent study of agricultural spraying of chlorpyrifos in Washington State, polyurethane foam samplers recorded approximately three times higher air concentrations than OVS tubes.[39] If air concentrations were three times higher in Egyptian cotton fields, then our inhalation dose estimates would be increased accordingly, but the dermal route of exposure would still contribute >80% to total dose for all job categories.

Inhalation and dermal dose comparisons

Discussion of the relative contributions of inhalation and dermal exposure to dose for pesticide applicators has been complicated by a lack of distinction between the terms 'exposure' and 'dose'. The International Programme on Chemical Safety[38] defines exposure as 'contact between an agent and a target... at an exposure surface over an exposure period'; whereas,

dose is defined as 'the amount of agent that enters a target after crossing an exposure surface'. This distinction is particularly important when comparing inhalation and dermal exposures, since the absorption rates for these routes can be markedly different. Thus, calculation of the relative contributions of the inhalation and dermal routes requires determination of total absorbed dose.

Only a few studies have taken this approach. Durham *et al.* conducted sequential trials of power (airblast) spraying of parathion in orchards, estimating total dose from urinary metabolites.[17] Separating the trials into inhalation-only or dermal-only exposures, they found that dermal exposure contributed an average of 87% to the total dose. Fenske and Elkner estimated total absorbed chlorpyrifos doses for eight termiticide applicators and found that dermal exposure contributed anywhere from 52 to 90% to total dose, depending on the types of personal protection used and whether or not applicators worked in enclosed spaces.[18] Hines and Deddens calculated GM inhaled chlorpyrifos doses of 1.5–2.4 μg/kg/day for termiticide applicators conducting three different types of work tasks. TCPy was measured in morning void urine samples, but total doses were not calculated, so no conclusion could be drawn in regard to the contribution of inhalation to total dose.[15] Thomas *et al.* collected urine samples on multiple days from applicators using either 2,4-D or chlorpyrifos.[32] Absorbed dose values were calculated for the 2,4-D applicators, but not for the chlorpyrifos applicators. The authors stated that only two study subjects who used chlorpyrifos met the criteria for absorbed dose calculation, and both of these subjects had pre-application TCPy levels that exceeded those measured after applications.

Many reports of dermal and respiratory exposures for pesticide applicators have compared exposures (pesticide deposition on skin and clothing) rather than doses when commenting on the relative importance of the inhalation and dermal routes. Such comparisons can be misleading. For example, Wojeck *et al.* reported that respiratory exposure was less than 1% of total exposure for citrus workers applying ethion, implying that inhalation exposures were insignificant.[13] However, skin absorption of ethion has been estimated at only 3.3% by Feldmann and Maibach,[35] so respiratory exposure might well have contributed about 20% to total dose for these workers.

Several other studies illustrate the lack of clarity that often accompanies exposure assessments for pesticide applicators. Grover *et al.* collected both inhalation exposure data and biomonitoring data, but did not calculate inhalation dose.[16] Instead, they reported that 2,4-D inhaled by workers during ground rig applications on wheat was only 2% of potential exposure. However, calculations from data in the article indicate that the inhalation route contributed >50% to total dose for two of eight workers and that the median contribution from inhalation was approximately 8%. Conversely, Al-Jaghbir *et al.* measured both dermal and inhalation exposure for six workers spraying tomato crops with dimethoate in Jordan.[14] They reported that the maximum dose among these workers was 18.2 mg/day, but this value was based solely on inhalation exposure, ignoring any contribution from the dermal route.

In short, how workers are exposed to pesticides can be specific to place and task, and can change over time. Furthermore, the relative contributions of dermal and inhalation exposures can vary according to volatility, aerosol particle size, dermal absorption efficiency, personal protective equipment use, and whether work is primarily in enclosed spaces or outdoors. Both total dose (i.e. internal dose estimated from biological monitoring) and the dose from at least one route of exposure need to be determined in order to characterize the relative contributions of the inhalation and dermal routes.

The challenges presented by the exposures observed in this population are not completely novel. A recent study of pesticide handlers in Washington State documented the critical role of protective footwear in reducing exposures.[40] A study in Florida greenhouses demonstrated that protection of the lower body dramatically reduced skin exposure from contact with treated foliage.[41] Fluorescent tracer evaluation of skin exposure has been shown to be an effective means of communicating risks and motivating workers to adopt safer practices.[42,43] Direct engagement with the Egyptian Agricultural Ministry and the at-risk workers is needed to develop practical solutions to improve pesticide safety in Egyptian cotton production.

Conclusions

The results from this study lead us to draw several conclusions. Reduction in dermal exposures is the best strategy for reducing chlorpyrifos doses in the Egyptian cotton workers who apply pesticides for Egypt's Ministry of Agriculture. Applicators are at greatest risk and should be the focus of interventions. Chemical protective clothing for the lower portion of the body would greatly reduce dermal exposure, especially for applicators and others who enter the field. Chemical-resistant footwear and gloves should be used to further reduce dermal exposures. Routine preventive maintenance on the backpack mistblowers will reduce leakage and contribute to minimizing applicator exposures. Using fluorescent tracers in spray tanks for training would benefit worker understanding of dermal exposures by visualizing pesticide residue on skin and clothing.

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