Measurement of Children's Exposure to Pesticides: Analysis of Urinary Metabolite Levels in a Probability-Based Sample

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The Minnesota Children's Pesticide Exposure Study is a probability-based sample of 102 children 3–13 years old who were monitored for commonly used pesticides. During the summer of 1997, first-morning-void urine samples (1–3 per child) were obtained for 88% of study children and analyzed for metabolites of insecticides and herbicides: carbamates and related compounds (1-NAP), atrazine (AM), malathion (MDA), and chlorpyrifos and related compounds (TCPy). TCPy was present in 93% of the samples, whereas 1-NAP, MDA, and AM were detected in 45%, 37%, and 2% of samples, respectively. Measured intrachild means ranged from 1.4 µg/L for MDA to 9.2 µg/L for TCPy, and there was considerable intrachild variability. For children providing three urine samples, geometric mean TCPy levels were greater than the detection limit in 98% of the samples, and nearly half the children had geometric mean 1-NAP and MDA levels greater than the detection limit. Interchild variability was significantly greater than intrachild variability for 1-NAP ($p = 0.0037$) and TCPy ($p < 0.0001$). The four metabolites measured were not correlated within urine samples, and children's metabolite levels did not vary systematically by sex, age, race, household income, or putative household pesticide use. On a log scale, mean TCPy levels were significantly higher in urban than in nonurban children (7.2 vs. 4.7 µg/L; $p = 0.036$). Weighted population mean concentrations were 3.9 [standard error (SE) = 0.7; 95% confidence interval (CI), 2.5, 5.3] µg/L for 1-NAP, 1.7 (SE = 0.3; 95% CI, 1.1, 2.3) µg/L for MDA, and 9.6 (SE = 0.9; 95% CI, 7.8, 11) µg/L for TCPy. The weighted population results estimate the overall mean and variability of metabolite levels for more than 84,000 children in the census tracts sampled. Levels of 1-NAP were lower than reported adult reference range concentrations, whereas TCPy concentrations were substantially higher. Concentrations of MDA were detected more frequently and found at higher levels in children than in a recent nonprobability-based sample of adults. Overall, Minnesota children's TCPy and MDA levels were higher than in recent population-based studies of adults in the United States, but the relative magnitude of intraindividual variability was similar for adults and children. *Key words*: children's health, exposure assessment, Minnesota Children's Pesticide Exposure Study (MNCPES), National Health and Nutrition Examination Survey (NHANES), National Human Exposure Assessment Survey (NHEXAS), organophosphate pesticides, urinary biomarkers. *Environ Health Perspect* 109:583–590 (2001). [Online 22 May 2001]
http://ehpnet1.niehs.nih.gov/docs/2001/109p583-590adgate/abstract.html

The potential health effects associated with children's exposure to pesticides are the subject of increasing concern. Children may be exposed to unsafe levels of pesticides in the air they breathe, the food they eat, the water they drink, and the surfaces they touch (*1–4*). Concerns about dietary exposures in the general population resulted in the passage of the Food Quality Protection Act of 1996 [FQPA (*5*)]. Under FQPA, realistic evaluation of the potential health risks to children from pesticides requires information about the full range of children's exposures. Unfortunately there is a scarcity of data available to assess children's actual exposure to pesticides, which makes it difficult, if not impossible, to assess children's health risks realistically (*6*).

Urinary biomarkers of pesticides and their metabolites have been used to characterize body burden levels for adult populations as well as trends over time in the United States (*7,8*) and Europe (*9*). Under FQPA, organophosphorus (OP) pesticides and carbamates are subject to increased scrutiny because they are used widely in agricultural and residential settings and they inhibit cholinesterase, an enzyme essential for proper functioning of the nervous system (*10*). Most metabolites of OPs and carbamates have relatively short biological half-lives, generally on the order of days, and are excreted primarily in the urine (*11,12*). Past studies of children's OP pesticide exposure either have been conducted outside the United States (*13*) or have focused on special populations, such as children of agricultural worker families (*14*), which are presumed to be more highly exposed than average. There is a lack of probability-based studies that provide baseline data on the distribution of children's exposures in urban and nonurban settings.

As part of the Minnesota Children's Pesticide Exposure Study (MNCPES), which was a probability-based sample of children 3–13 years of age (*15*), urine samples were collected and analyzed for metabolites of commonly used pesticides. The MNCPES was a Phase III special study that was part of the National Human Exposure Assessment Survey (NHEXAS) (*16*). The multiphase study was designed to identify and select children with a known probability by applying a predetermined set of eligibility criteria and adjusting for subject nonresponse within each phase. The sampling process preferentially (but not exclusively) selected a higher proportion of households reporting more frequent pesticide use as well as children more likely to experience exposures to four target pesticides: the herbicide atrazine and the organophosphates chlorpyrifos, diazinon, and malathion. It was postulated that children living in these homes and participating in the MNCPES were more likely to have measurable concentrations of pesticide metabolites in their urine (*15*). Individual-level statistical weights were developed to adjust for oversampling these nominally higher-pesticide-use households. These weights were incorporated into the analysis presented here so inferences could be drawn about the prevalence and magnitude of pesticide exposure for children within the census tracts sampled.

