Chlorpyrifos exposure in farmers and urban adults: metabolic characteristic, exposure estimation, and potential effect of oxidative damage

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Abstract

Chlorpyrifos is a widely used organophosphorus pesticide that efficiently protects crops against pests. However, recent studies suggest that severe exposure to chlorpyrifos may present adverse health effects in human. To analyze the exposure level and metabolic characteristics of chlorpyrifos pesticide in urban adults and farmers with/without occupation pesticide contact, the occurrence of urinary chlorpyrifos and methyl chlorpyrifos (CP-me), as well as their metabolite, 3,5,6-trichloro-2-pyridinol (TCPy), was determined in farmers of an agricultural village in China, and in urban adults of a nearby town. The geometric mean (GM) concentrations of TCPy, which is the major marker of chlorpyrifos exposure, were 4.29 and 7.57 $\mu\text{g/g-creatinine}$ in urban adults and farmers before pesticide application, respectively. Chlorpyrifos spraying significantly increased the concentrations of urinary TCPy. In the first day after spraying, a GM concentration of 43.7 $\mu\text{g/g-creatinine}$ was detected in the urine specimens from farmers, which decreased to 38.1 and 22.8 $\mu\text{g/g-creatinine}$ in the second and third day after chlorpyrifos spraying. The ratio of TCPy and its parent compounds, i.e. chlorpyrifos and CP-me, was positively associated with the sum concentration of urinary chlorpyrifos, CP-me, and TCPy, suggesting the increasing metabolic efficiency of chlorpyrifos to TCPy at higher chlorpyrifos exposure levels. To estimate the farmers' occupational exposure to chlorpyrifos pesticide, a new model based on the fitted first-order elimination kinetics of TCPy was established. Occupational chlorpyrifos exposure in a farmer was estimated to be 3.70 $\mu\text{g/kg-bw/day}$ (GM), which is an exposure level that is higher than the recommended guideline levels. Significant increase of urinary 8-hydroxydeoxyguanosine (8-OHdG) was observed on the first day after chlorpyrifos spraying, which indicates a potential oxidative damage in farmers. However, urinary 8-OHdG returned to its baseline level within two days.

Keywords: chlorpyrifos; biomonitoring; metabolic characteristic; exposure estimation; 8-hydroxydeoxyguanosine (8-OHdG)

INTRODUCTION

Organophosphorus pesticides (OPPs) are widely used in many countries for controlling various insect pest in agriculture and animal production (Akoto 2008; Wang et al., 2008; Wu et al., 2010; An et al., 2014). As a broad-spectrum OPP, chlorpyrifos is used to control insects in various crops (Giesy et al., 1999). In addition, chlorpyrifos is also one of the major insecticides used in residential settings, as it is contained in nearly 800 registered products on the market (Grabusky et al., 2004). In China, an annual chlorpyrifos production of 200000 tons was estimated in 2015 (John and Shaike, 2015). Due to the widespread usage, chlorpyrifos has been frequently detected in air, soil, and water environments in China (Li et al., 2011&2014; Zhang et al., 2012).

Chlorpyrifos and its homologue, methyl chlorpyrifos (CP-me), are active compounds in chlorpyrifos pesticides (Griffin et al., 1999; Morgan et al., 2005; Eaton et al., 2008). The in vivo metabolism pathway of chlorpyrifos and CP-me in animals is showed in Figure S1 in the supplementary data. Several studies found that 3,5,6-trichloro-2-pyridinol (TCPy) is the dominant metabolite of chlorpyrifos in humans (Sultatos et al., 1984; MacIntosh et al., 2001; Olsson et al., 2004; Alexander et al., 2006; Morgan et al., 2011; Liu et al., 2014; Trunnelle et al., 2014). As such, TCPy is used as the biomarker of chlorpyrifos exposure. For example, urinary TCPy was included in the U.S. National Report on Human Exposure to Environmental Chemicals, with a geometric mean concentration (GM) of 0.779 $\mu\text{g/L}$ in the U.S. adults during 2009–2010 (USCDC, 2015). Recently, TCPy was detected in 70% of urine specimens from Chinese infants (Liu et al., 2014), indicating wide exposure to chlorpyrifos among the Chinese. However, limited information is available on chlorpyrifos exposure in Chinese adults until now. Pesticide application might result

in an occupational exposure to chlorpyrifos in farmers. Increased urinary TCPy among farmers in the days following pesticide application has been reported in some cases (Alexander et al., 2006; Curwin et al., 2007b), suggesting potential residue in human body when farmers were exposed to chlorpyrifos at relatively high dose. Therefore, question will present if the daily intake model with urinary TCPy of only one day was used in estimating the chlorpyrifos exposure through pesticide application activity.

