The reliability of using urinary biomarkers to estimate children's exposures to chlorpyrifos and diazinon

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Abstract

A few studies have reported concurrent levels of chlorpyrifos and diazinon and their environmentally-occurring metabolites, 3,5,6-trichloro-2-pyridinol (TCP) and 2-isopropyl-6-methyl-4-pyrimidinol (IMP), in food and in environmental media. This information raises questions regarding the reliability of using these same metabolites, TCP and IMP, as urinary biomarkers to quantitatively assess the everyday exposures of children to chlorpyrifos and diazinon, respectively. In this study, we quantified the distributions of chlorpyrifos, diazinon, TCP, and IMP in several environmental and personal media at the homes and daycare centers of 127 Ohio preschool children and identified the important sources and routes of their exposures. The children were exposed to concurrent levels of these four chemicals from several sources and routes at these locations. Diazinon and IMP were both detected above 50% in the air and dust samples. Chlorpyrifos and TCP were both detected in greater than 50% of the air, dust, (solid) food, and hand wipe samples. TCP was detected in 100% of the urine samples. Results from our regression models showed that creatinine levels (<0.001) and dietary ($p<0.001$) and inhalation ($p<0.10$) doses of TCP were each significant predictors of urinary TCP, collectively explaining 27% of the urinary TCP variability. This information suggests that measurement of urinary TCP did not reliably allow quantitative estimation of the children's everyday environmental exposures to chlorpyrifos.

Keywords: chlorpyrifos, diazinon, TCP, IMP, children

Introduction

Chlorpyrifos and diazinon are organophosphate (OP) insecticides that were commonly used in the United States (U.S.) to control insect pests (i.e., ants, cockroaches, fleas, and termites) at residences and other places such as daycares, schools, and parks where children spend their time (USEPA 2002 and 2008). The US Environmental Protection Agency (USEPA) ended most residential uses and other similar uses where children could be potentially exposed to chlorpyrifos and diazinon at the end of 2001 and 2004, respectively. These insecticides continue to be applied on agricultural crops to control for insect pests in the U.S. (Eaton et al. 2008; USEPA, 2002 and 2008).

In the environment, chlorpyrifos can degrade into several metabolites including 3,5,6-trichloro-2-pyridinol (TCP), diethylphosphate (DEP), and diethylthiophosphate (DETP; Racke 1993; Adgate et al. 2001). Diazinon can also break down into several environmental metabolites such as 2-isopropyl-6-methyl-4-pyrimidinol (IMP), DEP, and DETP. A few studies have recently reported concurrent levels of these two OP insecticides and their environmentally-occurring metabolites in food and in environmental media at residences and child daycare centers (Wilson et al. 2003; Morgan et al. 2005; Zhang et al. 2008; Wilson et al. 2008 and 2009). This information suggests that children are probably being concurrently exposed to low levels of chlorpyrifos and diazinon and to their environmentally-occurring metabolites in their everyday environments.

The toxicokinetics of chlorpyrifos and diazinon in exposed humans and other mammals is well characterized (Nolan et al. 1984; Griffin et al. 1999; Garfitt et al. 2002; Timchalk et al. 2005 and 2007; Eaton et al. 2008). Chlorpyrifos once absorbed into the

body is rapidly metabolized into more polar products and is eliminated in the urine as TCP, DEP and/or DETP (Nolan et al. 1984; Hines and Deddens 2001; Timchalk et al. 2007; Eaton et al. 2008). After absorption, diazinon is also quickly metabolized into more polar products and is renally excreted as IMP, DEP and/or DETP (Garfitt et al. 2002; Poet et al. 2004; CDC 2005). TCP and IMP are commonly used as specific urinary biomarkers to assess the non-occupational (non-acute) exposures of humans to chlorpyrifos and diazinon, respectively.

Very few data exist, however, on the toxicokinetics of environmental-occurring OP metabolites in exposed humans or other mammals (Bakke and Price 1976; Timchalk et al. 2007). Timchalk et al. (2007) recently reported that 24 male Sprague-Dawley rats orally gavaged (140 *u*mol/kg) with TCP, DETP, or DEP eliminated 100%, 65%, or 86% of the doses, respectively, unchanged in their urine. Bakke and Price (1976) also showed that one sheep orally dosed with 100 mg/kg of TCP (by capsule) excreted about 90% of the dose unchanged in its urine. These studies suggest that humans directly exposed to environmentally-occurring OP metabolites are likely absorbing into their bodies and excreting a substantial amount unchanged in their urine. This information raises questions regarding the reliability of using the urinary metabolites, TCP and IMP, to quantitatively estimate the exposures of children to chlorpyrifos and diazinon, respectively, at low levels in their home and daycare environments (Figure 1).

In this work, the objectives were to quantify the distributions of chlorpyrifos, diazinon, TCP, and IMP in environmental and personal media at a large set of preschool children's homes and daycare centers participating in the 2001 Ohio (OH) phase of the CTEPP study, to identify the important sources and pathways of the children's exposure

to each chemical, to estimate the children's potential exposures and potential absorbed doses to chlorpyrifos, diazinon, TCP, and IMP by each exposure route, and based on these data to determine if TCP and IMP were reliable urinary biomarkers to quantitatively assess the children's low-level, non-acute exposures to chlorpyrifos and diazinon.

Materials and methods

Study design

The Children's Total Exposure to Persistent Pesticides and Other Persistent Organic Pollutants (CTEPP) study investigated the aggregate exposures of 257 preschool children and their adult caregivers to chemicals commonly found in their everyday environments in North Carolina (phase I, 2000) and OH (phase II, 2001). An in-depth description of the CTEPP study design can be found in Wilson et al. (2004). In a previous publication, we quantified the exposures of 129 North Carolina CTEPP preschool children to chlorpyrifos and TCP in environmental and personal media at their homes and daycare centers in 2000-2001 (Morgan et al. 2005).