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We thank the families who participated in the survey for their cooperation and M. Bollenbeck for his assistance with the data analysis. MNCPES was funded in part through the NHEXAS Pesticides and PAH Module Cooperative Agreement R821902 between the U.S. Environmental Protection Agency and the consortium of Research Triangle Institute/Environmental and Occupational Health Sciences Institute (RTI/EOHSI), by U.S. EPA STAR (Science to Achieve Results) Grant R825283 to the University of Minnesota, and by a grant from the Legislative Commission on Minnesota Resources.

Received 20 October 2000; accepted 12 March 2001.

This study measured four specific urinary metabolites: 1-naphthol (1-NAP), atrazine metcapturate (AM), malathion dicarboxylic acid (MDA), and 3,5,6-trichloro-2-pyridinol (TCPy). These compounds are the primary urinary metabolites of the parent compounds naphthalene or carbaryl (1-NAP), atrazine (AM), malathion (MDA), and chlorpyrifos, chlorpyrifos-methyl, or triclopyr (TCPy) (*12*). Naphthalene is used in mothballs and found in cigarette smoke and petroleum products, and carbaryl is a carbamate insecticide used on turf and gardens (*7*). Atrazine is a herbicide used on a wide variety of crops in the United States, and malathion is an insecticide used on fruits and vegetables as well as in household products (*12,17*). Chlorpyrifos and chlorpyrifos-methyl have had wide use on fruits and vegetables and as treatments inside and outside homes, although many residential uses were recently restricted (*18*). TCPy is the primary environmental degradation product of chlorpyrifos, chlorpyrifos-methyl, and/or triclopyr and may persist in soil for up to a year (*19*). Because of the numerous uses of chlorpyrifos and chlorpyrifos-methyl as well as the ubiquity and stability of TCPy in the environment, urinary TCPy represents total intake of both "environmental" TCPy and parent compounds. Until more is known about the adsorption, distribution, and metabolism of TCPy, the relative contribution of environmental TCPy to total urinary TCPy remains unknown.

In this article we report measured urinary metabolite levels and the variability of 1-NAP, AM, MDA, and TCPy within and between individual children, and within and between sociodemographic subgroups, including urban and nonurban children. Weighted distributions of urinary metabolite levels for the general population of children in the census tracts sampled are also presented. Additionally, we compare these population distributions to adult levels and examine the implications of our findings for exposure analysis and risk assessment.

Methods

Study design. MNCPES used a cross-sectional design, and exposure-related measurements were obtained for each subject during a one-week sampling period between May and September 1997. The sampled population consisted of children 3–13 years of age living in households located in either the cities of Minneapolis and St. Paul (designated urban households), or Rice and Goodhue Counties (designated nonurban households), located just south of the Twin Cities metropolitan area. A detailed description of the study design strategy, eligibility criteria, household and subject sample selection, field sampling methods, monitoring outcomes, and the development and application of statistical weights has been published (*15,20*). To maintain a probability-based structure within a complex study design, probabilities of subject selection by investigators (based on application of eligibility criteria described below) and subject response (participation rate) were assessed at each step. This information was used to develop statistical weights to adjust the population distributions for the oversampling of some subpopulations. The probability-based sample was obtained through a systematic process that occurred over three phases: identification, household screening, and intensive exposure monitoring. Table 1 summarizes how participation rates were calculated over the two steps within each of the three phases: the number of children listed in the "number eligible" column reflects both the process of subject selection within each phase and the number of participants electing to partake in the previous step(s).

Identification. We selected 2,303 telephone numbers from a commercially available list of residences (Genesys Systems, Inc., Fort Washington, PA) predicted to have age-eligible children based on birth records and other publicly available data. Because of concerns that the list might underrepresent families from lower socioeconomic strata (SES), we sampled telephone numbers from lower-SES census tracts proportional to their rate of occurrence in the 1990 Census. Of the initial 2,303 telephone numbers, 2,211 were determined to be residential, 2,057 were judged eligible for screening, and telephone screening was completed for 1,388 of these households.

Household Screening. Using a combination of selection criteria (i.e., residence located in target areas, age-eligible child present, reported use of pesticides, and use of a well as a water source in nonurban households) and probability sampling, we deemed 477 families eligible to participate; 294 completed the screening-phase survey of in-home pesticide storage and use (*21*). In the MNCPES survey design, a larger proportion of households with "more frequent pesticide use" and with more than one eligible child were selected for the household-screening phase, and families with private wells in nonurban areas were preferentially selected. On the basis of screening results, those classified as having a greater potential for exposure to target pesticides were selected at a higher rate for the intensive-monitoring phase of MNCPES. Identification of children considered likely to have higher exposures was based on scoring, which integrated information from a household roster, screening questionnaire, and the pesticide inventory (*20*). The scoring factors used to assess exposure, from highest to lowest weight, were use of a primary pesticide (the OPs chlorpyrifos, malathion, and diazinon) in the household in the previous year; any pesticide use inside or outside the home in the previous 6 months; adult occupational pesticide contact; primary pesticides present but not reported used in the previous year; and use of only nonprimary pesticides in the previous year.

Intensive monitoring. Of the 181 eligible families, 174 completed a baseline questionnaire (7 refused), and after selection appointments were made with 109 to begin intensive monitoring. Of these, 102 families

Table 1. Summary of participation rates for MNCPES.