Chlorpyrifos and CP-me was found to inhibit cholinesterase (USEPA, 2000) and cause disorder of the neurotransmission and reproduction in animals (Lemus et al., 2000). Statistically significant correlation between urinary TCPy and adverse health end points was found only in limited studies in recent years. As reported by Farahat et al. (2011), inhibition of blood butyrylcholinesterase and acetylcholinesterase was observed in farmworkers exposed to chlorpyrifos, while inverse correlation between urinary TCPy and serum total thyroxine and thyrotropin in the U.S. was proposed in another epidemiological study (Fortenberry et al., 2012). The ability of chlorpyrifos to induce oxidative stress in cell and animals experiments has been proved (Saulsbury et al., 2009; Ma et al., 2013). However, epidemiological evidence on the oxidative damage effect of chlorpyrifos is still insufficient.

In the current study, urine specimens from adult farmers were collected daily before and after the spraying activity of chlorpyrifos pesticide. Urinary chlorpyrifos and CP-me, as well as the major metabolites, TCPy and chlorpyrifos-oxon (CP-oxon) were measured and compared with those in nearby urban residents. The objectives of this study were to (i) determine the concentrations of urinary chlorpyrifos and metabolites in Chinese adults and evaluate the effects of pesticide spraying; (ii) establish an exposure estimation model for the chlorpyrifos exposure induced by

pesticide spraying on the basis of the toxic kinetics of chlorpyrifos and the detected urinary TCPy in farmers; and (iii) discuss the potential effect of oxidative damage induced by chlorpyrifos exposure.

1. MATERS AND METHODS

2.1. Sample collection

Urine specimens (n=165) were collected in May, 2013 from adult farmers and urban residents in a village and a nearby town in Shandong Province, China. Among which, 120 samples from 20 adult farmers occupying in spraying activity (9 males and 11 females) were collected daily 3 days before and 3 days after chlorpyrifos pesticide was sprayed in their farmlands. Samples were collected on only 3 following days after pesticide spraying, because some farmers might contact pesticide again on the fourth day after spraying. During the same period, urine samples (n=45) were collected for 3 days from 15 adults (7 males and 8 females) living in a nearby town that is located approximately 25 km far from the village. First morning urines were collected through the use of 50 mL polypropylene (PP) tubes, shipped in dry ice to the laboratory within 6 h, and stored at -80 °C before analysis. Additional information on pesticide spraying is provided in Table S1 (Supplementary data). Urine collection was approved by the Institutional Review Board of Nankai University.

2.2. Reagents and standards

Standards of chlorpyrifos (purity: 99.9%), CP-me (99.9%), CP-oxon (99.9%), TCPy (99.2%), d₁₀-chlorpyrifos, creatinine (98%), ammonium acetate (98%) and β -glucuronidase from *Helix romantia* (129316 units/mL) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.). The structure and physiochemical properties of the target analytes are listed in Table S2.

2.3. Sample preparation.

The urine samples were analyzed for chlorpyrifos, CP-me, TCPy, and CP-oxon of its metabolites by liquid-liquid extraction (LLE), as described in a previous study (Wang and Kannan, 2013), with slight modifications. Briefly, 100 μ L of d_{10} -chlorpyrifos solution (100 ng/ml) were spiked into 2 ml of urine taken in a 15 ml PP tube. The sample was buffered with 300 μ L of 1M ammonium acetate buffer containing 77 units of β -glucuronidase (prepared by spiking 50 μ L of β -glucuronidase into 100 ml of 1.0 M ammonium acetate solution) and digested at 37 °C for 12 h in an incubator shaker. The mixture was extracted with 8 ml of ethyl acetate by shaking for 60 min and then centrifuged at 3000 r/min for 5 min. After the supernant was taken out, LLE was repeated once more with 5 ml of fresh ethyl acetate. Two supernants were combined and washed with 1 ml of Milli-Q water by shaking for 10 min, and then concentrated to near-dryness under a gentle nitrogen stream. Finally, 0.5 ml methanol was added and vortex mixed for analysis by UPLC-MS/MS.