In this current work, we examined the exposures of 127 OH CTEPP preschool children to chlorpyrifos and diazinon and to their metabolites TCP and IMP, respectively, in environmental and personal media at their homes and daycare centers in 2001. Preschool children, ages 2-5 years, were randomly recruited from homes and daycare centers in six OH counties between January 2001 and November 2001. A total of 127 preschool children were recruited successfully into this study. Of these participants, 69 and 58 of the children were in the home and daycare groups, respectively. The median

age of the preschool children was 48 months, and their ages ranged between 20 and 67 months.

This was an observational research study, as defined in 40 Code of Federal Regulations (CFR) Part 26.402. The study protocol and procedures to obtain the assent of the children and informed consent of their parents or guardians were reviewed and approved by an independent institutional review board and complied with all applicable requirements of the Common Rule regarding additional protections for children (Subpart D).

Field sampling

An in-depth description of the sampling methodology in the CTEPP study has been described in Wilson et al. (2004). Briefly, field sampling at the homes and daycare centers occurred between April 2001 and November 2001 in OH. The children from the home group had environmental and personal samples collected over a 48-h period at their residences. The children in the daycare group had these samples collected simultaneously over the 48-h period at both their homes and daycare centers. Environmental and personal samples were collected at 127 homes and 16 daycare centers. Environmental samples collected at these locations included soil, outdoor air, indoor air, and carpet dust. Personal samples collected by the adult caregivers from their children consisted of solid food, liquid food, hand wipes, and spot urine. Up to six spot urine samples (i.e., morning, after lunch, and before bedtime) were collected from each child over the 48-h sampling period. All samples were transported in coolers with blue ice by field staff to the laboratory and stored in freezers ($\leq$ -10^{o}C) until analyses. Additional data that were collected in this

study included household observations, pre- and post-monitoring questionnaires, and children's activity diaries and food diaries.

Sample analysis

Previous methods were developed from small pilot studies to analyze for the levels of chlorpyrifos, diazinon, and TCP in the environmental and personal samples (Chuang et al. 1999; Wilson et al. 2001, 2003, and 2004). By the OH phase of the CTEPP study, we were able to quantify levels of IMP in all study media, except for the food and urine samples, using the same analytical methods from our published pilot studies.

The extraction and analytical procedures for these four chemicals are described in detail in Morgan et al. (2005). The same laboratory and technical staff were responsible for the extraction and analyses of all samples. The surrogate recovery standard (SRS) for chlorpyrifos and diazinon was *p,p´*-DDE-d_4. No SRS was used for the analysis of TCP and IMP in the samples. The internal standard for these two pesticides was diazinon-d_{10}. The internal standard for TCP and IMP was 3,5,6-trichloro-2-pyridinol-^{13}C-^{15}N. Matrix spikes were used for each chemical in all relevant media. Samples were analyzed using a gas chromatograph/mass selective detector (Hewlett-Packard 6890/5973A) in selected ion monitoring mode. The limits of quantification (LOQs) were estimated based on the lowest calibration standard (2 ng/mL) with a signal-to-noise ratio above 2. The estimated LOQs for each chemical are listed in Table 1. The estimated instrumental limit of detection (LOD) was about half the reported LOQ.

Quality control

Quality control samples were used to assess the overall quality of sample collection, extraction, and analysis. Field blanks were collected for air, dust/soil, wipe, food, and urine samples. The blanks were all below the detection limits except for a few air samples that were slightly contaminated with chlorpyrifos (two of 14 samples; 2/14), IMP (1/14) or TCP (4/14) and one wipe slightly contaminated with TCP (1/14). The mean values for these field blanks were all at or below the LOD for each matrix, except for TCP in the air samples (0.1±0.4 ng/m^3) which was barely above the estimated LOD of 0.09 ng/m^3. The laboratory method blanks were all below the detection limits except for slight TCP contamination in one wipe (1/8), one solid food (1/9), and one urine (1/13) sample. The mean values for these laboratory blanks were all at or below the LOD for each matrix, except for TCP in the solid food samples (0.15±0.1 ng/g) which was slightly about the estimated LOD of 0.13 ng/g. Therefore, no background corrections were made for these samples. The matrix spikes for chlorpyrifos and diazinon had mean recoveries between 72% and 110% for all media. For TCP and IMP, mean recoveries for the matrix spikes were between 56% and 96% in these media. The SRS, p,p´-DDE-d_4, had mean recoveries between 75% and 100% for all media. Duplicate samples (aliquots of the same sample) were analyzed for each chemical in soil/dust, food, and urine (TCP, only). The mean relative percent difference of all duplicate samples by matrix was less than 10% for each chemical. Analytical duplicates (repeat analysis of the same extract) were analyzed for each chemical in soil/dust, air, food, wipes, and urine (TCP, only). The mean relative

percent difference of all analytical duplicates by matrix was less than 6% for each chemical.

Statistical analyses

All results that were less than the LOD were assigned the value of LOD divided by the square root of two, except for the liquid food results. Because the majority of the analytes in the liquid food samples in this study were barely detectable on the chromatographs, we used a more conservative approach by assigning the results below the LOD with the value of LOD divided by 10 (Morgan et al. 2004; 2005). Descriptive statistics (mean, median, range, and percentiles [25^{th}, 75^{th}, and 95^{th}]) were calculated for chlorpyrifos, diazinon, TCP, and IMP in each medium (except urine) at the children's homes and daycare centers. Descriptive statistics were also computed for TCP in the urine samples as unadjusted (ng/mL) and creatinine-adjusted (ng/mg) for children overall and by group (home and daycare). Creatinine-adjusted values were calculated using the following equation: Creatinine-adjusted value (ng/mg) = 100 mL/dL x urine concentration (ng/mL)/creatinine concentration (mg/dL). The Wilcoxon (two-sample) test was used to determine if there were significant differences in concentrations of an OP insecticide and its degradation product for media between the two locations or the two groups of children.

