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Concentrations of selective metabolites of organophosphorus pesticides in the United States population

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Abstract

We report population-based concentrations (stratified by age, sex, and composite race/ethnicity variables) of selective metabolites of chlorpyrifos (3,5,6-trichloro-2-pyridinol; TCPY), chlorpyrifos methyl (TCPY), malathion (malathion dicarboxylic acid; MDA), diazinon (2-isopropyl-4-methyl-6-hydroxypyrimidine; IMPY), methyl parathion (*para*-nitrophenol; PNP), and parathion (PNP). We measured the concentrations of TCPY, MDA, IMPY, and PNP in 1997 urine samples from participants, aged 6–59 years, of the National Health and Nutrition Examination Survey, 1999–2000. We detected TCPY in more than 96% of the samples tested. Other organophosphorus pesticide metabolites were detected less frequently: MDA, 52%; IMPY, 29%; and PNP, 22%. The geometric means for TCPY were 1.77 μg/L and 1.58 μg/g creatinine. The 95th percentiles for TCPY were 9.9 μg/L and 8.42 μg/g creatinine. The 95th percentiles for MDA were 1.6 μg/L and 1.8 μg/g creatinine. The 95th percentiles for IMPY and PNP were 3.7 μg/L (3.4 μg/g creatinine) and 5.0 μg/L (4.2 μg/g creatinine), respectively. Multivariate analyses showed that children aged 6–11 years had significantly higher concentrations of TCPY than adults and adolescents. Similarly, adolescents had significantly higher TCPY concentrations than adults. Although the concentrations between sexes and among composite racial/ethnic groups varied, no significant differences were observed.

Keywords: Chlorpyrifos; Urine; Biological monitoring; Reference range; Organophosphorus pesticide

1. Introduction

In 1999, an estimated 415,000 tons of pesticides were applied in the United States (Donaldson et al., 2002). Organophosphorus (OP) pesticides, which have a common mechanism of action as a cholinesterase inhibitor (Mileson et al., 1998), are among the most widely used insecticides (Donaldson et al., 2002). Although many residential uses of OP pesticides have been eliminated (US EPA, 2002), OPs remain highly used agricultural insecticides. Nearly 40 OP pesticides are registered with the US Environmental Protection Agency (US EPA) for use in the United States (US EPA, 2003). Some of the most commonly used OP pesticides are chlorpyrifos (Dursban), methyl parathion, diazinon (Dianon), and malathion.

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Many studies have evaluated human exposures to OP pesticides and the behavioral, dietary, and living conditions predictive of these exposures (Adgate et al., 2001; Berkowitz et al., 2003; Fenske et al., 2002a, c; Kieszak et al., 2002; Wilson et al., 2003; Morgan et al., 2004). Other studies have focused primarily on adverse health outcomes associated with exposures to OP pesticides (Kaloianova et al., 1989; Young et al., 2005; Padungtod et al., 1998; Richter et al., 1984, 1992a, b; Savitz et al., 1997). More recently, fetal and childhood exposures to OP pesticides have been highlighted because of the high potential for children to become exposed through the home environment or diet (Berkowitz et al., 2004; Whyatt et al., 2003, 2004; Eskenazi et al., 2004; National Research Council, 1993; Perera et al., 2003; Young et al., 2005). These studies document widespread exposure to OP pesticides in adults, children, and fetuses, despite attempts to reduce these exposures through decreased food tolerances as a result of the Food Quality Protection Act (FQPA) (1996) and the elimination of certain residential uses. However, the extent of these exposures throughout the US population cannot be easily extrapolated from the existing data in the literature because most of the studies reported have focused on at-risk populations. Thus, the success of the FQPA in reducing overall exposures in adults and children may be better evaluated by monitoring exposure trends from population-based data.

We report urinary concentrations of pesticide-specific metabolites of OP pesticides in approximately 2000 individuals of the general US population aged 6–59 years in 1999 and 2000. Specifically, we report urinary concentrations of 3,5,6-trichloropyridinol (TCPY), a metabolite of chlorpyrifos and chlorpyrifos methyl (Nolan et al., 1984); malathion dicarboxylic acid (MDA), a metabolite of malathion (Bradway and Shafik, 1977); 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMPY), a metabolite of diazinon (Iverson et al., 1975; Mucke et al., 1970); and *para*-nitrophenol (PNP), a metabolite of methyl parathion, parathion (Abu-Qare and Abou-Donia, 2000), *O*-ethyl-*O*-4-nitrophenyl phenylphosphonothioate (EPN), and other industrial chemicals. The data that we report are representative of the civilian, noninstitutionalized US population and are stratified by age, sex, and race/ethnicity.

2. Materials and methods

The National Health and Nutrition Examination Survey (NHANES), conducted by the National Center for Health Statistics of the Centers for Disease Control and Prevention (NCHS/CDC), is designed to measure the health and nutrition status of the civilian noninstitutionalized US population (CDC, 2003a). In 1999, NHANES became a continuous survey, fielded on an ongoing basis. Each year of data collection is based on a representative sample, which covers all ages of the civilian noninstitutionalized population. Public-use data files will be released in 2-year groupings (cycles). National population estimates for OP metabolites and estimates for the three largest race/ethnicity subgroups in the US population (non-Hispanic white, non-Hispanic black, and Mexican American) are derived from the first 2-year cycle of the survey, NHANES 1999–2000.

The sampling scheme for NHANES is based on a complex multistage area probability design, which includes selection of primary sampling units (counties), household segments within the counties, and sample persons from selected households. In 1999 and 2000, people aged 12–19 years and 60 years and older, non-Hispanic blacks, and Mexican Americans were oversampled. Low-income white Americans were oversampled in 2000. Data were collected through a household interview and a standardized physical examination that was conducted in a mobile examination center. Urine specimens were collected from each participant 6 years of age and older during one of three daily examination periods. Sociodemographic information and medical histories of the survey participant and the family were collected during the household interview.