2.4. Instrumental analysis

The separation and detection of four target analytes, (i.e. chlorpyrifos, TCPy, CP-me and CP-oxon) were accomplished with an acquity ultrahigh-performance liquid chromatography system (UPLC, Milford, MA, U.S.) equipped with a 2777 autosampler, interfaced with a Xevo TQ-S triple quadrupole mass spectrometer (ESI-MS/MS, Waters, Milford, MA, U.S.). Ten microliters of the sample were injected into an analytical column (BEH Shield RP18, 3.0 mm $\times$ 100 mm, 1.7 μ m, Waters, MA, U.S.), which was connected to a Javelin guard column (VanGuard BEH C18, 1.7 μ m). Two different gradient elutions at a flow rate of 0.4 mL/min were used for the analysis of TCPy and rests, which are described in detail in Table S3. The MS/MS was operated in multiple reaction monitoring (MRM) negative ion modes for

TCPy and positive ion modes for other analytes. The MS/MS parameters were optimized by the infusion of individual analytes, as shown in Table S4.

Urinary creatinine was used to adjust the concentration of the target analytes. The analysis of creatinine was performed according to our previous study (Wang, et al., 2012).

Determination of 8-OHdG in urine was performed using a commercial Human Hydroxylysine (Hyl) ELISA Kit (Westang Bio, Shanghai, China), according to the method of Lee et al. (2007). The final reported urinary 8-OHdG levels were adjusted by individual urinary creatinine concentration.

2.5. *Quality Assurance and Quality Control (QA/QC).*

Contamination arising from laboratory materials and solvents was evaluated by analyzing procedural blanks. Recoveries of target analytes in spiked procedural blanks at the levels of 5 and 10 ng ranged from 54.3% to 63.3% and from 69.6% to 80.1%, respectively (Table S5). Analytes quantification was achieved by using an isotope-dilution method based on the responses of d₁₀-chlorpyrifos (for chlorpyrifos, CP-me, and CP-oxon) and ¹³C₆-MeP (for TCPy). The limit of quantification (LOQs), which was calculated from the value of the lowest acceptable calibration standard and a nominal sample volume of 2 mL, was 0.05 ng/mL for chlorpyrifos and CP-oxon and 0.10 ng/mL for CP-me and TCPy. A calibration standard and methanol were injected after every 20 samples to check the drift in instrumental sensitivity and the carry-over of target analytes between samples, respectively. The relative standard deviation (RSD) of check-draft analysis of samples was <10% and no target analytes were detected in the solvent blank and analyzing blanks. Instrumental calibration was verified by injection eight calibration standards (at concentrations ranging from 0.50 to 100 ng/mL for TCPy and 0.05 to 10 ng/mL for chlorpyrifos, CP-me and CP-oxon), and the

regression coefficients (R) of all calibration curves were ≥ 0.99 .

2.6. Data analysis

Data were acquired by using the MassLynx V4.1 software package (Waters Corporation, Milford, MA, U.S.). Repeated measures ANOVA and one way ANOVA were performed with SPSS 16.0 (IBM Inc.). For data analysis, concentrations below the LOQ were assigned a value equal to the LOQ divided by the square root of 2, in the calculation of GM values. In the calculation of the sum concentration of different analytes, a value of “0” was assigned to samples with concentration below the LOQ. Correlations between analytes were assessed by Pearson’s correlation analysis. A value of $p < 0.05$ was considered significant.

3. RESULTS AND DISCUSSION

3.1. Urinary concentrations of chlorpyrifos, CP-me, and TCPy in farmers and urban adults

The concentrations of total forms (free form plus conjugated forms) of urinary chlorpyrifos, CP-me, and TCPy in farmers and urban adults were shown in Table 1. Prior to pesticide spraying, chlorpyrifos and CP-me were detected in almost all urine samples from farmers, with GM concentrations of 0.367 $\mu\text{g/g-creatinine}$ (for chlorpyrifos) and 0.403 $\mu\text{g/g-creatinine}$ (for CP-me) (Table 1). TCPy, which is the major metabolite of chlorpyrifos and CP-me, was detected in all samples, with a GM concentration of 7.57 $\mu\text{g/g-creatinine}$ and a maximum concentration of 106 $\mu\text{g/g-creatinine}$ detected in a female farmer. Non-occupation exposure to chlorpyrifos through vegetable, air, water, food, and infant toy for common populations has been proposed in the previous studies (Gurunathan et al., 1998; Cabras and Angioni, 2000; Wang et al., 2008; Li et al., 2014; Yuan et al., 2014). This might explain the wide exposure of this pesticide in farmers with no occupational exposure.