Type of participation	Number eligible[a]	Number participating	Participation rate (%)	Cumulative rate (%)
Determined if household or business phone	2,303	2,211	96.0	96.0
Completed telephone screening	2,057	1,388	67.5	64.8
Agreed to in-home screening	477	348	73.0	47.3
Completed in-home screening	335	294[b]	87.8	41.5
Completed baseline questionnaire	181	173	95.6	39.7
Kept monitoring appointment	109	102[c]	93.6	37.1

[a]Number of subjects eligible for each phase based upon application of selection criteria described in the text as well as participant response in each previous phase. See Adgate et al. (*20*) for a full description of the selection process. [b]14 additional cases were completed after the deadline for selection of monitoring subjects. [c]Seventy-two urban and 30 nonurban households.

Table 2. Percent relative SD and bias of field quality control samples of 1-NAP, AM, MDA, and TCPy.

Analyte	*n*[a]	Percent relative SD	Characterized concentration (µg/L)[b]	Concentration in MNCPES control samples (µg/L)	Bias	Mean percent bias
1-NAP	7	29	4.2 ± 1.3	3.4	–0.8	–19
AM	8	7.0	9.0 ± 1.1	5.8	–3.2	–36
MDA	8	8.0	10.3 ± 1.5	15	4.7	46
TCPy	6	23	10.3 ± 2.1	11	0.7	6.8

[a]Number of field quality control samples with known reliable results. [b]No standard reference materials exist for these metabolites in urine. Concentration characterized over time from a pool of urine with all four metabolites at approximately 8 µg/L.

with children were enrolled and subsequently completed the intensive-monitoring phase of the study [the 7 remaining families were not monitored because of constraints on the number of monitoring appointments available per week (*15*)]. During this phase a combination of personal exposure measurements (i.e., air, duplicate diet, hand rinse), environmental measurements (i.e., residential indoor/outdoor air, drinking water, dust on residential surfaces, soil), and data on children's activity patterns were collected, in addition to blood and urine measurements (*22*). During the week-long monitoring period, individual children (sometimes with help from their parents) completed time-activity diaries, and a follow-up questionnaire was administered at the completion of monitoring.

Response rates and sampling weights. As shown in Table 1, subject response rates ranged from 68% to 96% within each of the three phases, with a compound response rate of 37% for the children who completed all three phases. On the basis of the sampling scheme, we developed weights for households and children because not all members of the population had an equal probability of selection. Sampling weights were computed based on probabilities of selection, were adjusted for nonresponse, and incorporated potential pesticide usage scoring factors and household pesticide inventory information (*20,21*). Therefore, weighted summary statistics can be used to provide unbiased estimates of the general population in the census tracts sampled.

Urine sampling and analysis. First-morning-void samples were collected on days 3, 5, and 7 of the week-long monitoring period to bracket duplicate diet sample collection. Samples were split into two subfractions, frozen, and shipped by overnight express mail to the National Center for Environmental Health at the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, where they were stored at –70°C pending chemical analysis.

We determined concentrations of 1-NAP and TCPy by capillary gas chromatography and tandem mass spectrometry using an isotope dilution technique with ^{13}C and/or ^{15}N-labeled internal standards (*23*). Similarly, we determined AM and MDA by liquid chromatography and tandem mass spectrometry using an isotope dilution technique with ^{13}C or deuterium-labeled internal standards (*24*). For most of the analytical runs, the analytical detection limits (DLs) were 1.3 µg/L for 1-NAP, 1.4 µg/L for TCPy, and 1.0 µg/L for AM and MDA. Lower DLs were obtained in some of the analytical runs because of improved instrumentation and increased operator experience: 1.0 µg/L for 1-NAP (n = 62), 0.53 µg/L for AM (n = 135), and 0.49 µg/L for MDA (n = 67).

We determined urinary creatinine concentrations using the established colorimetric enzymatic method available on Vitros CREA slides (Ortho Clinical Diagnostics, Raritan, NJ). We made reflectance measurements using a Kodak Ektochrome 250 analyzer (Eastman Kodak Co., Rochester, NY) at 3.85 and 5 min. The difference in the two reflectance measurements was directly proportional to the creatinine concentration of the urine.

We used field blanks and spiked samples to assess the quality of sample collection and analysis procedures. Field blanks consisted of deionized 18 mega-ohm water poured from a bulk container to a urine sample cup in the residences of randomly selected children. Eleven of the 13 field blanks contained concentrations of pesticide metabolites below the DLs. Two field blank samples had detectable levels of 1-NAP and TCPy, but levels were just above the DL.

Using methods developed by CDC investigators (*25*), samples from a characterized pool of urine containing 1-NAP, AM, MDA, and TCPy at approximately 8 µg/L were transported frozen to the field for use as field quality control samples. These samples were thawed and transferred to a sample cup in the residence of randomly selected children and then returned to the laboratory with participant samples. The CDC technicians conducting the analyses were blind to the sample types. Precision, characterized by calculating percent relative standard deviation of repeat measurements, and bias (deviation from the true value) of pre-characterized metabolite concentrations in the field quality control samples are summarized in Table 2. Overall precision and bias were within the range of MNCPES acceptance criteria, and results were adjusted for recovery by using isotope dilution mass spectrometry.

