National Study of Exposure to Pesticides among Professional Applicators: An Investigation Based on Urinary Biomarkers

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Epidemiologic studies of pesticides have been subject to important biases arising from exposure misclassification. Although turf applicators are exposed to a variety of pesticides, these exposures have not been well characterized. This paper describes a repeated measures study of 135 TruGreen applicators over three spraying seasons via the collection of 1028 urine samples. These applicators were employed in six cities across the United States. Twenty-four-hour estimates (μg) were calculated for the parent compounds 2,4-D, MCPA, mecoprop, dicamba, and imidacloprid and for the insecticide metabolites MPA and 6-CNA. Descriptive statistics were used to characterize the urinary levels of these pesticides, whereas mixed models were applied to describe the variance apportionment with respect to city, season, individual, and day of sampling. The contributions to the overall variance explained by each of these factors varied considerably by the type of pesticide. The implications for characterizing exposures in these workers within the context of a cohort study are discussed.

KEYWORDS: Occupational exposure; biological monitoring; urine; pesticides; exposure assessment methods; mixed models

INTRODUCTION

Pesticides are ubiquitous, and the sources of exposure are varied and include residues from food and water, indoor and outdoor air, household dust, applications to lawns and gardens, and some occupations. Although experimental and epidemiological investigations have provided important information about the human health effects associated with chronic exposure to pesticides, much remains unknown. Epidemiologic studies conducted to assess the chronic effects of pesticides have been fraught with difficulties in characterizing exposures (1, 2). For example, case-control studies that have relied on self-reported data are particularly vulnerable to recall bias. Surrogate measures of pesticide exposure such as living on a farm or in rural area, or the use of pesticide application records, have also been shown to result in substantial exposure misclassification (3, 4). Such misclassification would likely serve to attenuate associations between pesticide exposure and adverse health outcomes; however, the effects of measurement error on risk estimates can be unpredictable. Improvements in the ability to characterize pesticide exposures are needed to accurately describe health risks, particularly those associated with long-term exposures.

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Biological markers, such as urinary metabolites, hold promise as they can provide a direct and objective means to assess not only exposure but, perhaps more importantly, absorbed dose. Studies that incorporate biomarker analyses with prospective follow-up will provide the most useful estimates of risks (or lack of risk) of cancer or neurobehavioral effects associated with pesticide exposures. Such studies are best carried out within occupational groups that have both anticipated high and variable levels of exposure.

Professional turf applicators represent such a workforce as they are exposed to levels of herbicides and insecticides as well as some fungicides that are recognized to be several orders of magnitude higher than those in the general population. Previous epidemiologic investigations provide some support for increased cancer risks among these workers. In a retrospective cohort mortality study of 32600 lawn care workers (5), there was no statistically significant difference in overall mortality when compared to the general population (Standardized Mortality Ratio (SMR) = 0.76, 95% confidence interval (CI) = 0.55, 1.01). However, there was suggestive evidence of an increased risk of non-Hodgkin’s lymphoma (NHL) among male lawn applicators, particularly among those employed for at least 3 years (SMR = 7.11, CI = 1.78, 28.42). Mortality from bladder cancer was significantly increased, but two of the three observed deaths had no direct occupational contact with pesticides (i.e., office workers). No other cause of death was significantly elevated among lawn applicators as a group or among those employed...
for three or more years. Whereas the findings from this study are somewhat limited by small sample size, the increased risks of NHL are consistent with associations found among farmers exposed to pesticides (6). However, it is important to note that the characterization of exposure in the study by Zahm et al. (5) was subject to important sources of measurement error. Namely, like most other longitudinal studies of pesticide exposures, individual measures could not be made within this study due to lack of spraying application data, and exposure had to be inferred from the number of days worked and pesticides purchased at each branch location. The resulting inability to classify exposure differences across and within occupational groups may preclude the detection of any real associations.

The elevated lymphoma finding and the likelihood for exposure misclassification underscore the need for more detailed exposure data, particularly given that the phenoxy herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) was widely used by these workers and continues to be used internationally. In addition to being linked to non-Hodgkin’s lymphoma elsewhere (7–9), 2,4-D also has been associated with other forms of cancer that include multiple myeloma (10) and prostate cancer (11). Turf applicators also are exposed to a variety of other herbicides including dicamba (benzoic acid herbicide, 3,6-dichloro-2-methoxybenzoic acid), and other phenoxy herbicides such as 4-chloro-2-methylphenoxyacetic acid (MCPA) and mecoprop (2,4-chloro-2-methylphenoxy)propionic acid (MCPP). There are even fewer published studies on exposures and associated health effects of these herbicides.