In urban adults who live in a nearby town, the concentration level of urinary TCPy (GM: 4.29 $\mu\text{g/g-creatinine}$) was slightly lower than that detected in farmers in the absence of pesticide spraying, but with less significance (Table S6). The highest concentration of TCPy, i.e. 38.4 $\mu\text{g/g-creatinine}$, was detected in a sample from a female donor. For the parent compounds of TCPy, i.e. chlorpyrifos and CP-me, the GM concentrations of urban adults were 0.229 and 0.261 $\mu\text{g/g-creatinine}$, respectively. High detection rate of chlorpyrifos (>93.3%), CP-me (>86.7%), and TCPy (100%) indicates the widespread exposure to chlorpyrifos pesticide among urban adults.

Urinary TCPy is generally accepted as a biomarker of chlorpyrifos exposure (Farahat et al., 2011; Fortenberry et al., 2012). The GM concentrations of urinary TCPy in Chinese urban adults and farmers (prior to pesticide spraying) detected in the current study were higher than the reported urinary TCPy in the infants from Southeast China (GM concentration of 1.33 $\mu\text{g/L}$) (Liu et al., 2014). Similar levels of urinary TCPy were also detected in people living in agricultural regions of Thailand (Panuwet et al., 2008; Rohitrattana et al., 2014).

Pesticide spraying significantly increased urinary TCPy farmers in the following 3 days after pesticide spraying. On the 1st, 2^{ed}, and 3rd day after spraying activity, GM urinary TCPy were determined as 43.7, 38.1, and 22.8 $\mu\text{g/g-creatinine}$, respectively. Repeated measures suggest a highly significant effect ($p=0.001$, $F=6.391$) of spraying activity on urinary TCPy in farmers (Table S7). Results of one way ANOVA also indicate that, urinary TCPy in farmers on the three following days after pesticide spraying was significantly higher than those in farmers before spraying and in the urban adults ($p<0.01$), while the difference of urinary TCPy between urban adults and farmers before pesticide spraying is insignificant (Table S6). Similar results have been reported in other studies on chlorpyrifos pesticide occupational exposure of farmers

(Alexander et al., 2006; Curwin et al., 2007b). As reported by Alexander et al. (2006), pesticide spraying operation increased the GM concentration of urinary TCPy in farmers from MN, U.S., from 4.2 $\mu\text{g/g-creatinine}$ (one day before spraying) to 10.5 $\mu\text{g/g-creatinine}$ (one day after spraying). Phung et al. (2012) found that after pesticide application, mean concentration of urinary TCPy in rice farmers increased from 2.8 $\mu\text{g/g-creatinine}$ to 47.5 $\mu\text{g/g-creatinine}$. In this study, a maximum concentration of 294 $\mu\text{g/g-creatinine}$ was detected in a urine sample from a female farmer in the first day after pesticide spraying, which indicates high exposure to chlorpyrifos pesticides for some farmers. The occurrence of the parent compounds of TCPy, i.e. chlorpyrifos and CP-me, in farmers were not detected in those studies. In the samples of the present study, the increase in urinary chlorpyrifos and CP-me after pesticide spraying was insignificant compared with that of urinary TCPy (Table 1).

CP-oxon, which is another metabolite of chlorpyrifos, was detected in limited samples (detection rate <10% in all groups), with concentrations near the LOQ. Previous animal experiment indicated that 10 to 20% of chlorpyrifos exposed in rats was metabolized to CP-oxon, which can hydrolyzed soon to TCPy (Sunaga et al., 1989). Considering the low detection rate of urinary CP-oxon, the occurrence of this analyte was not further discussed in this study.

Gender difference of urinary TCPy was found in farmers especially for the samples collected on the 1st and 2^{ed} day after pesticide spraying. For example, GM concentration of 64.5 and 57.4 $\mu\text{g/g-creatinine}$ of urinary TCPy were detected in females on the 1st and 2^{ed} day after spraying, which were higher than the corresponding concentrations detected in males, i.e. 27.2 and 22.7 $\mu\text{g/g-creatinine}$ (Table 1). However, result of repeated measures suggests limited statistical significance ($p=0.276$, $F=1.276$) for the factor of gender (Table S7), which was further

certificated by the test of one way ANOVA (Table S8). In this study, male and female farmers worked together during the pesticide spraying activity, with no observable difference on working hours or intensity. Gender difference might be relevant as the difference of males and females in metabolism and in body fat distribution. In addition, washing clothes after spraying is assumed to lead to an extra pesticide exposure for the females, which needs to be further verified in future studies.