NHANES 1999–2000 was conducted in 26 locations throughout the United States and included examinations of 9282 people. For the OP metabolites, measurements were conducted on a subset of participants who were selected by NCHS/CDC based on a random one-half sample of children aged 6–11 years in 1999 and 2000, a random one-quarter sample of people aged 12–59 years in 1999, and a random one-third sample of people aged 12–59 years in 2000. Because the subset was a random selection from the entire set, the ability of the samples tested to accurately represent the US population was maintained (CDC, 2003a).

2.1. Laboratory methods

During the physical examinations, "spot" or "grab" urine specimens were collected from participants, aliquoted, and stored cold (2–4 °C) or frozen until shipment. Urinary creatinine concentrations were determined using an automated colorimetric method based on a modified Jaffe reaction (Jaffe, 1886) on a Beckman Synchron AS/ASTRA clinical analyzer (Beckman Instruments, Inc., Brea, CA) at the Fairview University Medical Center (Minneapolis, MN). Samples collected for OP pesticide measurements were shipped on dry ice to CDC's National Center for Environmental Health. Urine samples were analyzed for pesticide-specific metabolites of OP pesticides using two analytic methods. TCPY and PNP were measured using a modification of the method of Hill et al. (1995a). Briefly, 5 mL of

urine was spiked with an isotopically labeled internal standard mixture and incubated with β-glucuronidase/sulfatase to liberate conjugated metabolites. The hydrolysate was acidified, extracted with *n*-butyl chloride: diethyl ether (4:1), and then back-extracted into 3 N sodium hydroxide solution. The metabolites were derivatized to their respective chloropropyl ethers using 1-chloro-3-iodropane in *n*-butyl chloride. The ethers were extracted into toluene and then passed through a silica gel solid-phase (SPE) extraction cartridge. The extract was concentrated and then analyzed using gas chromatography—positive chemical ionization—tandem mass spectrometry. IMPY and MDA were measured using the method of Olsson et al. (2004). Briefly, 2 mL of urine was spiked with isotopically labeled analogues. The urine samples were subjected to an enzyme hydrolysis using β-glucuronidase/sulfatase. The hydrolysates were extracted using OASIS mixed-mode SPE cartridges. The cartridges were washed with 5% methanol in a 0.1% acetic acid solution, and the metabolites were eluted using methanol. The extracts were concentrated and analyzed using high-performance liquid chromatography—atmospheric pressure chemical ionization—tandem mass spectrometry. All metabolites were quantified using isotope dilution calibration. Metabolite concentrations were adjusted using creatinine concentrations to correct for variable urine dilutions in the "spot" urine samples. Both laboratories and methods were certified according to guidelines set forth in the Clinical Laboratory Improvement Amendment (1988).

2.2. Demographic covariates

Age was reported at the time of the household interview as the age in years at the last birthday. Age categories used in our statistical analyses were 6–11 years, 12–19 years, and 20–59 years. A composite race/ethnicity variable based on self-reported race and ethnicity was created to define three major racial/ethnic groups: non-Hispanic black, non-Hispanic white, and Mexican American. Individuals from other racial/ethnic groups were included in the total estimates reported in this publication; however, no separate demographic breakdown was provided.

Traditionally, creatinine concentrations have been used to adjust spot urine samples for variable dilution caused by the different hydration states of the sample donor. Because creatinine concentrations vary with age, sex, and race/ethnicity, creatinine adjustment in diverse populations would not be valid for comparisons of OP metabolite concentrations among the demographic groups. To overcome this limitation and allow for an appropriate comparison of OP metabolite concentrations among the demographic groups, creatinine was also used as a covariate in statistical models (Barr et al., 2004, 2005). By using this model, we appropriately corrected for covariate effects on the creatinine concentrations while eliminating the variability caused by urine dilution of spot samples.

2.3. Statistical analysis

Survey-specific sample weights calculated for the one-third random subset were used in statistical analyses. Parametric statistics were performed only on TCPY because it was the only metabolite whose frequency of detection was greater than or equal to 60%. Geometric means (GMs), least squares geometric means (LSGMs), and percentiles of urinary OP metabolite concentrations were calculated using SAS release 8 (SAS Institute, Cary, NC) and SUDAAN release 7.5.6 (RTI International, Research Triangle Park, NC). For concentrations below the analytic limits of detection (LOD),[1] a value equal to the LOD divided by the square root of two was used (Hornung and Reed, 1990). Because the LODs of MDA and IMPY varied depending upon sample volume and analytic parameters, their distribution percentiles were presented only if the value was greater than or equal to the mean LOD. SUDAAN incorporates the NHANES sampling weights and adjusts for the complex sample design of the survey. Sample weights take into account the unequal probabilities of selection, resulting from the cluster design and the planned oversampling of certain subgroups. Oversampling of adolescents, the elderly, non-Hispanic blacks, and Mexican-Americans necessitated the use of sampling weights in all analyses to produce national estimates of prevalence and associated variances.

The LSGM TCPY concentrations for each demographic group were corrected for effects of all covariates, including creatinine. Differences in LSGMs among demographic groups were considered to be significant when $P<0.05$.

3. Results

The distribution of TCPY in the NHANES samples analyzed is presented in Table 1. These values are presented as both volume-based and creatinine-adjusted concentrations to allow for comparisons with similar data in the literature. TCPY was detected in 96% of the samples tested.

The LSGMs for TCPY in children, adolescents, and adults were 3.7, 2.1, and 1.6 μg/L, respectively (Fig. 1).