Statistical analysis. For summary statistical calculations, highly dilute urine samples (< 0.3 g/L creatinine; n = 4) were excluded, and samples with values that were less than the DL were assigned half the limit of detection. All urine metabolite concentrations were reported as mass per unit volume (µg/L). SAS was used for tabulations and for weighted analysis of variance between groups (PROC GLM) (*26*). SUDAAN (*27*) was used for all weighted summary statistical and sociodemographic comparisons, so that weighted means, for example, were obtained by multiplying a child's mean metabolite concentration by the weighting factor for that child and then dividing by the sum of the weights for all children. Reported p-values were not adjusted for multiple comparisons.

Results

At least one urine sample was collected for 90 of the 102 children who participated in the week-long intensive monitoring phase of MNCPES: 87 children provided three samples each, two children provided two, and one child provided one sample. As shown in Table 3, males and females were randomly distributed among the children providing urine samples. The weighted mean age of the children providing samples was 7.4 years [range 3–13, standard error (SE) = 0.40], and mean age did not vary significantly between urban (n = 62) and nonurban (n = 28) subjects nor between children who provided urine samples and those who did not. We obtained 266 urine samples, and 24 (9%) had missing data for creatinine or at least one metabolite, which was caused by analytical problems, such as matrix interference, or insufficient sample volume.

Table 4 summarizes the unweighted distribution of analytical results for 1-NAP, AM, MDA, TCPy, and creatinine in these urine samples. TCPy was present at detectable levels in nearly all samples, 1-NAP was present at detectable levels in more than half of the samples, MDA was present at detectable levels in more than a third of the samples, and AM was detected infrequently. The distribution of creatinine results is also presented because few baseline distributional data exist in the scientific literature on children for this commonly used adjustment for urinary dilution.

Intra- and interchild variability. The distribution of metabolite levels was right skewed for all metabolites, as shown by the intrachild arithmetic means, standard deviations, and CVs presented in Table 5 (AM excluded because of infrequent detection). For the 87 children with three samples, intrachild mean levels ranged from 1.4 µg/L

Table 3. Age distribution of children providing urine samples by sex and household location.

	Number of children in each age group											
Age (years)	3	4	5	6	7	8	9	10	11	12	13	Total
Sex												
Male	3	4	5	3	4	2	3	6	7	7	1	45
Female	5	3	5	11	5	4	6	1	3	2	0	45
Location												
Urban	4	4	8	10	6	5	6	6	7	6	0	62
Nonurban	4	3	2	4	3	1	3	1	3	3	1	28

for MDA to 9.2 µg/L for TCPy. Mean intrachild CVs were 74% (range 0–160) for 1-NAP, 59% (range 0–158) for MDA, and 55% (range 5.9–134) for TCPy. The average intrachild range for the 89 children with 2 or 3 samples was 5.0 µg/L (range 0–53) for 1-NAP, 2.6 µg/L (range 0-22) for MDA, and 9.0 µg/L (range 0.9-36) for TCPy. Intrachild ranges were at least as great as the overall pooled population means for 1-NAP, MDA, and TCPy. Figure 1 displays TCPy levels in all children providing samples (n = 90) sorted from lowest to highest mean levels, and demonstrates the wide variability in metabolite levels in this population of children. Because of the skewed distribution of all detectable metabolites, subsequent statistical comparisons were conducted using log-transformed values: a Box-Cox procedure confirmed that the log transformation was appropriate for both the intrachild and population distributions of these metabolites (*28*).

A tabulation of the number of samples with detectable concentrations is presented in Table 6 for the 87 children who provided 3 urine samples: The number of children with valid analytical results in all 3 samples ranges from 80 to 83. Concentrations of AM were lower than the DL in almost all children, but all children had at least one TCPy measurement greater than the DL. The percentage of children with detectable metabolite concentrations in all three samples ranged from 0% for AM to 83% for TCPy. Ninety-eight percent of the intrachild geometric mean (GM) TCPy levels were greater than the DL, and slightly less than half of the children had GM 1-NAP and MDA concentrations greater than the DL. Only three children had GM AM levels above the DL.

To examine the correlation between individual metabolites within urine samples, we calculated bivariate correlation coefficients for all possible pairs of metabolites with concentrations greater than the detection limit (AM was excluded because of the high proportion of nondetectable samples). Pearson's r values for log-transformed metabolite concentrations were low and not statistically significantly different from 0: –0.19 (p = 0.24) for 1-NAP versus TCPy; 0.14 (p = 0.39) for 1-NAP versus MDA; and 0.22 (p = 0.16) for MDA versus TCPy.

A weighted analysis of variance of log-transformed metabolite levels indicated that interchild variability was significantly greater than intrachild variability for 1-NAP and TCPy (1-NAP, F = 1.66, p = 0.0037; TCPy, F = 2.79, p < 0.0001). For MDA, interchild variability was greater than intrachild variability, but only marginally so, perhaps as a result of the relatively larger number of nondetects (F = 1.23, p = 0.13). Intrachild TCPy variability increased with increasing concentration, which was determined by examining the relations between the log intrachild variance and the log intrachild mean using weighted regression (Slope = 2.2, SE = 0.21, t = 10.3, p < 0.01). A similar pattern was observed for 1-NAP (Slope = 3.0, SE = 0.4, t = 7.5, p < 0.01) and MDA levels (Slope = 3.0, SE = 0.20, t = 15.3, p < 0.01) for metabolite concentrations greater than the DL.