3.2. *Distribution profile of urinary chlorpyrifos, CP-me and TCPy in farmers and urban adults*

As mentioned above, although urinary TCPy in farmers increased significantly after pesticide spraying, the changes of urinary chlorpyrifos and CP-me were insignificant (Table 1), thereby suggesting the varied distributions of chlorpyrifos, CP-me, and TCPy in different populations. As show in Figure S2, profile of the parent compounds, chlorpyrifos and CP-me, accounted for about 8%–9% among three target analytes in urban adults and in farmers before pesticide spraying. On the first day after pesticide spraying, the profiles of urinary chlorpyrifos and CP-me in female and male farmers decreased considerably to 0.8%–1.2% for each compound.

TCPy is commonly considered the major metabolite of chlorpyrifos and CP-me (ATSDR, 1997; Choi et al., 2006; USCDC, 2015). Therefore, a ratio of TCPy concentration to the sum concentration of TCPy, chlorpyrifos and CP-me, i.e. $f = \frac{[TCPy]}{[\sum CPs]}$, was used to evaluate the degree of phase I metabolism of chlorpyrifos and CP-me. This relationship might be observed when the natural logarithmic concentration (ln) of f was related to the corresponding ln $[\sum CPs]$ in samples (Figure 1). This indicates that with the increasing chlorpyrifos exposure, the metabolic efficiency to TCPy might increase.

3.3. *Elimination rate of chlorpyrifos in farmers after pesticide spraying*

After pesticide spraying, the elimination of chlorpyrifos in farmers can be assessed on the basis of the changes of the urinary TCPy in the following three days (Figure 2A). Compared with the baseline level of urinary TCPy before pesticide spraying (GM=7.57 $\mu\text{g/g-creatinine}$, Table 1), GM urinary TCPy increased to 43.7 $\mu\text{g/g-creatinine}$ in the samples on the first day after spraying; this value reduced to 38.1 and 22.8 $\mu\text{g/g-creatinine}$ in the samples on the second and third days after spraying, respectively. According to the questionnaire survey, the farmers who volunteered in this study were not occupational exposed to any chlorpyrifos pesticide when they didn't conduct the pesticide spraying activity. Therefore, the occurrence of urinary TCPy in the samples on the second and third days after pesticide spraying, which was still significantly higher than the baseline level, is also due to the activity of pesticide spraying. The elevated urinary TCPy detected in farmers on the second and third days after pesticide spraying also support the notion that parts of exposed chlorpyrifos and CP-me might concentrate in the human body and be eliminated in the following days.

According to the data of human experiments, metabolism from chlorpyrifos to TCPy is very efficient (Nolan et al., 1984; Griffin et al., 1999; Sams and Jones, 2011), and first-order elimination kinetics, as shown in Equation 1, can be applied to describe the elimination of TCPy in the human body (Nolan et al., 1984),

$$\ln C_t = -kt + \ln C_0 \quad (1)$$

where C_0 and C_t are the concentrations of urinary TCPy at the original time and at t hours, k is kinetics coefficient, and t is the time from pesticide spraying. As shown in Figure 2B, the changes in the GM concentrations of TCPy in the urine samples of the three following days after pesticide spraying can be described well by a first-order elimination kinetics, i.e. $\ln C = -0.016 t + 4.18$. According to this kinetics model,

urinary TCPy needs approximately 136 h to resume to its baseline level before pesticide spraying. This value is very similar to that calculated in another study (Phung et al., 2012), which reported that urinary TCPy in farmers returned to the baseline level at 144 h after pesticide application. The half-life ($t_{1/2}$) of TCPy in farmers after pesticide spraying can be calculated as 43.3 h, according to a formula of “ $t_{1/2} = \frac{\ln 2}{k}$ ”. This $t_{1/2}$ is higher than those of the oral exposure to chlorpyrifos in human volunteer studies (Nolan et al., 1984; Griffin et al., 1999; Sams and Jones, 2011) but similar to that from dermal exposure experiments in humans (Griffin et al., 1999). The relatively long half-life of TCPy might be related to the much higher lipophilicity of chlorpyrifos and CP-me (with log K_{ow} of 4.96 and 4.13, Table S2) than TCPy (with log K_{ow} of 1.22). As reported by animal experiments, chlorpyrifos can concentrate in the body tissues of rats, particularly in adipose tissues (Tanvir et al., 2015). Therefore, in addition to those eliminated as urinary TCPy, chlorpyrifos (or CP-me) exposed in farmers might also concentrate in human body tissues, which weakening the increase in the urinary levels of chlorpyrifos and CP-me.