The estimated potential exposures (ng/day) and potential absorbed doses (ng/kg/day) of these 127 OH children to chlorpyrifos, TCP, diazinon, and IMP were

calculated for the inhalation, dietary, and indirect ingestion routes based on the following equations:

Inhalation: $$E_{inh} = \frac{[(C_{di} * t_{di}) + (C_{do} * t_{do}) + (C_{hi} * t_{hi}) + (C_{ho} * t_{ho}) + (C_{away} * t_{away})] * V}{t_{di} + t_{do} + t_{hi} + t_{ho} + t_{away}}$$

Dietary: $$E_{diet} = \frac{[(C_{dl} * M_{dl}) + (C_{ds} * M_{ds}) + (C_{hl} * M_{hl}) + (C_{hs} * M_{hs})]}{N_f}$$

Indirect: $$E_{ind} = \frac{[(D_{dd} * M_d * t_{di}) + (D_{ds} * M_s * t_{do}) + (D_{hd} * M_d * t_{hi}) + (D_{hs} * M_s * t_{ho})]}{t_{di} + t_{do} + t_{hi} + t_{ho}}$$

Table 2 defines each variable used in the above equations for each route of exposure. The children's potential absorbed doses were calculated by dividing $E_{inh,}$ $E_{diet,}$ or E_{ind} by their body weight and multiplying by the fraction absorbed each day. We used a 70% absorption rate for chlorpyrifos and a 60% absorption rate for diazinon through the ingestion (dietary and indirect) route of exposure based on published human studies conducted by Nolan et al. (1984) and Garfitt et al. (2002) respectively. Since the absorption rate for chlorpyrifos (~2%) and diazinon (~1%) through the dermal route is low in humans, we did not estimate the children's exposure to these insecticides by this route (Nolan et al. 1984 and Garfitt et al., 2002). We used a 50% absorption rate when human data were lacking on the fraction of a pollutant absorbed into the body by an exposure route (Ross et al. 2001 and Eaton et al. 2008). The estimated excreted amounts of urinary TCP (ng/kg/day) by each child over a 24-h period was calculated by multiplying their urinary TCP concentration (ng/mL) by their daily urine output assumed to be 22 mL/kg/body weight (Miller and Stapleton 1989; Szabo and Fegyverneki).

Pearson correlation coefficients were first used to examine the pairwise relationships between the response variable (i.e., the natural logarithm [ln] of the excreted amounts of urinary TCP), and the independent variables (i.e., ln creatinine and ln of inhalation, dietary ingestion, and indirect ingestion doses of chlorpyrifos and TCP). Pearson correlations were also used to examine the pairwise relationships in log space between the independent variables to assess potential collinearity.

Because of the potential impact of multicollinearity between measurements of chlorpyrifos and TCP, we used separate multivariate linear regression models for chlorpyrifos and TCP. As such, [ln] excreted urinary TCP was separately regressed on the children's estimated potential absorbed doses to chlorpyrifos or TCP by the inhalation, dietary ingestion, and indirect ingestion routes after adjusting for urinary creatinine concentrations. Multivariate regression analysis was performed using a sequential, step-wise backward elimination procedures in the PROC REG procedure in SAS. The step-wise procedure was used to generate the "best" model to characterize the relationship between the ln of the excreted urinary TCP and the ln of the potential absorbed doses of chlorpyrifos or TCP by each route. At each step, we looked at the level of significance of the independent variable and its effect on the r^2 of the model. There were two criteria for keeping independent variables in our model: a variable that had a $p \leq 0.10$, or a variable that when excluded from the model changed the r^2 by 10% or more. Thirteen children that had recent pesticide applications were excluded from these above analyses as creatinine was not measured in these spot samples due to insufficient urine volumes. Urinary creatinine concentrations ranged from 17-250 mg/dl for these children,

and all values were included in these analyses as supported by published research by Barr et al., 2005. All analyses were performed using SAS version 9.1 (SAS Cary, NC).

Results

Tables 3 and 4 present the distributions of chlorpyrifos, diazinon, TCP, and IMP in the environmental and personal media (except urine), respectively. Chlorpyrifos and TCP were both detected the most often in the outdoor air (>70%), indoor air (>97%), carpet dust (>98%), and solid food (>65%) samples at the children's homes and daycare centers. Diazinon and IMP were both detected the most often in the outdoor air (>68%), indoor air (>90%) and carpet dust (>86%) samples at both locations. In these environmental media, the ratios of chlorpyrifos to TCP (CPF/TCP) and diazinon to IMP (DZN/IMP) were generally higher indoors than outdoors which suggested that these insecticides were likely degrading faster in outdoor environments. The median ratios of CPF/TCP were 2.8 and 3.0 in the indoor air samples and 1.0 and 0.5 in the outdoor air samples at the homes and daycare centers, respectively. The median ratios of DZN/IMP were also greater in the indoor air (2.0 and 2.0) than the outdoor air (0.5 and 0.5) samples at the homes and daycare centers, respectively. In the carpet dust samples, the median ratios of CPF/TCP were 1.3 at homes and 3.0 at daycares. Similarly, the median ratios of DZN/IMP in the carpet dust samples were 1.4 at homes and 2.4 at daycares. For the solid food samples, the median ratios of CPF/TCP were identical (0.1) at both locations. This was an important result as the median levels of TCP were at least 10 times greater than the median levels of chlorpyrifos in the solid food samples at the homes (1.9 *vs.* 0.2 ng/g)

or the daycare centers (1.5 *vs.* 0.1 ng/g), and the distributions were significantly different ($p<0.0001$).