Sociodemographic covariates and questionnaire responses. Mean log metabolite levels did not vary significantly when stratified by sex or by age (< 6 years versus > 6 years). Weighted mean TCPy concentrations, however, were significantly higher on a log scale in urban than in nonurban children (t = 2.1; p = 0.036), and mean log 1-NAP and MDA levels were both marginally higher in urban areas (Table 7). Statistical comparisons for AM were precluded because only 5 children had detectable levels in their urine, although four of the five were from urban areas.

The mean log metabolite levels did vary between racial and income subgroups, but no clear race- or income-related trends are evident for these metabolites. For example, log 1-NAP levels for children from households with incomes between $30,000–$50,000 were significantly higher than levels in children from households with incomes > $75,000 ($t$ = 2.28, p = 0.025), and white children had significantly higher levels than nonwhites (t = 2.7, p = 0.009). Log MDA levels, however, were significantly higher in nonwhite children when compared to white children (t = 2.15, p = 0.035), and children from households with incomes in the $30,000–$50,000 range had lower log MDA

Table 4. Unweighted summary statistics of the distribution of pesticide metabolites (µg/L) and creatinine (g/L) for all urine samples collected from 90 children.

Analyte	n[a]	Frequency of detection (%)[b]	Range	Mean[c]	SE	GM[c]	GM SE
1-NAP	258	45.3	< DL-55	3.0	0.34	1.4	1.1
AM	262	2.3	< DL-16	0.55	0.10	—[d]	—[d]
MDA	262	36.6	< DL-23	1.4	0.18	0.7	1.1
TCPy	261	93	< DL-45	9.2	0.48	6.4	1.1
Creatinine	263	100	0.32-3.4	1.1	0.031	1.0	1.0

GM, geometric mean.
[a]Number of successful chemical analyses, with a maximum potential n = 266. Variation in numbers reflects lack of a valid analytical result for a specific metabolite within samples as well as exclusion of 4 very dilute samples (creatinine values less than 0.3 g/L). [b]For metabolites with two DLs (see "Methods"), frequencies were calculated based on the DL associated with each sample. [c]Pesticide metabolite values < the DL assigned half the DL. [d]Insufficient percent detectable for calculation.

Table 5. Summary of intrachild metabolite levels (µg/L): mean, SD, and percent coefficient of variation (CV) for 1-NAP, MDA, and TCPy in children with valid analytical results in three urine samples (n = 80 for 1-NAP; n = 83 for MDA and TCPy).

	Intrachild mean[a] (µg/L)			Intrachild SD (µg/L)			Intrachild CV[b]		
Analyte	Mean	Min	Max	Mean	Min	Max	Mean (%)	Min (%)	Max (%)
1-NAP	2.9	<1.3	20	2.8	0	31	74	0	160
MDA	1.4	<1.0	8.4	1.4	0	12	59	0	158
TCPy	9.2	<1.4	37	4.8	0.5	19	56	5.9	134

Abbreviations: Min, minimum; Max, maximum.
[a]Values < the DL assigned one-half the DL associated with each sample to calculate mean and SD. [b]CV calculated by dividing intrachild SD by intrachild mean.

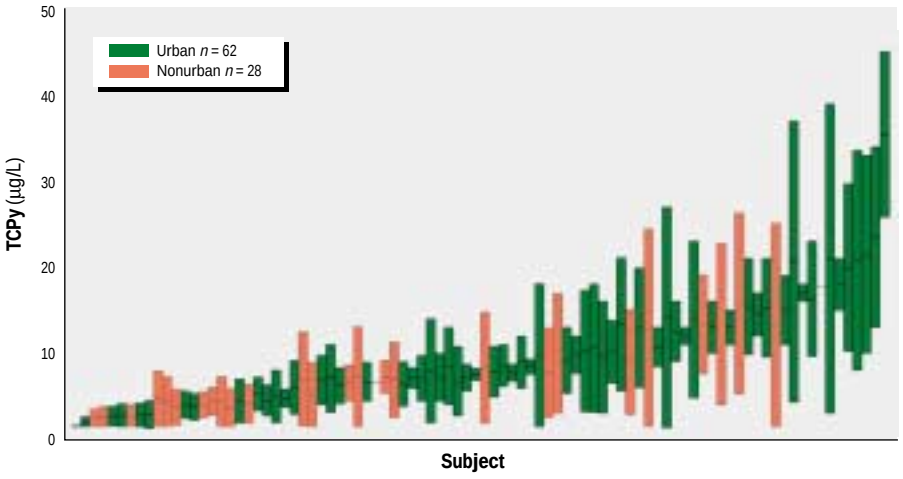

Figure 1. Mean and range of intrachild TCPy levels for urban and nonurban subjects sorted from lowest to highest concentration.

levels when compared to children from households with incomes < $30,000 ($t$ = 2.02, p = 0.047), $50,000–$75,000 (t = 1.84, p = 0.07), and > $75,000 ($t$ = 2.68, p = 0.009). Children from households with incomes between $50,000–$75,000 had significantly lower log TCPy levels compared to households with incomes < $30,000 ($t$ = 2.56, p = 0.012) and $30,000–$50,000 (t = 2.55, p = 0.013).