3.4. Exposure estimation of chlorpyrifos exposed by pesticide spraying.

As a widely used organophosphorus pesticide, human exposure to chlorpyrifos has been estimated various studies on the basis of the urinary level of the TCPy. In most studies (Morgan et al., 2005; Alexander et al., 2006; Curwin et al., 2007a; Farahat et al., 2011; Phung et al., 2012; Liu et al., 2014; Rohitrattana et al., 2014), the intake of chlorpyrifos was commonly estimated according to the following equation:

$$EI = \frac{C_{TCPy} \times P \times R_{mw} \times CF}{BW} \quad (2)$$

where EI is the estimated intake of chlorpyrifos ($\mu\text{g/kg-bw/day}$); C_{TCPy} is the concentration of urinary TCPy in the sampling day ($\mu\text{g/kg-bw/g-creatinine}$); P is the

daily elimination of urinary creatinine (g-creatinine/day), which is calculated as the product of the detected concentration of urinary creatinine in this study and a urine elimination volume of 2.0 L/day (Guo et al., 2011); R_{MW} is the adjustment factor of the molecular weight (MW) between TCPy and chlorpyrifos ($\frac{MV_{chlorpyrifos}}{MV_{TCPy}} = 1.77$); CF of 1.4 is the correction factor of urinary TCPy, from reciprocal of 70% (approximately 70% of absorbed chlorpyrifos is excreted as urinary TCP) (Nolan et al., 1984); and BW is body weight (kg). Equation (2) has been used in studies of occupational and non-exposure to chlorpyrifos in farmers and common populations (Alexander et al., 2006; Curwin et al., 2007a; Farahat et al., 2011; Phung et al., 2012; Liu et al., 2014; Rohitrattana et al., 2014). In the current study, the GM EI of the baseline level in farmers and urban adults were calculated as 0.261 $\mu\text{g/kg-bw/day}$ and 0.150 $\mu\text{g/kg-bw/day}$, respectively, which are at the same level with that reported in recent studies on Asian countries (Liu et al., 2014; Rohitrattana et al., 2014), but lower than those in the U.S. populations reported in 2006 (Alexander et al., 2006) and 2007 (Curwin et al., 2007a) before the ban of domestic use of chlorpyrifos in the US. According to Equation 2, the increase of EI induced by pesticide spraying (ΔEI) was calculated as 1.47 $\mu\text{g/kg-bw/day}$ (Table 2).

The model of Equation 2 was established based on the assumption that “*the daily exposed chlorpyrifos can be metabolized to TCPy and eliminated through urine within 24 h*”. This model is suitable for estimating the low dose daily intake of pesticide for the common people, but might be unsuitable for estimating chlorpyrifos intake during a pesticide spraying activity. As mentioned above, after pesticide spraying, approximately 136 h is needed before urinary TCPy can resume its baseline level in farmers. This indicates that within 5–6 days after pesticide spraying, urinary TCPy might reflect the chlorpyrifos from the spraying activity. Therefore, the value estimated

by Equation 2 does not reflect the daily intake dose but the daily elimination dose of chlorpyrifos. As shown in Figure 3, to obtain a relatively accurate ΔEI , the daily elimination doses of chlorpyrifos on the following days after pesticide spraying should be added, until the urinary TCPy resumes its baseline level. Accordingly, ΔEI should be estimated as follows,

$$\Delta EI = \frac{P' \times R_{mw} \times CF}{BW} \times \int_0^{t'} (C_{TCPy} - C_{base}) dt \quad (3)$$

where P' is the elimination kinetics coefficient of urinary creatinine (g-creatinine/h), which can be roughly expressed as " $P/24$ ", and t' is the time at which C_{TCPy} resumes its baseline level (C_{base}). Based on the first-order elimination kinetics (Equation 1), $C_{TCPy} = C_0 \times e^{-kt}$ and $t' = \frac{\ln C_0 - \ln C_{base}}{k}$ could be obtained and substituted into Equation 3. Accordingly, Equation 3 can be further transformed to Equation 4 as follows:

$$\begin{aligned} \Delta EI &= \frac{P' \times R_{mw} \times CF}{BW} \times \int_0^{t'} (C_0 \times e^{-kt} - C_{base}) dt \\ &= \frac{P' \times R_{mw} \times CF}{BW} \times \frac{[(C_0 - C_{base}) - C_{base} (\ln C_0 - \ln C_{base})]}{k} \end{aligned} \quad (4)$$

As shown in Table 2 and Figure 3, the GM ΔEI calculated according to Equation 4 is 3.70 $\mu\text{g/kg-bw/day}$, which is nearly thrice that calculated by Equation 2 (1.47 $\mu\text{g/kg-bw/day}$).

