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 3.2 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)] 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 intra-individual variability was similar for adults and children. Key words: children's health, exposure assessment, Minnesota Children's Pesticide Exposure Study (MCPEX), 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 (MCPEX), 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 MCPEX 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 MCPEX 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.
This study measured four specific urinary metabolites: 1-naphthol (1-NAP), atrazine metabolite (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 found in mothballs and found in cigarette smoke and petroleum products, and carbaryl is a carbamate insecticide used on turf and gardens. Chlorpyrifos is a carbamate insecticide used on fruits and vegetables as well as residential uses. Malathion is a carbamic acid, and carbaryl is a compound used in both home and gardening applications. In the United States, and malathion is an insecticide used on foods 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 in (15,20). To maintain a probability-based structure within a complex study design, we used a random-digit selection process (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).

| Type of participation | Number eligiblea | Number participating | Participation rate (%) | Cumulative rate (%) |
|-----------------------|------------------|----------------------|------------------------|---------------------|
| Determined if household or business phone | 2,231 | 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 | 294b | 87.8 | 41.5 |
| Completed baseline questionnaire | 161 | 173 | 95.6 | 39.7 |
| Kept monitoring appointment | 109 | 102c | 93.6 | 37.1 |

| Analyte | n² | Percent relative SD | Characterized concentration (μg/L)² | 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 ± 1.1                          | 11                                            | 0.7  | 6.8             |

*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. Additional cases were completed after the deadline for selection of monitoring subjects. Seventy-two urban and 30 nonurban households.

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

### Summary of participation rates for MNCPES.

- **Completed telephone screening**: 2,057 (67.5%), 1,388 (64.8%)
- **Agreed to in-home screening**: 477 (73.0%), 348 (47.3%)
- **Completed in-home screening**: 335 (87.8%), 294 (41.5%)
- **Completed baseline questionnaire**: 161 (95.6%), 173 (39.7%)
- **Kept monitoring appointment**: 109 (93.6%), 102 (37.1%)

### 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...
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 13C and/or 15N-labeled internal standards (23). Similarly, we determined AM and MDA by liquid chromatography and tandem mass spectrometry using an isotope dilution technique with 13C 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 Ektachrome 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 among 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. For the 87 children with three samples, intrachild mean levels ranged from 1.4 µg/L
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 ranged 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 the corresponding intrachild variability for all three metabolites (1-NAP and TCPy: F = 1.66, p = 0.037; TCPy: F = 2.75, 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 = 0.15). 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 = 2.0, SE = 0.4, t = 2.28, p = 0.036), and mean log 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.

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 = 2.5, 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 levels.
levels when compared to children from households with incomes < $30,000 (t = 2.02, p = 0.047), $30,000–$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 M N C P E S 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 non-specific: 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 children in which 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 M N C P E S 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.

**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, M N C P E S 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 M N C P E S 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 N ational H eatlth and N utrition Examination Survey III (N H A N E S 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 N ational H uman E xposure A ssessment S urvey (N H E X A S – M D) (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 NHEXAS 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 NHEXAS 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 2.1 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
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, M DA, 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 M DA, 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).

Individual exposure levels were recorded by weekly (administered by study staff at the end of the week of monitoring) were employed in this study (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 descriptively measured data that appear to explain variability in individual exposure levels were recorded on 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 affect to 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 M DA (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 napthalene; chlorpyrifos, chlorpyrifos-methyl, or tri cyclyp; 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 M DA, respectively, whereas metabolites of atrazine were detected 45% 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 M DA 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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