In the post-monitoring questionnaires, about 70% of the daycare directors reported past applications of one or more insecticides at their centers. For the adult caregivers (usually the parent), approximately 50% of them reported using one or more insecticides in the past at their homes. The majority of these above respondents, however, were unable to provide the specific names of the insecticide(s) they had used to control for various insect pests at these locations.

Table 5 presents the distributions of the urinary TCP concentrations for these children overall and by the home group and by the daycare group. TCP was detected in 100% of the children's urine samples. The median urinary TCP concentration for all children was 5.1 ng/mL, and the maximum value was 15.3 ng/mL. The median TCP concentrations in the urine samples were slightly higher for children in the home group (5.3 ng/mL) compared to the daycare group (4.4 ng/mL). However, there were no statistically significant differences in the distributions for TCP in the children's urine samples between the home group and the daycare group ($p>0.05$). These results suggest that these preschool children were exposed to and absorbed chlorpyrifos, and likely environmental TCP, into their bodies.

Table 6 presents the estimated median potential exposures (ng/day) and potential absorbed doses (ng/kg/day) of the 127 children to chlorpyrifos, TCP, diazinon, and IMP for the inhalation, indirect, and/or dietary routes of exposure. The estimated median potential absorbed doses of the children to chlorpyrifos and TCP by each route were 0.4 and 0.1 ng/kg/day (inhalation), 2.9 and 25.3 ng/kg/day (dietary ingestion), and 0.1 and

0.1 ng/kg/day (indirect ingestion), respectively. Dietary ingestion, primarily through the consumption of solid food, was the dominant route of the children's exposures for both chlorpyrifos (2.9 ng/kg/day) and TCP (25.3 ng/kg/day). In contrast, the estimated median potential absorbed doses of the children to both diazinon and IMP were highest for the inhalation route (0.2 and 0.3 ng/kg/day) respectively, followed by the indirect ingestion route (0.04 and 0.02 ng/kg/day). It remains unclear whether dietary ingestion was an important route of the children's exposure to IMP since we were unable to quantify for the levels of this chemical in the solid and liquid food samples in this study.

Table 7 presents the Pearson correlation coefficients for chlorpyrifos and TCP by each exposure route. In this table, our data show that excreted amounts of urinary TCP were significantly correlated with the children's potential dietary doses of TCP ($r=0.30$, $p<0.0001$) and creatinine in urine ($r=0.31$, <0.0001). The results also highlight positive correlations between most of the chlorpyrifos and TCP exposure measurements; only two of nine correlations were not significant (indirect chlorpyrifos vs. dietary TCP, $r=0.06$ and $p=0.51$; inhalation chlorpyrifos vs. dietary TCP, $r=-0.01$ and $p=0.96$.

The results from our full regression model for chlorpyrifos shows an $r^2=0.12$ which indicated that about 12% of the variability of excreted amounts of urinary TCP was explained by the children's potential absorbed doses of chlorpyrifos through inhalation, dietary, and indirect routes and by the creatinine levels. As a result of the step-wise elimination, our final reduced model for chlorpyrifos showed that only creatinine remained in this model and explained 10% of the variability of the excreted amounts of the children's urinary TCP. In contrast, the results from our full regression model for TCP shows an $r^2=0.27$ which indicated that approximately 27% of the variability of the

excreted amounts of urinary TCP was explained by the children's potential absorbed doses to TCP through the three exposure routes and by creatinine. Our final reduced model shows that TCP through the dietary and inhalation routes and creatinine were significant parameters and explained 27% of the variability of the excreted amounts of TCP in the children's urine. However in this model, TCP through the inhalation route was only marginally significant ($p<0.10$) compared TCP through dietary ingestion which was highly significant ($p<0.0001$). These results suggest that TCP, particularly through dietary ingestion, significantly contributed to the variability of the excreted amounts of TCP in the children's urine.

Discussion

For many years, TCP and IMP have been recognized by the scientific community as gold standard biomarkers of human exposure to chlorpyrifos and diazinon, respectively. However, scientists are beginning to question the reliability of using these urinary biomarkers to quantitatively estimate the non-occupational (non-acute) exposures of children or adults to these two OP insecticides. A few studies have recently found concurrent levels of these OP insecticides and their environmentally-occurring metabolites, TCP and IMP, in environmental media and in food in non-occupational settings (Wilson et al. 2003; Morgan et al. 2005; Zhang et al. 2008; Wilson et al. 2008 and 2009). Wilson et al. (2003) found measurable levels of both chlorpyrifos and TCP in several media including carpet dust, solid food, and liquid food at the homes and daycare centers of nine children in NC in 1997. In our previous CTEPP-NC study conducted in 2000-2001, we also reported measurable levels of both chlorpyrifos and TCP in several

media including solid food, liquid food, carpet dust, indoor air, outdoor air and wipe samples at the homes and daycares of 129 preschool children in NC (Morgan et al., 2005). An important study finding was that the median levels of TCP were at least 12 times higher than the median levels of chlorpyrifos in solid food samples at both locations. In this work, the 2001 CTEPP-OH study, we also found concurrent levels of chlorpyrifos and TCP in the same media as mentioned above at the homes and daycares of 127 children in OH. In addition, we found concurrent levels of diazinon and IMP in carpet dust, indoor air, outdoor air, and hand wipe samples at these same locations.