[1]The analytic limits of detection, determined as $3s_0$ where s_0 is the standard deviation at zero concentration, were 0.4 μg/L for TCPY, 0.4 μg/L for PNP, an average 0.31 μg/L for MDA (maximum value 2.6, standard deviation 0.45, median 0.45), and an average of 1.6 μg/L for IMPY (maximum value 7.1, standard deviation 1.46, median 1.2).

Table 1
Weighted quantiles of urinary TCPY concentrations in the NHANES 1999–2000 study population

Analyte	Demographic category	*N*	Weighted detection frequency (%)	Geometric mean	10th	25th	50th	75th	90th	95th
TCPY (μg/L)	All[a]	1994	91	1.77 (1.56–2.01)	<LOD	0.87 (0.79–0.99)	1.7 (1.5–2.0)	3.5 (2.7–4.5)	7.3 (5.4–9.4)	9.9 (7.6–14)
	6–11 years	481	97	2.88 (2.13–3.88)	0.78 (0.63–0.97)	1.2 (1.1–1.7)	2.7 (1.8–4.2)	6.9 (3.7–9.4)	11 (7.7–17)	16 (10–24)
	12–19 years	681	97	2.37 (2.00–2.81)	0.79 (0.70–0.89)	1.2 (1–1.5)	2.1 (1.6–2.6)	4.5 (3.1–5.7)	8 (5.7–12)	12.5 (8.4–23)
	20–59 years	832	89	1.53 (1.36–1.73)	<LOD	0.75 (0.62–0.88)	1.5 (1.2–1.6)	2.8 (2.4–3.7)	5.9 (4.3–7.9)	8.6 (6.3–12)
	Males	972	92	1.92 (1.67–2.21)	0.45 (<LOD-0.63)	1 (0.81–1.1)	1.9 (1.6–2.2)	3.5 (2.9–4.6)	7.3 (5.6–9.4)	9.9 (7.9–14)
	Females	1022	90	1.63 (1.41–1.88)	<LOD	0.77 (0.65–0.87)	1.5 (1.2–1.7)	3.3 (2.5–4.7)	7.2 (4.9–9.7)	10 (6.9–16)
	Mexican Americans	697	87	1.61 (1.37–1.9)	<LOD	0.87 (0.61–1.1)	1.67 (1.3–2.1)	3.2 (2.6–3.8)	5 (4–6.4)	7.4 (5.5–12)
	Non-Hispanic blacks	521	93	2.17 (1.71–2.76)	0.56 (<LOD-0.80)	1 (0.84–1.2)	1.9 (1.5–2.5)	4.2 (2.8–7.3)	9.4 (6.7–12)	13 (9.6–25)
	Non-Hispanic whites	602	91	1.76 (1.52–2.03)	0.42 (<LOD-0.6)	0.88 (0.76–1.1)	1.6 (1.5–2.0)	3.4 (2.6–4.6)	7.1 (4.7–9.6)	10 (6.9–16)
TCPY (μg/g creatinine)	All[a]	1994	91	1.58 (1.4–1.77)	<LOD	0.87 (0.76–0.97)	1.47 (1.31–1.7)	2.85 (2.2–3.4)	5.43 (4.3–6.6)	8.4 (6.3–12)
	6–11 years	481	97	3.11 (2.39–4.05)	0.86 (0.54–1.36)	1.64 (1.2–2.1)	3.2 (2.1–4.4)	6.37 (4.2–8.2)	10.1 (6.8–16)	14 (8.7–22)
	12–19 years	681	97	1.6 (1.40–1.83)	0.61 (0.55–0.7)	0.93 (0.82–1.1)	1.45 (1.2–1.7)	2.58 (2.0–3.6)	4.82 (3.9–5.6)	6.2 (5.0–9.8)
	20–59 years	832	89	1.41 (1.26–1.58)	<LOD	0.79 (0.7–0.89)	1.33 (1.2–1.5)	2.37 (2.0–2.9)	4.25 (3.3–5.6)	6.4 (5.0–11)
	Males	972	92	1.48 (1.3–1.67)	0.47 (0.4–0.56)	0.8 (0.7–0.91)	1.44 (1.3–1.6)	2.52 (2.1–3.2)	4.95 (4.0–6.3)	7.6 (5.7–11)
	Females	1022	90	1.69 (1.49–1.91)	<LOD	0.91 (0.82–1)	1.51 (1.3–1.7)	2.96 (2.4–3.7)	5.63 (4.2–7.2)	8.4 (6.3–13)
	Mexican Americans	697	87	1.46 (1.27–1.67)	<LOD	0.86 (0.74–0.99)	1.44 (1.2–1.7)	2.38 (2.1–2.9)	3.82 (3.3–5.1)	5.8 (4.4–9.0)
	Non-Hispanic blacks	521	93	1.47 (1.18–1.84)	0.43 (0.41–0.51)	0.73 (0.59–0.92)	1.33 (1–1.8)	2.86 (1.8–4.4)	5.88 (4.3–8.9)	8.9 (5.9–14)
	Non-Hispanic whites	602	91	1.66 (1.46–1.89)	0.54 (0.46–0.68)	0.91 (0.80–1.0)	1.55 (1.3–1.7)	2.93 (2.1–3.7)	5.5 (4.2–6.9)	8.4 (6.1–13)

Upper and lower 95th confidence intervals of each quantile are shown in parentheses. These data are shown as total population data and divided into demographic subgroups based upon race/ethnicity, sex, and age group.

TCPY, 3,5,6-trichloro-2-pyridinol; *N*, sample size; 10th, 10th percentile of distribution; 25th, 25th percentile of distribution; 50th, 50th percentile or median of distribution; 75th, 75th percentile of distribution; 90th, 90th percentile of distribution; 95th, 95th percentile of distribution.

[a]All population data including those individuals not grouped into one of the three composite race/ethnicity categories presented.