By selecting different endpoints (e.g. NOEL or NOAEL) and different safety factors, varied acute guidelines were recommended by different agencies. For example, a guideline of 5 $\mu\text{g/kg-bw/day}$ was recommended by the USEPA(2000), whereas 3 $\mu\text{g/kg-bw/day}$ was recommended by ASTDR (1997). As shown in Table 2, the baseline EI level of chlorpyrifos is commonly lower than these recommended guidelines. However, ΔEI of chlorpyrifos induced by pesticide spraying (GM 3.70

µg/kg-bw/day), indicates an exposure level higher than the recommended guideline levels. In addition, a maximum Δ EI of 19.3 µg/kg-bw/day was estimated in a female farmer (Table 2), indicating a very high risk to human health induced by pesticide spraying.

3.5. Potential relationship between chlorpyrifos exposure and urinary 8-OHdG level in farmers

Oxidative stress induced by chlorpyrifos has been identified in cell and animals experiments (Saulsbury et al., 2009; Ma et al., 2013). As a common biomarker of oxidative stress to DNA, 8-OHdG was determined in urine specimens of this study (Wu et al., 2004). In 120 specimens from 20 farmers, the urinary 8-OHdG was detected in the range of 8.38~253 µg/g-creatinine, with a GM of 35.7 µg/g-creatinine. No statistically significant associations were observed between urinary 8-OHdG and the target analytes (TCPy, chlorpyrifos, CP-me, or sum of them) in farmers. This might be due to the relatively great individual variation of 8-OHdG elimination, which might be affected by age, gender, and personal habits such as cigarette smoking (Wu et al., 2004). However, significant increase of urinary 8-OHdG in farmers was found on the first day after pesticide spraying. As shown in Figure 4, GM urinary 8-OHdG increased from the baseline level (about 30 µg/g-creatinine) to 61.3 µg/g-creatinine in specimens on the first day after pesticide spraying, which then decreased to the baseline level in the second and third days after pesticide spraying. In a previous study, positive correlation between urinary 8-OHdG and other organophosphorus pesticides metabolites, such as dimethyl phosphate and dialkyl phosphates, has been observed in indoor insecticide sprayers (Lee et al., 2007) and agricultural workers (Kisby et al., 2009). Our result validates that, occupational exposure to organophosphorus pesticide, chlorpyrifos, might result in an increase in

urinary 8-OHdG, which causes an oxidative stress in humans. However, this stress damage seems to be repaired soon with the elimination of chlorpyrifos and metabolites.

4. CONCLUSION

In summary, detected urinary chlorpyrifos, CP-me and TCPy with high frequency indicates a widely exposure to chlorpyrifos pesticide for the Chinese urban adults and farmers in this study. For farmers who applied chlorpyrifos pesticide, concentration of urinary TCPy increased significantly on the following days after pesticide spraying, indicating high occupational exposure to chlorpyrifos pesticide in these farmers. According to the first-order elimination kinetics, a new exposure model was developed and the occupational exposure to chlorpyrifos pesticide in the farmers, which was close to or even higher than the recommended guideline level in human, was estimated. In addition, increase of urinary 8-OHdG, a biomarker for DNA damage, was also found in farmers after the pesticide spraying. All above indicate the high risk to human health of farmers induced by chlorpyrifos pesticide application.

Supplementary data

Supplementary data associated with this article can be found in the online version.

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FIGURE LEGENDS

Figure 1. Correlation between phase I metabolism coefficient ($f_1 = \frac{[TCPy]}{[\sum CPs]}$) of chlorpyrifos and sum concentration of urinary CP, CP-me and TCPy ($[\sum CPs] = [CP] + [CP-me] + [TCPy]$).

Figure 2. GM concentrations of urinary TCPy in farmers as related to pesticide spraying time(A) and the first order elimination kinetics curve fitted according to the data of the following 3 days after pesticide spraying, with time of 18 h, 42 h, and 66 h from spraying put in to the equation (B). Three outliers of urinary TCPy, i.e. 106 µg/g-creatinine in a farmer before spraying, 294 and 258 µg/g-creatinine in a farmer on the 1st and 2^{ed} day after spraying were identified by using Boxplot method.