A longitudinal study (Pesticide Exposures of Preschool Children Over Time [PEPCOT]) of the pesticide exposures of 101 preschool children in 50 homes was recently conducted between 2003 and 2005 in North Carolina (Wilson et al. 2009). One objective of the PEPCOT study was to investigate the changes in young children's exposures to chlorpyrifos and diazinon in residential settings, as a result of the phased withdrawal of these pesticides from the market for use in residential settings beginning in 2000 (chlorpyrifos) and 2001 (diazinon). Wilson et al. (2009) showed that the PEPCOT children's exposures to chlorpyrifos and diazinon were substantially reduced from those found in our earlier CTEPP-NC study (2000-2001), with the children's aggregate median potential absorbed doses decreased by as much as 62%. However, the levels of TCP and IMP continue to be measurable and widespread in the children's surroundings and in their diets. When only the aggregate intake of chlorpyrifos or diazinon was considered, the urinary output of their metabolites, TCP or IMP, was shown to be many times in excess, sometimes by a factor of 20 or more. When the intake dose of TCP or IMP in food and environmental media were included in this estimate, the discrepancy in the

urinary TCP output could be explained for chlorpyrifos, but an unexplained excess in urinary IMP output still remained for diazinon. The PEPCOT results suggest that the decreased aggregate intake of chlorpyrifos and diazinon observed for the PEPCOT children compared to the CTEPP children is probably related to the USEPA's mitigation efforts in phasing out most residential uses and similar uses of these two insecticides. Nevertheless, this information indicates that children are still being exposed at their homes to measurable, albeit low, levels of these four chemicals, particularly in food and/or in other media such as dust and indoor air. It is important to note that several agricultural uses of these pesticides still remain, although the tolerances have been set at lower levels than those allowed before the discontinuance of their use in residential settings.

Many factors can influence the urinary excretion of these OP insecticides and their environmental metabolites, including the behavior of the individuals, the degree of absorption by the body, the metabolic transformation and route of elimination, and the possible existence of intake routes that may not have been considered. If no significant metabolism of the OP insecticide occurs in the environment, then the specific urinary OP metabolite may serve as a good biomarker of exposure. However, the results reported later for the PEPCOT study (Wilson et al. 2009) support our findings in the CTEPP study that measurements of the specific metabolites, TCP or IMP, in the urine of young children may sometimes be insufficient quantitative indicators of their exposures to chlorpyrifos and diazinon, respectively. More research is needed to understand the limitations and proper ways to use these OP metabolites to quantitatively assess

children's exposures to chlorpyrifos and diazinon in exposure and epidemiological studies.

The results above are significant because past human observational measurement studies have not accounted for direct exposures of individuals to environmentally-occurring OP metabolites in foods or at residential settings and other similar settings (Lu et al. 2005; Eaton et al. 2008; Raina and Sun 2008; Zhang et al. 2008). Based on the rat study conducted by Timchalk et al. (2007), this information indicates that children directly exposed to TCP or IMP in their surroundings are likely absorbing into their bodies and excreting a substantial amount of these environmental metabolites unchanged in their urine. This information also suggests that the children's observed urinary TCP and/or IMP concentrations reflect not only their exposures to the parent insecticides but also to their environmental metabolites from several sources, pathways, and routes in their everyday environments. These studies have provided important insights into the issues and challenges in using TCP and IMP as reliable urinary biomarkers to quantitatively estimate the non-occupational (non-acute) exposures of humans to chlorpyrifos and diazinon, respectively. A major concern is that past human observational measurement studies may have overestimated the non-occupational exposures of children or adults to chlorpyrifos and diazinon when relying on TCP and IMP as urinary biomarkers (Rigas et al. 2001; Eaton et al. 2008). More research would be useful to understand the toxicokinetics of environmental OP metabolites and other environmental metabolites of current-use pesticides in mammalian systems.

There were several limitations to our interpretation of the data in the OH phase of the CTEPP study. On average, we accounted only for approximately 33% of the

children's median excreted amounts of urinary TCP (126.6±76.0 ng/kg/day) based on their estimated median aggregate potential absorbed doses (41.3 ng/kg/day) to chlorpyrifos and TCP. These results suggest that we may be missing other important sources or pathways of the children's exposures to chlorpyrifos and/or TCP at their homes and daycare centers. In addition, these children could have been exposed to small amounts of chlorpyrifos-methyl in their diets. Chlorpyrifos-methyl is an agricultural OP insecticide that can also degrade into TCP in the environment (Koch and Angerer 2001; FDA, 2006). Approximately 80,000 lbs of chlorpyrifos-methyl were applied annually in the U.S., mainly on stored grains such as wheat, oats, barley and rice to control for insect pests until the end of 2004 (USEPA 2000). However, the amounts of chlorpyrifos-methyl used in the U.S. pale in comparison to the amounts of chlorpyrifos used prior to the residential-use phase-out (chlorpyrifos 21-24 million pounds annually – roughly 300 times the 80,000 lb of chlorpyrifos-methyl). Furthermore, chlorpyrifos-methyl was not registered in the U.S. for nonagricultural use, and was unlikely to have been used in the home and daycare environments of the participating children. Although residues of chlorpyrifos-methyl have been detected in foods, particularly in breads, crackers, and cereals (FDA, 2006), it is unlikely to have contributed greatly to the CTEPP children's diets. We did not quantify the levels of chlorpyrifos-methyl in this study. Finally, we did not originally determine the storage stability of chlorpyrifos or diazinon in any media or the potential hydrolysis of these insecticides during sample preparation (i.e., homogenization of food). However, Chuang et al. (unpublished data), conducted a storage stability study before the CTEPP study was conducted and showed that chlorpyrifos-^{13}C-^{15}N spiked into duplicate diet solid food samples before homogenization