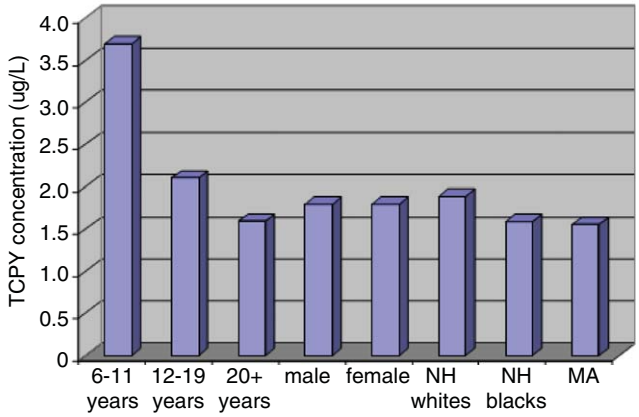

Fig. 1. Least squares geometric mean concentrations of TCPY in demographic subgroups of the US population. Child concentrations were significantly higher than those of adolescents and adults. Adolescent concentrations were significantly higher than those of adults.

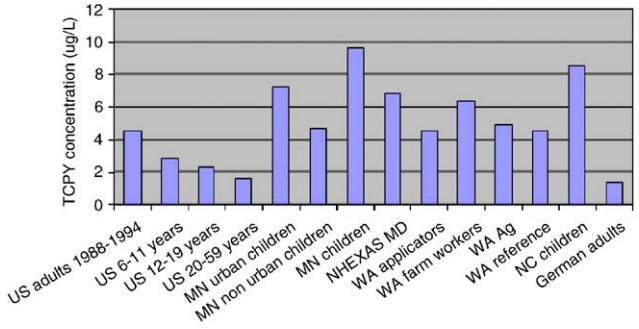

Fig. 2. Comparison of TCPY concentrations in the literature.

Children had significantly higher concentrations than adolescents or adults. Adolescents had significantly higher concentrations than adults. No differences between males (1.8 μg/L) and females (1.8 μg/L) or among racial/ethnic groups (non-Hispanic whites, 1.9 μg/L; non-Hispanic blacks, 1.6 μg/L; and Mexican Americans, 1.6 μg/L) were observed.

MDA (52%), IMPY (29%), and PNP (22%) were detected much less frequently than TCPY. The distributions of both volume-based and creatinine-adjusted MDA in NHANES urine samples are presented in Table 2. The 95th percentiles of the distributions of IMPY and PNP are shown in Table 3.

We previously reported concentrations of dialkylphosphate metabolites of OP pesticides that were measured in the same samples (Barr et al., 2004). These metabolites are derived from the phosphate portion of the pesticide that is common to about 75% of the US EPA-registered OP pesticides. TCPY concentrations were significantly correlated with both diethylphosphate (DEP; $r = 0.22$, $P < 0.0001$) and diethylthiophosphate (DETP; $r = 0.29$, $P < 0.0001$), metabolites of *O*,*O*-diethyl-substituted OP pesticides such a chlorpyrifos. TCPY concentrations were also significantly correlated with both dimethylphosphate (DMP; $r = 0.11$, $P = 0.007$) and dimethylthiophosphate (DMTP; $r = 0.12$, $P = 0.01$), metabolites of *O*,*O*-dimethyl-substituted OP pesticides such as chlorpyrifos methyl. MDA concentrations were significantly correlated with DMTP ($r = 0.16$, $P < 0.0001$).

4. Discussion

4.1. Chlorpyrifos/chlorpyrifos methyl

Chlorpyrifos is the OP pesticide that has been the most studied. Its toxicokinetics in rats (Bakke and Price, 1976; Sunaga et al., 1989; Timchalk et al., 2002), sheep (Bakke and Price, 1976; Ivey and Palmer, 1981), and humans (Nolan et al., 1984) have been thoroughly investigated. The half-life of chlorpyrifos in rat blood was approximately 5 h and the half-life of TCPY in urine was approximately 24 h (Sunaga et al., 1989). About 90% of the total rat dose was excreted in the urine, primarily as TCPY or its conjugates. In human male volunteers, approximately 70% of the oral dose and 3% of the dermal dose of chlorpyrifos were excreted in the urine as TCPY or its conjugates (Nolan et al., 1984); the low percentage of the dermal dose excreted was primarily a result of poor dermal absorption of chlorpyrifos rather than differential metabolism. The maximum TCPY concentrations observed were 6 h after oral dosing (930 μg/L) and 24 h after dermal dosing (63 μg/L). Since these groundbreaking studies were published, detailing the toxicokinetics of chlorpyrifos in mammals, urinary TCPY has frequently been measured as an indicator of chlorpyrifos exposure in occupational (Chang et al., 1996; Fenske and Elkner, 1990; Jitsunari et al., 1989), paraoccupational (Fenske et al., 2002c), and environmental (Adgate et al., 2001; Aprea et al., 1999; Berkowitz et al., 2004; Byrne et al., 1998; Eskenazi et al., 2004; Fenske et al., 2002b; Hill Jr. et al., 1995b; Koch and Angerer, 2001; Krieger et al., 2001; MacIntosh et al., 1999; Meeker et al., 2004; Murphy et al., 1983; Olsson et al., 2003; Koch et al., 2001; Berkowitz et al., 2003) settings (Table 4; Fig. 2).