Turf applicators also are exposed to insecticides that have the potential to affect human health. In North America, bifenthrin and imidacloprid are two insecticides that are used extensively; these two insecticides are fairly new, having replaced the commonly used insecticide chlorpyrifos in 2000. To date, there have been no published reports of health effects of bifenthrin, which is a type I pyrethroid. However, studies of workers spraying other types of pyrethroids have reported increased symptoms ranging from abnormal facial sensations to dizziness, headache, nausea, and appetite loss (12, 13). Imidacloprid is considered slightly mutagenic with a low risk of carcinogenic effects. However, there is scant information available on its human health effects with the exception of some reports of poisoning (14, 15). To date, occupational exposure to these insecticides has been described in only a small sample (n = 8) of Australian pest control sprayers (bifenthrin) (16) and among 10 greenhouse workers (imidacloprid) (17). The assessment of exposure to imidacloprid was made using data collected from air sampling; therefore, no direct measure of absorbed dose has been published to date.

The objective of this study is to describe the body burden associated with exposure to selected herbicides and insecticides in professional turf applicators. An important strength of this study is the availability of repeated urine metabolite measures for each applicator. This allows us to examine the extent to which geographic region, spraying season, or day of work may contribute to the variability in urinary pesticide levels. The repeated measures design of this study also provided us the opportunity to characterize exposures with respect to between- and within-worker variability. The corresponding implications of our findings, as they pertain to characterizing acute and long-term health risks among turf applicators, are discussed.

MATERIALS AND METHODS

Study Population. The study population was drawn from employees of TruGreen, which was first established in Ohio in 1969. This company, headquartered in Memphis, TN, has become the world’s largest lawn and landscape company and serves more than an estimated 3.4 million residential and commercial customers across the United States with lawn care, tree and shrub care, and landscaping services (http://www.trugreen.com). Currently, TruGreen has approximately 200 branches and 40 franchises located throughout the United States and Canada.

The design of the study consisted of two phases, which are described in much greater detail by Harris and Wells (2). The first phase in 2003 was a pilot study of 22 tree and shrub and turf applicators in the Richmond, VA, branch of the company. These workers signed informed consent forms and provided 12- and 24-h urine samples during the summer and fall of 2003. The Richmond workers were given $200 following submission of the equivalent of 10 12-h and 14 24-h urine samples. The extensive urine sampling was conducted in this group to allow for future analysis of toxicokinetic and toxicodynamic profiles of the various pesticides under study.

In 2004, the national study was initiated and we selected five additional branch locations across the United States. The branches were chosen to reflect national and, therefore, geographic differences in pesticide programs (i.e., different pesticides, concentrations, or formulations used) and the timing of the applications (due to climatic conditions). Only those branches that sprayed both herbicides and insecticides and could provide at least 20 employees were considered. Logistically, site selection also was made so that the spraying season was as long as possible (April—November 2004) such that the requisite fieldwork could be carried out with a small number of staff. In consultation with the company’s corporate managers, five branches were selected for inclusion in the study. These branches were Sterling, VA; Plano, TX; Puyallup, WA; Plainfield IL; and Salt Lake City, UT. Subsequently, meetings were held with individual branch and operations managers, and potential participants were given both verbal and written information about the background, aims, and procedures of the study. Only those workers who were 18 years of age or older and had potential contact with pesticides as part of their job were eligible to participate. As such, office workers were not eligible. A total of 113 employees from the 5 locations provided informed consent. Subjects were compensated $20 per sampling week (i.e., $10/sample; $60 for three seasons) and were allowed to keep the soft-sided cooler pack used to keep the urine samples cool during sample collection.

In these five branches, urine samples were collected during three spraying seasons in 2004: the spring (April and May) and fall (October and November) herbicide sprays, as well as the summertime (June and July) insecticide spray. Participants were provided a 3 L urine collection container (Simport Plastics Limited), a soft-sided cooler bag, and two frozen ice packs. Total urine output was collected for two consecutive 24-h periods during the herbicide sprays and for four consecutive 12-h periods (insecticide), following a minimum of 3 consecutive workdays.

In all branches samples were processed by two individuals, the Principal Investigator and the Project Manager. Upon collection, total sample volume was recorded and specific gravity was measured using a Leica AR200 digital hand-held refractometer (Leica catalog no. 13950000). Each sample was divided into three 40-mL aliquots (in 50-mL Corning graduated plastic tubes, Corning catalog no. 430828) and two 100-mL aliquots (in 125-mL Nalgene rectangular HDPE bottles, Nalgene catalog no. 200700004), packaged in accordance with Federal dangerous goods shipment guidelines, and overnight shipped in insulated diagnostic shippers (Safe-Pak item STP-320) on ice packs to the Environmental Health Laboratory at Virginia Commonwealth University. Upon arrival, samples were immediately frozen and were stored at –20 °C until analysis.

To evaluate completeness of urine collection, one 40-mL aliquot from all 24-h urine samples was analyzed for creatinine content by Scientific Testing Laboratories (Richmond, VA). Urine samples were analyzed for MCPA, mecoprop, bifenthrin metabolite (MPA), imidacloprid and its metabolite 6-chloronicotinic acid (6-CNA), dicamba, and 2,4-D using a method developed at the Division of Consolidated Laboratory Services, Virginia Department of General Services (DGS).