did not degrade during the analytical procedures. The chlorpyrifos was also stable in <-10°C freezers for up to 16 months. In the CTEPP study, as well as in the PEPCOT study, very similar freezer storage conditions (i.e., <-10°C) were used for the children's duplicate diet solid food samples at this same laboratory. Although Lu et al. (2005) and Weerasekera et al. (2009) found that fruit juices spiked with chlorpyrifos and diazinon showed substantial degradation of the spiked pesticides on short-term refrigerated storage and/or sample preparation, little degradation of chlorpyrifos occurred in dust samples stored in similar conditions. This degradation is highly pH-dependent. Nishioka et al. (unpublished data) found small amounts of degradation of the OP pesticides during acid hydrolysis of food samples. However, acid hydrolysis was not used in analysis of food samples in the CTEPP study (nor in the published PEPCOT study). On this basis and on the storage stability study by Chuang et al. mentioned above, we do not believe that significant degradation of chlorpyrifos occurred during preparation, analysis, and storage of the CTEPP food samples. Nevertheless, this information suggests that environmental factors such as temperature, time, and pH can impact the stability of OP insecticides in some media, and should be considered in future studies.

Conclusions

The results from the OH phase of the CTEPP study show that these preschool children were exposed to concurrent levels of chlorpyrifos and TCP and diazinon and IMP from several sources, pathways, and routes at their homes and daycare centers. It is possible that the CTEPP children who were directly exposed to TCP or IMP in their everyday environments were likely absorbing into their bodies and eliminating a

substantial amount unchanged in their urine. Therefore, our study results have provided important insights into the current challenges, issues, and limitations in using TCP, and likely IMP, to quantitatively assess the non-occupational (non-acute) exposures of humans to chlorpyrifos and diazinon, respectively.

Conflict of interest

The authors declare no conflict of interest.

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Table 1. Estimated limits of quantification for the target analytes in environmental and personal samples.

Chemical	Environmental				Personal			
	Soil (ng/g)	Outdoor Air (ng/m^3)	Indoor Air (ng/m^3)	Carpet Dust (ng/g)	Hand Wipe (ng/cm^2)	Solid Food (ng/g)	Liquid Food (ng/mL)	Urine (ng/mL)
Chlorpyrifos	1.0	0.2	0.2	4.0	0.01	0.2	0.1	n/a[c]
TCP[a]	0.4	0.2	0.2	4.0	0.01	0.3	0.2	2.0
Diazinon	1.0	0.2	0.2	4.0	0.01	0.2	0.1	n/a
IMP[b]	0.4	0.2	0.2	4.0	0.01	----[d]	----	----

[a]3,5,6-Trichloro-2-pyridinol
[b]2-Isopropyl-6-methyl-4-pyrimidinol
[c]Not applicable
[d]Not quantifiable in this media using this method

Table 2. Variables used to calculate the potential exposures of each child to chlorpyrifos, TCP, diazinon, and IMP by route of exposure.

Variable	Definition	Unit
	Inhalation Equation	
E_{inh}	Potential exposure of each child over a 24-h period	ng/day
C_{di}, C_{hi}	Concentration of chemical in the indoor air samples at daycare and home	ng/m^3
C_{do}, C_{ho}	Concentration of chemical in the outdoor air samples at daycare and home	ng/m^3
C_{away}	Indoor air concentration of chemical in locations where children spent time away from daycare/home	ng/m^3
t_{di}, t_{hi}	Time spent indoors at the daycare and home	h/d
t_{do}, t_{ho}	Time spent outdoors at the daycare and home	h/d
t_{away}	Time spent indoors at locations other than at daycare and home	h/d
V	Ventilation rate for a child[a]	m^3/d
	Dietary Ingestion Equation	
E_{diet}	Potential exposure of each child over a 24-h period	ng/day
C_{dl}, C_{hl}	Concentration of chemical in the liquid food samples at daycare and home	ng/mL
C_{ds}, C_{hs}	Concentration of chemical in the solid food samples at daycare and home	ng/g
M_{dl}, M_{hl}	Total volume of the liquid food samples collected at daycare and home	mL
M_{ds}, M_{hs}	Total weight of the solid food samples collected at daycare and home	g
N_f	Number of days that the food samples were collected for each child	d
	Indirect Ingestion Equation	
E_{ind}	Potential exposure of each child over a 24-h period	ng/day
D_{dd}, D_{hd}	Concentration of chemical in the dust sample at daycare and home	ng/g
D_{ds}, D_{hs}	Concentration of chemical in the soil sample at daycare and home	ng/g
t_{di} , t_{hi}	Time spent indoors at the daycare and home	h/d
t_{do}, t_{ho}	Time spent outdoors at the daycare and home	h/d
M_d, M_s	Child's estimated daily dust and soil ingestion rates[b]	g/d

[a]Ventilation rate was 6.8 m^3/day for children less than 36 months of age and 8.3 m^3/day for children aged 36 months or higher.
[b]Children were assigned a daily dust or soil ingestion rate of high (0.100 g/day), medium (0.05 g/day), or low (0.025 g/day) based on their activity levels.

Table 3. Levels of chlorpyrifos, TCP, diazinon, and IMP in environmental media collected at the children's homes and daycare centers.