Background TCPY concentrations from environmental exposures in children and adults are reasonably comparable across studies, including our study. Median concentrations were typically less than 10 μg/L or 5 μg/g creatinine. In many studies, children tended to have higher concentrations than adults; however, these differences may have been a result of creatinine "overcorrection" or due to real differences in exposure, absorption, metabolism, or excretion. Urinary TCPY was measured in a 1000-sample nonrepresentative subset of adult participants of NHANES III conducted in

Table 2
Weighted quantiles of urinary MDA concentrations in the NHANES 1999–2000 study population

Analyte	Demographic category	*N*	Weighted detection frequency (%)	50th[a]	75th	90th	95th
MDA (μg/L)	All[b]	1920	52	<LOD	0.54 (0.46–0.71)	1.3 (1.1–1.7)	1.6 (1.3–2.3)
	6–11 years	453	56	0.49 (0.38–0.7)	1.2 (0.86–1.5)	2.8 (2.1–3.9)	2.8 (1.9–5.5)
	12–19 years	660	54	<LOD	0.38 (0.3–0.46)	0.84 (0.56–1.5)	1.6 (1.3–2.2)
	20–59 years	807	51	<LOD	0.5 (0.44–0.65)	1.1 (0.93–1.6)	1.3 (1.0–2.0)
	Males	937	50	<LOD	0.45 (0.42–0.53)	1.1 (0.89–1.3)	1.6 (1.3–2.2)
	Females	983	55	<LOD	0.71 (0.5–0.82)	1.6 (0.95–2.2)	1.6 (1.2–2.8)
	Mexican Americans	680	61	<LOD	0.67 (0.38–0.95)	1.3 (0.98–1.7)	1.6 (1.3–2.0)
	Non-Hispanic blacks	498	50	<LOD	0.48 (0.34–0.64)	1 (0.76–1.5)	1.7 (0.93–3.2)
	Non-Hispanic whites	579	53	<LOD	0.53 (0.44–0.76)	1.4 (1.04–1.8)	1.6 (1.3–2.7)
MDA (μg/g creatinine)	All[b]	1920	52	<LOD	0.49 (0.43–0.65)	1.1 (0.91–1.3)	1.8 (1.3–2.5)
	6–11 years	453	56	0.44 (0.30–0.63)	1 (0.73–1.3)	2.2 (1.4–3.1)	3.7 (2.3–4.6)
	12–19 years	660	54	<LOD	0.38 (0.30–0.44)	0.73 (0.62–0.89)	1.2 (0.8–1.6)
	20–59 years	807	51	<LOD	0.48 (0.40–0.58)	1 (0.80–1.2)	1.6 (1.1–2.2)
	Males	937	50	<LOD	0.44 (0.38–0.50)	0.98 (0.78–1.1)	1.4 (1.2–1.8)
	Females	983	55	<LOD	0.63 (0.46–0.77)	1.3 (0.93–1.9)	2.1 (1.6–3.6)
	Mexican Americans	680	61	<LOD	0.57 (0.36–0.88)	1.1 (0.88–1.5)	1.7 (1.3–2.3)
	Non-Hispanic blacks	498	50	<LOD	0.45 (0.30–0.64)	0.83 (0.58–1.1)	1.1 (0.93–1.8)
	Non-Hispanic Whites	579	53	<LOD	0.5 (0.42–0.71)	1.2 (0.93–1.6)	2 (1.4–2.8)

Upper and lower 95th confidence intervals of each quantile are shown in parentheses. These data are shown as total population data and divided into demographic subgroups based upon race/ethnicity, sex, and age group.
MDA, malathion dicarboxylic acid; <LOD, less than the analytical limit of detection; *N*, sample size; 50th, 50th percentile or median of distribution; 75th, 75th percentile of distribution; 90th, 90th percentile of distribution; 95th, 95th percentile of distribution.

[a]LODs varied based upon sample volume and analytical parameters. For determining this distribution, we used the measured values, or if the measured value was below the LOD, the actual LOD divided by the square root of two. For presentation, we report only the distribution percentiles equal to or greater than the mean LOD (0.31 μg/L). The standard deviation about the mean LOD was 0.45 μg/L and the LODs ranged from 0.01 to 2.6 μg/L.

[b]All population data including those individuals not grouped into one of the three composite race/ethnicity categories presented.

1988–1994. Hill et al. (1995b) reported that TCPY was detected in 82% of the samples measured for NHANES III. Although we detected TCPY much more frequently in the data that we report, this detection is an artifact of the lower LOD for our study. In fact, if the same LOD is applied to both studies, the frequency of detection is lower for NHANES 1999–2000 (75%).

The median concentration of TCPY in NHANES III (3.0 μg/L) was about double the concentration of adults in our study (1.4 μg/L), even when creatinine-adjusted data were compared (2.2 μg/g creatinine vs 1.3 μg/g creatinine). Because NHANES III was a nonrepresentative study, the results of the two studies cannot be easily compared. However, because of the regulatory reassessments and subsequent reduction in selected food tolerances (US EPA, 2002), the approximate 50% reduction in urinary TCPY across NHANES studies may be due to regulatory action or voluntary reduction

Table 3
Weighted quantiles of urinary PNP and IMPY concentrations in the NHANES 1999–2000 study population

Analyte	Demographic category	*N*	Weighted detection frequency (%)	95th (μg/L)	95th (μg/g creatinine)
IMPY[a]	All[b]	1994	29	3.7 (3.4–4.2)	3.4 (3.0–4.2)
	6–11 years	481	22	3.6 (3.3–4.3)	5.1 (3.2–10)
	12–19 years	681	31	4.2 (2.9–4.9)	2.5 (2.3–3.3)
	20–59 years	832	30	3.7 (3.3–4.2)	3.3 (2.8–4.0)
	Males	972	31	3.8 (3.4–4.3)	3.2 (2.5–3.8)
	Females	1022	27	3.5 (2.9–4.2)	3.9 (3.0–4.4)
	Mexican Americans	697	24	4.2 (2.9–4.8)	3.9 (2.8–6.4)
	Non-Hispanic blacks	521	36	4.5 (3.6–6.6)	4.2 (2.6–7.2)
	Non-Hispanic whites	601	28	3.6 (3.3–4.1)	3.3 (2.8–4.0)
PNP	All[b]	1989	22	5 (3.3–9.0)	4.2 (2.6–7.6)
	6–11 years	479	26	4.2 (2.7–6.4)	4.2 (3.3–6.7)
	12–19 years	680	25	5.7 (2.6–19)	4 (1.6–7.3)
	20–59 years	830	21	4.5 (2.5–9.2)	4.3 (2.4–10)
	Males	971	23	4.4 (2.6–11)	3.4 (1.9–7.6)
	Females	1018	22	5.2 (3.0–9.5)	6.9 (3.5–12)
	Mexican Americans	695	34	21 (5.0–31)	17 (4.8–35)
	Non-Hispanic blacks	518	30	4.8 (2.7–9.0)	3.7 (2.2–5.2)
	Non-Hispanic whites	602	19	4.2 (2.2–9.5)	3.8 (2.1–7.6)