Levels in "high use" households. It is notable that the mean log intrachild metabolite concentrations did not vary significantly between children from "high pesticide use" households [i.e., one of the four target MNCEPS pesticides present and used inside the home in the previous 12 months (*20*)] and children from households where pesticides use was less frequently reported. However, the scoring process used to place homes in the "high use" category was nonspecific: reported use of any single pesticide resulted in inclusion in this group, so a reported use does not necessarily correlate with a specific metabolite.

To further explore the variability in exposure we examined questionnaire responses for the urban and non-urban groups, including the 10 nonurban children residing on working farms. These questionnaires included queries about recent pesticide exposures: *a*) the number of days a child was present while pesticides were being mixed or prepared [from the time–activity diary (TAD)]; *b*) the number of days a child was present while pesticides were being applied (TAD); *c*) whether chemicals for control of fleas, roaches, ants, or other insects were used inside the home in the previous month [from the follow-up questionnaire (FQ)]; and *d*) whether chemicals for control of fleas, roaches, ants, or other insects were used on the exterior or foundation of the home in the previous month (FQ).

Five children who provided three urine samples reported being present while pesticides were mixed for 1 day during the monitoring week. Three of the five children were from nonurban households, but none were from working farms. Weighted log TCPy levels were noticeably higher (11.5 µg/L, SE = 2.9) in these five children compared to levels in children who were not around pesticides being mixed (6.9 µg/L, SE = 0.58). The levels of 1-NAP, AM, and MDA did not vary systematically between children present during mixing and those who were not present during mixing.

Sixteen children reported being present during pesticide applications inside or outside the home during the monitoring week. Ten of the 16 children were from nonurban households, and three were from working farms. The number of the days they were present during pesticide applications varied from 1 to 3 days: There were 9 children (2 from working farms) present for 1 day, 4 children (0 from working farms) present for 2 days, and 3 children (1 from a working farm) present for 3 days. Log metabolite levels did not vary systematically between children present during applications and those who were not present during applications.

Forty-five of the 90 households reported pesticide use inside the home in the previous month, while 18 reported pesticide use outside the home in the previous month. The mean log metabolite levels did not vary systematically between children from households reporting indoor or outdoor use in the past month and those reporting no use.

Weighted population distribution. Table 8 summarizes the weighted distributions of 1-NAP, AM, MDA, and TCPy for all urine samples, as well as comparable data from studies performed in adults. As a consequence of the weighting, the MNCPES metabolite results estimate mean and variability in a population of more than 84,000 children in the census tracts sampled. As can be seen by comparing these results with those in Table 4, the weighted and unweighted distributions are similar in terms of percent greater than the detection limit, mean, and variability. The weighted 95% confidence interval (CI) of the means are skewed right and relatively small compared to the overall range of 1-NAP, MDA, and TCPy. Although the weighted estimates of central tendency vary within relatively tight bounds, the upper bound of the distributions is known with less certainty. Nonetheless, this is a reasonable estimate of the mean and shape of the distribution for these metabolites in the more than 84,000 children represented by this sample.

Table 6. Summary of urinary metabolite concentrations (µg/L) > the detection limit (DL) for children with valid analysis in three urine samples.

	Number of children					
Metabolite	With valid analysis in 3 urine samples[a]	With 0 samples > DL	With 1 sample > DL	With 2 samples > DL	With 3 samples > DL	With GM metabolite concentration > DL (unweighted %)[b]
1-NAP	80	14	37	15	14	38 (48%)
AM	83	78	4	1	0	3 (3.6%)
MDA	83	30	21	26	6	38 (46%)
TCPy	83	0	3	11	69	81 (98%)

[a]Eighty-seven children provided three urine samples. [b]GMs calculated with values < the DL assigned one-half the DL associated with each sample.

Table 7. Weighted log mean differences between pesticide metabolite levels (µg/L) for urban and nonurban children.

Analyte	Urban GM[a] (n)	Nonurban GM (n)	Mean difference[b]	SE of mean difference[b]	t-Statistic	p-Value
1-NAP	1.7 (58)	1.2 (22)	0.33	0.21	1.6	0.13
MDA	0.77 (58)	0.61 (25)	0.24	0.14	1.7	0.099
TCPy	7.2 (60)	4.7 (23)	0.43	0.20	2.1	0.036

[a]Group GM calculated from intrachild GM values presented in Table 6. n = the unweighted number of children in each location category. [b]Log scale.

Discussion

This is one of the first probability-based samples of children's urinary pesticide levels conducted in the United States. One to three first-morning-void urine samples were obtained over 5 days from 90 children between the ages of 3 and 13 and analyzed for metabolites of commonly used pesticides. To increase the likelihood of obtaining detectable concentrations of target pesticides in environmental and biological samples, MNCPES oversampled households reporting frequent pesticide use. Since this oversampling was intentional, and probabilities of selection and response were assessed systematically in each phase of the study, it is possible to extrapolate our results to obtain estimates of the population distribution of metabolites for similar-age children in the sampled census tracts.