Medium	Location	N	%[a]	Mean	SD[b]	GM[c]	Min.	Percentiles 25th	50th	75th	95th	Max.
Soil (ng/g)												
Chlorpyrifos	Home	127	39	N/A[d]	----	N/A	<[e]	<	<	3.9	13.8	2,900
	Daycare	16	38	N/A	----	N/A	<	<	<	1.3	6.2	6.2
TCP	Home	127	80	4.0	15.3	0.8	<	0.2	0.7	2.0	8.9	127
	Daycare	16	81	1.2	1.6	0.6	<	0.2	0.6	1.3	6.3	6.30
Diazinon	Home	127	34	N/A	----	N/A	<	<	<	1.0	4.7	28,500
	Daycare	16	19	N/A	----	N/A	<	<	<	<	7.1	7.1
IMP	Home	125	41	N/A	----	N/A	<	<	<	0.4	2.1	162
	Daycare	16	38	N/A	----	N/A	<	<	<	0.4	1.4	1.4
Outdoor Air (ng/m^3)												
Chlorpyrifos	Home	112	75	0.4	0.8	0.2	<	<	0.2	0.4	1.4	6.5
	Daycare	14	71	0.2	0.1	0.1	<	<	0.1	0.2	0.4	0.4
TCP	Home	118	88	0.3	0.5	0.2	<	0.1	0.2	0.4	1.0	4.9
	Daycare	15	87	0.2	0.1	0.2	<	0.1	0.2	0.3	0.5	0.5
Diazinon	Home	127	75	1.2	7.3	0.2	<	<	0.2	0.4	1.5	78.9
	Daycare	16	69	0.3	0.5	0.2	<	<	0.1	0.2	2.3	2.3
IMP	Home	126	85	1.2	4.9	0.4	<	0.1	0.4	0.8	2.0	49.6
	Daycare	15	93	3.5	11.4	0.3	<	0.1	0.2	0.8	44.5	44.5
Indoor Air (ng/m^3)												

Chlorpyrifos	Home	125	98	6.4	14.8	2.2	<	0.9	1.7	4.8	23.3	98.0
	Daycare	22	100	5.2	5.6	2.9	0.5	1.1	2.1	8.9	12.6	21.7
TCP	Home	123	100	2.1	5.0	0.8	0.1	0.4	0.6	1.6	9.3	42.0
	Daycare	21	100	1.5	1.3	1.0	0.2	0.5	0.7	2.7	3.8	3.9
Diazinon	Home	125	98	11.8	51.4	1.3	<	0.5	1.0	2.2	46.4	483
	Daycare	22	100	11.6	21.0	2.1	0.3	0.4	1.0	10.2	58.9	59.6
IMP	Home	125	96	1.4	3.6	0.6	<	0.4	0.5	1.0	5.4	27.4
	Daycare	22	91	2.2	4.0	0.7	<	0.4	0.5	2.2	13.4	14.9
Carpet Dust (ng/g)												
Chlorpyrifos	Home	120	100	871	5,030	70.4	3.6	23.1	52.0	149	1,410	49,600
	Daycare	23	100	272	285	168	40.6	67.0	174	430	897	1,110
TCP	Home	120	99	153	348	51.0	<	23.7	41.0	89.8	824	1,960
	Daycare	23	100	93.8	105	62.3	15.3	35.8	57.7	141	194	503
Diazinon	Home	120	96	1,360	8,470	34.3	<	9.7	19.8	73.2	1,710	79,900
	Daycare	23	100	260	472	73.7	5.1	28.4	40.0	210	1,610	1,630
IMP	Home	120	88	84.0	313	16.4	<	6.2	14.3	40.7	270	2,450
	Daycare	23	87	73.0	155	18.8	<	7.8	16.7	38.2	312	696

[a]Percentage of samples with detectable levels of a chemical
[b]Standard deviation
[c]Geometric mean
[d] The mean and standard deviation (N/A) were not given for media in which fewer than half the samples had detectable levels
[e]The "<" symbol was assigned to samples that were below the limit of detection for a percentile

Table 4. Levels of chlorpyrifos, TCP, diazinon, and IMP in personal media collected at the children's homes and daycare centers.

Medium	Location	N	%[a]	Mean	SD[b]	GM[c]	Min.	Percentiles 25th	50th	75th	95th	Max.
Hand Wipe (ng/cm^2)												
Chlorpyrifos	Home	97	58	0.2	1.5	0.01	<[d]	<	0.01	0.03	0.2	14.8
	Daycare	29	62	0.03	0.1	0.01	<	<	0.01	0.02	0.1	0.6
TCP	Home	98	97	0.02	0.01	0.01	<	0.01	0.01	0.02	0.03	0.1
	Daycare	29	90	0.01	0.01	0.01	<	0.01	0.01	0.02	0.03	0.04
Diazinon	Home	97	35	N/A[e]	----	N/A	<	<	<	0.01	0.1	3.8
	Daycare	29	38	N/A	----	N/A	<	<	<	0.01	0.04	0.1
IMP	Home	98	30	N/A	----	N/A	<	<	<	0.004	0.02	0.1
	Daycare	29	24	N/A	----	N/A	<	<	<	<	0.02	0.04
Solid Food (ng/g)												
Chlorpyrifos	Home	125	66	0.4	0.6	0.2	<	<	0.2	0.4	1.6	3.5
	Daycare	29	69	0.2	0.2	0.1	<	<	0.1	0.2	0.6	0.9
TCP	Home	127	99	2.6	2.6	1.7	<	1.0	1.9	3.4	5.9	23.2
	Daycare	29	100	2.8	5.0	1.7	0.4	1.0	1.5	2.5	8.1	27.2
Diazinon	Home	125	15	N/A	----	N/A	<	<	<	<	0.2	0.7
	Daycare	29	24	N/A	----	N/A	<	<	<	<	0.2	0.2
IMP[f]	Home	----	----	----	----	----	----	----	----	----	----	----
	Daycare											
Liquid Food (ng/mL)												

Chlorpyrifos	Home	126	6	N/A	----	N/A	<	<	<	<	0.1	0.3
	Daycare	28	11	N/A	----	N/A	<	<	<	<	0.2	0.7
TCP	Home	126	33	N/A	----	N/A	<	<	<	0.1	1.2	2.3
	Daycare	28	54	0.2	0.3	0.1	<	<	0.1	0.2	0.8	1.5
Diazinon	Home	126	2	N/A	----	N/A	<	<	<	<	<	0.1
	Daycare	28	4	N/A	----	N/A	<	<	<	<	<	0.04
IMP[f]	Home	----	----	----	----	----	----	----	----	----	----	----
	Daycare											

[a]Percentage of samples with detectable levels of a chemical
[b]Standard deviation
[c]Geometric mean
[d]The "<" symbol was assigned to samples that were below the limit of detection for a percentile
[e]The mean and standard deviation (N/A) were not given for media in which fewer than half the samples had detectable levels
[f]The levels of IMP could not be quantified in this media

Table 5. Urinary TCP concentrations in children over a 48-h period.