Upper and lower 95th confidence intervals of each quantile are shown in parentheses. These data are shown as total population data and divided into demographic subgroups based upon race/ethnicity, sex, and age group.
PNP, *para*-nitrophenol; IMPY, 2-isopropyl-4-methyl-6-hydroxypyrimidine; <LOD, less than the analytical limit of detection; *N*, sample size; 50th, 50th percentile or median of distribution; 75th, 75th percentile of distribution; 90th, 90th percentile of distribution; 95th, 95th percentile of distribution.

[a]LODs for IMPY varied based upon sample volume and analytical parameters. For determining the 95th percentiles, we used the measured values, or if the measured value was below the LOD, the actual LOD divided by the square root of two. For presentation, we report only the 95th percentiles equal to or greater than the mean LOD (1.6 μg/L). The standard deviation about the mean LOD was 1.5 μg/L and the LODs ranged from 0.1 to 7.1 μg/L.

[b]All population data including those individuals not grouped into one of the three composite race/ethnicity categories presented.

in use of OP pesticides (Whyatt et al., 2003). Voluntary reduction in OP pesticide use or voluntary replacement of OP pesticides with less toxic pesticides possibly may be the primary contributor to a reduction in urinary TCPY. Most residential uses of chlorpyrifos were not fully eliminated until December 2001 (US EPA, 2002). The difference seen between NHANES III and NHANES 1999–2000 TCPY could also be a result of differences between the two study populations; thus, this comparison should be treated cautiously. Indeed, our observation should be verified by comparison with additional 2-year NHANES cycles before deciding that a real reduction in chlorpyrifos exposure as a result of regulatory action, or impending regulatory action, has occurred. The next two NHANES cycles may provide more information on the regulatory impact on

Table 4
TCPY concentrations in reported studies

Author	Study population	*N*	Detection frequency (%)	GM (μg/L)	Median (μg/L)	GM (μg/g creatinine)	Median (μg/g creatinine)
Takei (1981)	Adults	5	100	NR	27	NR	NR
Jitsunari[a] (1989)	Adult termine control workers	8	100	NR	NR	NR	250
Fenske[b] (1990)	Structural treatment applicators	7	100	NR	NR	NR	NR
	Preapplication	7	100	132	158	NR	NR
Hill (1995)	General population adults NHANES III	993	82	NR	3	NR	2.2
Chang (1996)	Pest control workers	16	100	320	340	190	180
Byrne[c] (1998)	Adults in homes before crack/crevice application	6	NR	5.8	5.8	NR	NR
	Adults in homes after crack/crevice application	6	NR	7.2	6.2	NR	NR
Aprea[d] (1999)	Adults (all)	42	88	NR	NR	2.8	NR
	Wine drinkers	17	100	NR	NR	3.5	NR
	Non wine drinkers	25	80	NR	NR	2.4	NR
MacIntosh (1999)	Adults (longitudinal samples)	346	96	5.1	5.3	4.5	4.6
Adgate (2001)	MN children	261	97	7	NR	NR	NR
Koch (2001)	Adults	50	100	NR	1.4	NR	1
Koch (2001)	Adult nonexposed	12	100	NR	2.2	NR	1.3
	Adult exposed	4	100	NR	5.3	NR	3.6
Kreiger[e] (2001)	All preapplication	14	100	4.7	7.5	NR	NR
	All post application	14	100	23.3	23.9	NR	NR
	People in homes with fogger discharge (preapplication)	4	100	1.6	1.6	NR	NR
	People in homes with fogger discharge (postapplication)	4	100	18.9	23.2	NR	NR
	People in homes with broadcast spray (preapplication)	5	100	6.3	8.1	NR	NR
	People in homes with broadcast spray (postapplication)	5	NR	25.1	27.1	NR	NR
	People in homes with crack/crevice treatment (preapplication)	5	NR	8.6	7.5	NR	NR
	People in homes with crack/crevice treatment (postapplication)	5	NR	25.4	20.7	NR	NR
Fenske (2002)	Applicators' children	49	20	NR	<8	NR	NR
	Farm workers' children	12	33	NR	<8	NR	NR
	Total agricultural children	61	23	NR	<8	NR	NR
	Reference children	14	29	NR	<8	NR	NR
Olsson (2003)	General population	115	56	9.7	NR	NR	NR
Berkowitz (2004)	Pregnant women in NY city	404	100	NR	7.6	NR	NR
Eskenazi (2004)	Pregnant women in rural California	485	77	NR	3.3	NR	NR
Meeker (2004)	Adult men	370	93	2	2.49	1.7	1.95

The geometric mean or median concentrations are shown to allow easier comparisons. Where noted, conversions to common units were made
NR, not reported; GM, geometric mean; TCPY, 3,5,6-trichloro-2-pyridinol.

[a]Estimated from graph.

[b]Assuming 1500 mL urine output per day or 1.5 g creatinine excreted per day.