Figure 3. Schematic diagram for the increase in EI induced by pesticide spraying (ΔEI) according to Equation 2 or Equation 3. ΔEI of chlorpyrifos according to Equation 3 was represented as the pink shadow area in diagram, while that estimated according to Equation 2 was represented as the green shadow area.

Figure 4. Levels of urinary 8-OHdG in farmers on different day before and after pesticide spraying. The lower and upper boundaries of each box represent the 25th and 75th percentiles, and the line within the box is the median concentration. The whiskers represent the 10th and 90th percentiles, and the dots are outlier samples.

Table 1. Concentrations of urinary CP, CP-me and TCPy in farmers and urban adults
($\mu\text{g/g-creatinine}$) ^a

	Chlorpyrifos	CP-me	TCPy
in urban adults (n=45 ^b)			
Geomean	0.229/0.259/0.204	0.261/0.307/0.225	4.29/5.07/3.68
GeoSD	2.22/2.35/2.06	2.37/2.27/2.07	2.92/2.74/3.03
Max	1.03/1.03/0.794	1.33/1.33/1.01	38.4/38.4/30.4
Detection rate	93.3%	86.7%	100%
in farmers before pesticide spraying (n=60 ^c)			
Geomean	0.367/0.352/0.390	0.403/0.400/0.410	7.57/8.90/6.02
GeoSD	3.08/2.84/3.51	2.68/2.31/3.27	2.23/2.08/2.35
Max	5.52/2.45/5.52	6.75/1.96/6.75	106/35.6/106
Detection rate	100%	100%	100%
in farmers on the 1st day after pesticide spraying (n=20 ^c)			
Geomean	0.449/0.484/0.410	0.474/0.463/0.489	43.7/64.5/27.2
GeoSD	2.71/3.08/2.27	2.31/2.66/1.86	3.52/3.08/3.48
Max	5.18/5.18/1.93	3.87/3.87/1.09	294/294/116
Detection rate	100%	95.0%	100%
in farmers on the 2^{ed} day after pesticide spraying (n=20 ^c)			
Geomean	0.497/0.720/0.312	0.421/0.545/0.305	38.1/57.4/22.7
GeoSD	3.06/2.75/2.92	2.97/3.15/2.48	3.60/3.08/3.58
Max	3.62/3.62/3.25	2.81/2.81/2.26	258/258/155
Detection rate	100%	75.0%	100%
in farmers on the 3rd day after pesticide spraying (n=20 ^c)			
Geomean	0.520/0.612/0.422	0.508/0.623/0.391	22.8/27.3/18.0
GeoSD	2.89/2.12/3.79	3.04/1.90/4.38	4.39/3.67/5.21
Max	7.66/2.85/7.66	8.99/1.88/8.99	114/112/114
Detection rate	100%	75.0%	100%

^a: Urinary concentrations of total forms (free plus conjugated forms) of analytes corrected by creatinine were shown in the table; “concentration in all samples/ concentration in samples from females / concentration in samples from males” were presented for each group;

^b: Urine of urban adults were collected from 8 females and 7 males;

^c: Urine of farmers were collected from 11 females and 9 males;

Table 2. Estimated intake (EI) of chlorpyrifos in farmers induced by pesticide spraying and in urban adults (µg/kg-bw/day).

	Model 1 ^a			Model 2 ^a		
	EI_{base}^b	EI_{1st-d}^b	ΔEI^b	$C_{0,TCP}$	k	ΔEI^c
In farmers						
GM (n=20)	0.261	1.63	1.47	1.05	0.016	3.70
Max ^d	4.50	11.4	6.90	8.93	0.037	19.3
In urban adults						
GM	0.150	--	--	--	--	--
Max ^d	1.25	--	--	--	--	--

^a Model 1 and 2 were shown as equation 2 and equation 4 in the text

^b EI_{base} was the baseline daily intake of chlorpyrifos calculated according to equation 2 using urinary C_{TCPy} shown in Table 1; EI_{1st-d} was the elimination amount of chlorpyrifos calculated according to equation 2 using C_{TCPy} in urine of the first day after pesticide spraying; ΔEI was calculated according to " $EI_{1st-d} - EI_{base}$ ";

^c ΔEI was calculated according to equation 4;

^d Maximum intake of chlorpyrifos induced by pesticide spraying was found in a female farmer aged 68 (with BW of 50 kg) and in a female urban adult aged 31 (with BW of 52 kg), respectively.

Figure 1

Figure 2

Figure 3

Figure 4