Urine	N	%[a]	Mean	SD[b]	GM[c]	Min.	Percentiles				Max.
							25th	50th	75th	95th	
ng/mL											
All Children	122	100%	5.6	3.4	4.6	1.2	2.9	5.1	7.3	12.3	15.3
Home Group	67	100%	6.1	3.7	4.9	1.2	3.0	5.3	9.1	12.9	15.3
Daycare Group	55	100%	5.1	2.8	4.3	1.4	2.7	4.4	6.9	11.2	12.8
ng/mg-creatinine[d]											
All Children	106	100%	7.7	4.3	6.5	1.3	4.3	6.7	10.4	16.1	20.1
Home Group	57	100%	8.5	4.5	7.1	1.3	5.2	7.7	11.7	17.8	19.2
Daycare Group	49	100%	6.8	3.9	5.9	1.7	4.0	5.8	8.2	14.1	20.1

[a]Percentage of samples with detectable concentrations of TCP
[b]Standard deviation
[c]Geometric mean
[d]Creatinine was not analyzed in the urine samples of children that had recent pesticide applications at their homes within seven days of field monitoring

Table 6. The children's estimated median potential exposures and potential absorbed doses to chlorpyrifos, TCP, diazinon, and IMP by exposure route.

Chemical	Potential exposure (ng/day)			Potential absorbed dose (ng/kg/day)[a]		
	Inhalation	Dietary Ingestion	Indirect Ingestion	Inhalation	Dietary Ingestion	Indirect Ingestion
Chlorpyrifos	14.6	77.9	2.67	0.4	2.9	0.1
TCP	5.1	858	1.6	0.1	25.3	0.1
Diazinon	8.0	<[b]	1.0	0.2	<	0.04
IMP	9.6	----[c]	0.9	0.3	----	0.02

[a] An absorption rate of 70% and 60% through the ingestion route of exposure was used for chlorpyrifos and diazinon, respectively, based on published human study data. A 50% absorption rate was used for a pesticide or environmental metabolite that lack sufficient human data by an exposure route.

[b]The "<" symbol indicated that fewer than half of the children had detectable levels of a chemical in media used to estimate this route of exposure

[c]Not estimated for this route of exposure

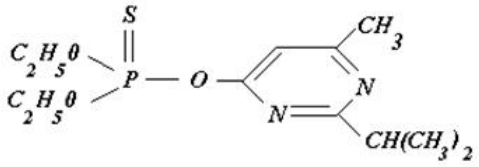
Table 7. Pearson correlations for estimating the children's potential absorbed doses of chlorpyrifos and TCP by route and excreted amounts of urinary TCP.[ab]

	Excreted Urinary TCP	Creatinine	Dietary Chlorpyrifos	Indirect Chlorpyrifos	Inhalation Chlorpyrifos	Dietary TCP	Indirect TCP	Inhalation TCP
Excreted Urinary TCP		0.31[c] <0.0001[d]	0.16 0.07	-0.04 0.71	-0.04 0.69	0.30 <0.0001	-0.01 0.88	0.02 0.85
Creatinine			0.08 0.39	0.13 0.19	0.10 0.33	-0.16 0.09	0.09 0.38	0.05 0.60
Dietary Chlorpyrifos				0.35 <0.0001	0.07 0.47	0.18 0.04	0.29 0.002	0.22 0.02
Indirect Chlorpyrifos					0.64 <0.0001	0.06 0.51	0.81 <0.0001	0.69 <0.0001
Inhalation Chlorpyrifos						-0.01 0.96	0.58 <0.0001	0.90 <0.0001
Dietary TCP							0.09 0.31	-0.07 0.45
Indirect TCP								0.57 <0.0001
Inhalation TCP								

[a]The natural logarithm was used for each independent variable and for the outcome variable (excreted amounts of urinary TCP)
[b]Units for all variables are in ng/kg/day, except for creatinine (mg/dl).
[c]Pearson correlation coefficients
[d]Level of significance (p value)

Figure Legend

Figure 1. Chemical structures of diazinon and chlorpyrifos and their corresponding urinary metabolites/environmental metabolites, IMP and TCP, respectively.



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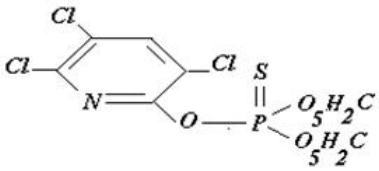

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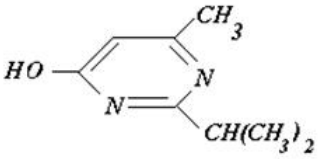
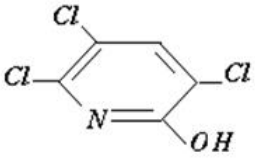


2-Isopropyl-6-methyl-4-pyrimidinol (IMP)



3,5,6-Trichloro-2-pyridinol (TCP)