[c]Assuming average weight of 70 kg/person and an average urine output of 1500 mL/day.

[d]Values below the limit of detection were excluded in analysis.

[e]Calculated using body weight reported.

chlorpyrifos exposures because their sample collection phase would have been conducted after the residential eliminations of chlorpyrifos were fully implemented.

General population TCPY data have been reported for European populations in Italy and Germany (Aprea et al., 1999; Koch and Angerer, 2001; Koch et al., 2001). The adult Italian data were derived from a small sample ($N = 42$) that included both wine-drinkers and non-wine-drinkers (Aprea et al., 1999). Their frequency of detection was 88% in the full sample and 100% for wine-drinkers. Their GM doubled (2.8 μg/g creatinine) the GM for our adult population, although they included only detectable measurements in their GM calculation. Interestingly, they found a statistical difference in urinary TCPY concentrations between the wine-drinkers and the non-wine-drinkers. The German population data were also determined on two small population subsets ($N = 12$; $N = 50$) of adults (Koch and Angerer, 2001; Koch et al., 2001). The median TCPY concentrations were 1.3 and 1.0 μg/g creatinine, both similar to the median concentration of adults in our population. TCPY was detected in all of the German population samples in both subsets.

In our study, we found TCPY in more than 95% of the samples tested, indicating widespread exposure to chlorpyrifos, chlorpyrifos methyl, environmental TCPY, or environmental chlorpyrifos/chlorpyrifos methyl oxons. Although reportedly a precursor to TCPY, the herbicide trichlopyr is not a major contributor to urinary TCPY because only a small percentage of the overall dose is excreted as TCPY or its conjugates (Carmichael et al., 1989). Because TCPY was more highly correlated with the dialkylphosphate metabolites of chlorpyrifos than with chlorpyrifos methyl, chlorpyrifos was likely a larger contributor to the overall urinary TCPY concentrations than was chlorpyrifos methyl. Because data on the contributions of environmental TCPY and OP oxons to urinary TCPY concentrations are limited or nonexistent, distinguishing with any certainty the percentage of urinary TCPY derived from actual exposure to chlorpyrifos is difficult. However, Wilson et al. (2003) reported urinary TCPY concentrations in children in daycare centers in North Carolina that were five times larger than the potential dose of chlorpyrifos estimated by using multimedia measurements. Similarly, an estimate of the potential dietary chlorpyrifos dose based upon the FDA's Market-Basket Surveys (1993–1997) accounted for less than 10% of the urinary TCPY in the Maryland–NHEXAS study (MacIntosh et al., 2001). Thus, if we assume that the primary source of chlorpyrifos exposure in our population was from the diet, and we apply a similar apportionment to our data, only 0.15–0.30 μg/g creatinine of the 1.47 μg/g creatinine median of urinary TCPY would be derived directly from exposure to chlorpyrifos.

Assuming that only 20% of the urinary TPCY is attributable to chlorpyrifos exposure (Wilson et al., 2003) and that 70% of the chlorpyrifos dose metabolizes to TCPY (Nolan et al., 1984), we can use the standard daily excretion rates of creatinine and average body weights to translate TCPY into a chlorpyrifos dose (Shurdut et al., 1998). Assuming that the average child weighs 26 kg, the average adolescent weighs 56 kg, and the average adult weighs 70 kg (CDC, 2000), the estimated daily excretion rates of creatinine in children, adolescents, and adults are 0.39, 1.1, and 1.4 g/day (Teitz, 1990), respectively. If we correct for the differences in molecular weight in chlorpyrifos (350.6 g/mol) and TCPY (198 g/mol), we can crudely estimate that the median chlorpyrifos doses for children, adolescents, and adults are 0.024, 0.008, and 0.008 μg/kg body wt/day. Similarly, the chlorpyrifos doses at the 95th percentile could be estimated as 0.06, 0.034, and 0.036 μg/kg body wt/day, respectively, for children, adolescents, and adults. These estimated doses are all well below the acute (100 μg/kg body wt/day) and chronic (300 μg/kg body wt/day) NOAEL levels for plasma cholinesterase depression. However, the estimated child dose at the 95th percentile is within a factor of two of the chronic population-adjusted dose[2] (cPAD) for children (0.1 μg/kg body wt/day). The median dose for children is about five times lower than the cPAD level.

4.2. Malathion, methyl/ethyl parathion, and diazinon

The LODs of MDA in individual samples varied based upon a variety of factors, including analyte recovery and sample volume (Fig. 3A). To evaluate the distribution percentiles of analytes with variable LODs, several different approaches can be used. In CDC's Second National Report on Human Exposure to Environmental Chemicals (CDC, 2003b), a distribution percentile was considered less than the LOD if it was less than the maximum LOD value of all MDA measurements. This conservative approach was used to eliminate any potential bias in the data distribution arising from the inclusion of large imputed values (i.e., LOD/square root 2 substituted for values below the LOD) in the statistical analysis; however, only the 95th percentile for children could be established. Because 75% of the samples reported as less than the LOD had LODs at or below the mean LOD value of 0.31 μg/L, we reported any distribution percentile greater than 0.31 μg/L. By using this approach, we were able to estimate the median and upper distribution percentiles. However, the

[2]The PAD is defined by the EPA as the reference dose (RfD) adjusted to include the FQPA safety factor. For children and females 13–50 years, the acute and chronic chlorpyrifos PADs are the RfDs divided by 3. The general population PAD is equivalent to the RfD.