Comparison of MNCPES with recent studies. The two largest studies that have reported measurements of urinary pesticide metabolites in children are not directly comparable to this study because they examined total alkyl- or dialkylphosphate metabolites, which are specific to OPs as a class but cannot be traced to specific parent compounds (*13*,*14*).

Most of the same urinary metabolites measured in this study were also measured as part of *a*) a probability-based cross-sectional sample of 1,000 adults conducted between 1988 and 1994 as part of the National Health and Nutrition Examination Survey III (NHANES III) (*7*) and *b*) a convenience sample of 80 adults from Maryland sampled up to six times over 1 year beginning in September 1995 as part of the National Human Exposure Assessment Survey (NHEXAS–MD) (*12*).

Table 8 summarizes and compares the distributions of metabolite levels in adults sampled once during NHANES III, adults sampled repeatedly as part of NHEXAS–MD, and the children sampled 1–3 times in MNCPES. In the NHANES III and NHEXAS–MD studies, 1-NAP was detected at a rate of 86%, with median concentration of 4.4 and 4.2 µg/L, respectively. The frequency of detection was 1.7 times greater than that observed in MNCPES, with median and 95th percentile values approximately four times the levels observed in MNCPES children. 1-NAP was the only metabolite for which the frequency of detection, median, and upper-bound urinary concentrations were higher for NHANES III and NHEXAS–MD adults than for MNCPES children. Levels of AM were not measured in the NHANES III population, and it was detectable in only 0.3% of adult samples obtained over 1 year in NHEXAS–MD, compared with 2.6% of children's samples measured during the summer months. Levels of MDA were detected in 36% of MNCPES children, but only in 6.6% of NHEXAS–MD adults. Median and 95th percentile MDA levels were approximately 4 times higher in MNCPES children than in NHEXAS–MD adults. Levels of TCPy were detectable in 97% of MNCPES children, 96% of NHEXAS–MD adults, and 82% of NHANES III adults, with median and 95th percentile values 2.4 and 1.4 times higher in MNCPES children than in NHANES III and NHEXAS–MD adults, respectively. For 1-NAP, MDA, and TCPy, the highest observed values in these studies were obtained in samples from adult subjects.

Study design limitations. MNCPES was a Phase III NHEXAS study, designed as a pilot to develop and evaluate methods and approaches for future large-scale national exposure studies (*16*). Several limitations of the design and issues of implementation provide important insights for investigators who will conduct future probability-based studies of children's exposures. In this study the use of a commercially available phone list produced a population with incomes approximately 40% greater than the median for the census tracts sampled, as well as a relatively low proportion of renters (*21*). Although inner-city census tracts were oversampled, and the population obtained reflected the racial and ethnic makeup of the census tracts as of 1990, this study obtained urine samples from relatively few nonwhites (n = 13) and Hispanics (n = 5). Additional studies need to be performed to obtain statistically robust and accurate characterization of these subpopulations to address issues of income- and race/ethnicity-related disparities in exposure and health effects. Lastly, this study measured metabolite levels in a relatively wide age range compared to other recent studies of children's pesticide exposures (*13,14*), which were confined to children 3–6 years of age; no studies published to date have reported metabolite levels in children < 3 years of age. Although metabolite levels did not vary systematically between children < 6 and older children in this study, obtaining data on exposure levels for children < 3 will require improved methods for both recruiting children and obtaining urine samples with sufficient volume for analysis of multiple metabolites.

Implications for exposure and risk assessment. Biomarkers of exposure can be used to characterize the relative magnitude of exposure within populations or population subgroups, as inputs to epidemiological investigations of health effects associated with chemical exposures, and as components of risk assessments and risk management decisions (*29*). In addition, they can be used to check the validity of models and pathways analyses. The results of this study supply a cross-sectional snapshot of exposure levels in a probability-based sample of children from urban and nonurban areas of Minnesota. These results can potentially provide a baseline for evaluating trends over time—e.g., TCPy levels to compare with levels observed in future studies examining the effect of the U.S. Environmental Protection Agency's recent regulatory decision limiting indoor and outdoor chlorpyrifos use (*18*).

Levels of 1-NAP were less frequently detectable and were lower in children compared to the NHANES III adult reference range, but MDA was detected more frequently and found at higher concentrations than in NHEXAS–MD adults. TCPy was detected more frequently in children than in the NHANES III adult reference range population, and levels were more than 2 times higher at both the median and 95th percentile compared to the adult reference range. Results from the adults in the NHEXAS–MD study indicated that TCPy levels vary seasonally, with the highest levels occurring in the summer, while 1-NAP levels did not vary seasonally. The MNCPES was conducted over a single summer, which is likely to represent the period of highest pesticide use in Minnesota.

In this study we measured urinary metabolite levels up to 3 times over a 5-day period. Individual metabolites did not vary in concert, and the data are consistent with a number of potentially overlapping explanations: relatively infrequent domestic applications, varying residue levels in the diet, and the rather short half-lives of these compounds in the body. Interchild variability for 1-NAP, TCPy, and, to a lesser extent, MDA was greater than intrachild variability, suggesting that relatively large sample sizes and

Table 8. Comparison of weighted 1-NAP, AM, MDA, and TCPy distributions from MNCPES children with distributions observed in adults sampled once in NHANES III (*7*) and repeatedly in NHEXAS–MD (*12*). All values in units of µg/L except as indicated.