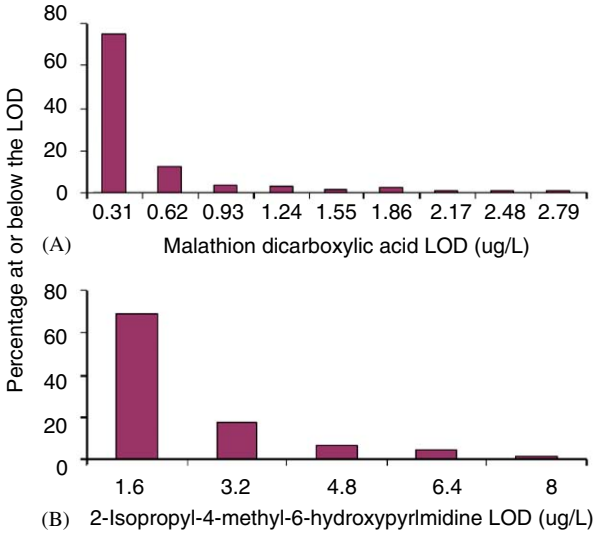

Fig. 3. Distribution of limits of detection for malathion dicarboxylic acid and 2-isopropyl-4-methyl-6-hydroxypyrimidine.

distribution was determined on data for which about half of the values were imputed using a wide array of LODs ranging from 0.01 to 2.6 μg/L. Thus, the distribution may have been inadvertently shifted to the right by using the higher imputed values. The same approach was used for IMPY, which also had variable LODs (Fig. 3B). However, 69% were at or below the mean LOD, thereby allowing us to establish the 95th percentile values for all demographic subgroups. This distribution estimation is subject to the same limitations of those for MDA.

Although less frequently measured in exposure assessment studies than TCPY, the concentrations for MDA, PNP, and IMPY have also been reported (Adgate et al., 2001; Olsson et al., 2003; Bradway and Shafik, 1977; Fenske and Leffingwell, 1989; Hryhorczuk et al., 2002; McCann et al., 2002; Baker et al., 2000; Barr et al., 2002). MDA was detected less frequently (0.5–32%) in studies investigating incidental exposures than in the general population data that we report. However, the variation in LODs among studies likely can explain the differences. Because malathion produces many metabolites including MDA (Bradway and Shafik, 1977), malathion monocarboxylic acid, DMP, DMTP, and dimethyldithiophosphate, estimation of population-based malathion exposure from MDA concentrations alone is likely overly conservative.

We detected IMPY in only about one-third of the samples tested. This detection frequency was less than that in a previously reported study (57%) (Baker et al., 2000). Our low frequency of detection was surprising, because diazinon was still a popularly used residential pesticide at the time that we collected samples for the study. The pending restriction on its residential use and increasing concerns about OP pesticide exposures could have resulted in less use. However, diazinon exposure in the US population likely was underestimated by measuring only IMPY. Diazinon reportedly produces two primary metabolites, including IMPY, which are present in approximate equal amounts in urine (Iverson et al., 1975). Other less abundant metabolites have also been noted (Iverson et al., 1975).

PNP concentrations have been reported in many studies; however, most of these studies involved occupational (Davies and Peterson, 1997; Durham et al., 1972; Roan et al., 1969) or accidental (Arteberry et al., 1961; Barr et al., 2002; Davies and Peterson, 1997; Hill Jr. et al., 2002; Hryhorczuk et al., 2002; McCann et al., 2002) exposures. Urinary PNP concentrations in our study are much less than those observed in occupational settings or as a result of misuse of methyl parathion. PNP was detected much more frequently (41%) in urine samples from a 1000-sample nonrepresentative subset of adult participants of NHANES III (1988–1994); however, the 95th percentile estimates were similar (Hill Jr. et al., 1995b). These differences may be attributable to differences between study populations or to a real reduction in exposures to methyl parathion and parathion. Fenske et al. (2002b) reported low levels of PNP in the urine of Washington reference and pesticide applicators' children and higher concentrations in children of farm workers; however, all median concentrations were less than the LOD (8 μg/L).

Our study has several limitations. In general, the measurements of urinary metabolites for pesticide exposure assessment can be limited because of potential contributions from nonpesticide sources (e.g., environmental degrades, other parent chemicals) (Morgan et al., 2004) and because of interperson variation in metabolism. For IMPY and PNP, almost three-quarters of the data were less than the LOD, which added much uncertainty to our 95th percentile estimations. Similarly, the use of imputed values, which varied based upon the LOD in our statistical analyses for MDA and IMPY, also introduced uncertainty into the distribution percentile estimates. Furthermore, MDA, IMPY, and PNP were detected in less than 60% of the samples tested; therefore, their geometric means could not be reliably calculated.

5. Conclusions

We report the US population-based reference data for TCPY and MDA; these data are stratified by age, sex, and a composite race/ethnicity variable. We found that children had significantly higher urinary concentrations than adolescents and adults. Adolescents had significantly higher concentrations of TCPY than adults. Sex and race/ethnicity did not significantly impact TCPY concentrations. Our data indicate that a majority of the

US population has some exposure to chlorpyrifos, chlorpyrifos methyl, or environmental degradates or metabolites. Our conservative, yet crude, estimates of daily chlorpyrifos doses from our data suggest that most children have chlorpyrifos doses below the current EPA cPAD limit. Similarly, about half of the population has some exposure to malathion. The concentrations that we report are similar to other data reported in the literature.

These data will serve many purposes in environmental public health. They will be used as reference range values by physicians and public health officials for comparing urinary levels of these metabolites to potentially exposed individuals or populations to assess their relative exposure status. These data will be used by many disciplines in environmental public health to track trends in exposure over time and to determine the effectiveness of public health efforts, including legislation such as the FQPA, to reduce exposures to all Americans, particularly to certain vulnerable or sensitive subgroups, such as children. These data will also help prioritize research gaps and needs for relating human exposures and adverse health outcomes; they will be used for comparing human urinary levels to urinary levels found in dosed animals that have exhibited adverse health outcomes.

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