Analyte/Study	n[a]	Percent > DL	Mean[b]	SE	Weighted 95% CI of the mean Lower	Upper	GM[b]	25th%	50th%	75th%	95th%	Max
1-NAP												
MNCPES	258	52[c]	3.9	0.7	2.5	5.3	1.6	< 1.3	1.0[c]	4.0	14	55
NHANES III	983	86	17	NR	NR	NR	NR	1.7	4.4	12	43	2,500
NHEXAS–MD	338	85	34	NR	NR	NR	4.2	1.6	4.2	9.3	52	2,500
AM												
MNCPES	262	2.6[c]	0.6	0.13	0.35	0.86	—[d]	< 1.0	< 1.0	<1.0	<1.0	16
NHEXAS–MD	348	0.3	—[d]	NR	NR	NR	—[d]	< 1.0	< 1.0	<1.0	<1.0	1.5
MDA												
MNCPES	262	36[c]	1.7	0.3	1.1	2.3	0.74	< 1.0	< 1.0	1.1	8.7	23
NHEXAS–MD	347	6.6	—[d]	NR	NR	NR	—[d]	< 1.0	< 1.0	<1.0	2.0	51
TCPy												
MNCPES	261	97[c]	9.6	0.9	7.8	11	7.0	4.4	7.2	11	26	45
NHANES III	993	82	4.5	NR	NR	NR	NR	1.3	3.0	5.9	13	77
NHEXAS–MD	346	96	6.8	NR	NR	NR	5.1	3.1	5.3	9.4	17	51

NR, not reported.
[a]Unweighted number of urine samples. [b]Pesticide metabolite values < the DL assigned one-half the DL. [c]Weighted frequencies calculated and weighted percentiles interpolated based on the DL associated with each sample. [d]Insufficient percent detectable to calculate.

multistage, probability-based designs are needed to accurately characterize the extremes of metabolite distributions for the general population of children.

Considerable intrachild variability was observed as well, with mean CVs ranging from 56% to 74% and maxima ranging from 134% to 160% for 1-NAP, MDA, and TCPy. The average intrachild range of values was 1.3 times the weighted population mean for 1-NAP, 1.5 times the weighted population mean for MDA, and approximately equal to the weighted population mean for TCPy. As with the more robust repeated measure design (up to 6 measurements over a year) used in the NHEXAS–MD study, this suggests that a single measurement of these metabolites is insufficient to characterize the relative magnitude of long-term exposure to parent compounds (*12*).

Both time–activity diaries (filled out daily by children, sometimes with parental assistance) and follow-up questionnaires (administered by study staff at the end of the week of monitoring) were employed in this study to obtain information on potential exposures. It is notable that the only descriptive data that appear to explain variability in individual exposure levels were recorded by children on their TADs, and that the two follow-up questionnaires did not explain any of the variability in urinary metabolite levels in study children.

Existing pharmacokinetic models for these compounds need to be developed and/or updated for the parent compounds measured in this study, and none of the existing models have been validated for children. Although we present the distribution of creatinine concentrations for these children, reported metabolite concentrations were not adjusted for creatinine excretion. At present it is not clear that creatinine adjustment will necessarily improve the correlation between exposure and dose (*30*), because this assumption has not been systematically validated in children (*31*). Although adjustment with creatinine appears to introduce additional variability in metabolite levels, it does not appear to affect the trends we have observed. For example, when metabolite levels in urban and nonurban children are compared using creatinine-adjusted data, urban children who provided 3 urine samples still had significantly higher TCPy concentrations than nonurban children (t = 2.39, p = 0.019), and there were still no significant differences between 1-NAP (t = 1.68, p = 0.097) and MDA (t = 1.42, p = 0.16) levels in urban and nonurban children. Derivation of exposure and dose of parent compounds must therefore account for intrachild variability in elimination, the relative contribution of pesticide metabolites in the environment to concentrations measured in the urine after adsorption, distribution, and metabolism, and variability potentially introduced by creatinine adjustment if that is used to compensate for urinary dilution.

Conclusions

We have presented individual and population-level urinary metabolite data indicating widespread exposure of children to the parent compounds of carbaryl or naphthalene; chlorpyrifos, chlorpyrifos-methyl, or triclopyr; and malathion. In a population representing more than 84,000 children, intrachild GM urinary metabolite levels measured over 5 days were greater than the detection limit 98%, 48%, and 46% of the time for TCPy, 1-NAP, and MDA, respectively, whereas metabolites of atrazine were detected 4% of the time. It is notable that metabolite levels were not significantly higher for subjects from households reporting higher than average pesticide use, likely due to a lack of recent applications. Metabolite levels varied widely between and within children, and metabolite concentrations were not correlated within a child's own urine samples. Although there was no systematic relationship between metabolite levels and most sociodemographic factors, TCPy levels were higher for urban compared to nonurban children for unknown reasons. Ninety-fifth percentile MDA and median and 95th percentile TCPy urinary concentrations were up to two times higher in children than levels observed in two comparable studies of adults. Overall, children's metabolite levels to these OP pesticides were greater than in recent population-based studies of adults in the United States.

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