Pesticide levels were estimated using a solid-phase extraction (SPE) followed by positive/negative ion electrospray ionization HPLC-MS/MS. Portions of frozen samples were thawed at room temperature prior to SPE on C-18 (Varian, Harbor City, CA) cartridges previously conditioned with MeOH and water. A 1-mL aliquot of the urine sample was diluted with 2 mL of 0.1% formic acid prior to loading and was eluted with 1 mL of methanol. The collected extract was then evaluated by gradient separation on an Agilent 1100 HPLC using a Phenomenex Synergi RP-18 column (Torrence, CA) followed by MS/MS analysis on a Bruker Esquire 3000-plus quadrupole ion trap mass spectrometer (Billerica, MA). To account for matrix effects,
calibration curves were generated using spiked urine samples, and extraction efficiencies of between 75 and 85% were obtained for all analytes in this study with $r^2 \geq 0.995$. Analytes were detected on-column in the low picogram range. The method was tested for robustness through blind studies, and all analytes were quantified within one standard deviation of the true value. This method would easily lend to automation and high-throughput analysis and is capable of quantifying levels of all analytes to 1 part per billion (ppb) (18).

Questionnaires were provided to the workers to collect information relevant to pesticide use. In terms of content, the questionnaire captured details on those factors that could increase or decrease pesticide exposure, as well as recognized correlates of exposure such as acres and volume sprayed. Other information collected with the self-reported questionnaire included demographic data, smoking status, number of years employed, protective equipment worn, frequency of uniform laundering, and personal history. From emerging data on the volume of daily pesticide use and total land area sprayed. Future analyses of how these factors influence absorbed dose are planned.

Statistical Analyses. Tabulations were performed to describe the number of workers and urine samples by location and season. The frequency of samples that were below the level of detection (LOD) and the distribution of exposures above the level of detection were obtained for both the 12- and 24-h urine samples.

Pesticide levels in parts per billion obtained from the 12- and 24-h samples were then used to estimate the total pesticide mass (in μg) over a 24-h period. Samples that were below the laboratory-reported LOD were categorized into three groups: unexposed, trace exposure, and above trace exposure (e.g., laboratory quantified results even though they were below the LOD). We did not modify pesticide levels for samples when the estimated concentration was 0 ppb as not all of the pesticides were used by the sprayers. For this reason, we decided it was more appropriate to treat these workers as unexposed rather than the common approach of assigning half the LOD.

For those with trace amounts of exposure, we statistically simulated values for these trace levels based on a frequency distribution that best represented values of the pesticide (in ppb) from trace levels to the level of detection. A series of distributions were then fit to the observed data to generate estimates of the values between trace and LOD. These distributions included normal, Weibull, exponential, gamma, and uniform. Goodness of fit tests compared the fit of the model to the observed data, and the distribution that best fit the data was identified. The Andersen–Darling test was used as the preferred goodness-of-fit test measure as it generally gives more weight to the tails of the distribution than the other tests used and makes use of the specific distribution when estimating extreme values unlike other goodness of fits such as the Kolmogorov–Smirnov (K-S) test, which is distribution free (19). The use of other goodness of fit tests generally identified the same distribution, suggesting robustness of results.

With the exception of MPA, the exposure levels between trace and LOD for each of the pesticides were best represented by the normal distribution. For MPA, the Weibull distribution best represented values between trace amount and the LOD. For each pesticide, the total estimate in micrograms was estimated by multiplying by the total volume of urine in milliliters and dividing by 1000. For 12-h urine samples, the total estimate in micrograms over the 24-h interval was derived from the sum of the microgram estimates over the two consecutive 12-h intervals. Pesticide concentrations were standardized by creatinine measures for the descriptive and statistical analysis.

Box plots were then constructed to describe the distributional properties of 24-h exposures (in μg) for each pesticide across the different spraying seasons. To minimize the undue influence of extreme outliers on the graphical representation of the box plots, the lower and upper tails of the plot extended to the 90th and 10th percentiles of the distribution, respectively, rather than the standard approach of using the maximum and minimum values. Box plots were not prepared for imidacloprid, its metabolite 6-CNA, or MPA, given that so few samples yielded values above the LOD.

The intraclass correlation coefficient (ICC) was then calculated to assess the variability in pesticide metabolite levels within and between workers. The ICC is a measure of reliability that can be used to describe the similarity in exposures within groups. The ICC has been derived within the framework of analysis of variance (ANOVA) models, and within this framework it can be expressed mathematically as

$$\text{ICC} = \frac{\alpha^2}{\alpha^2 + \sigma^2}$$

where $\alpha^2$ represents the between group variance and $\sigma^2$ represents the total variance. A single urine sample would be adequate to characterize exposure for measures that are highly reliable within individuals over time but show significant variation between individuals (i.e., ICC close to 1). On the other hand, measures with a low ICC highlight the need to collect repeated measurement for each individual. ICCs were calculated using PROC Mixed in SAS, and the model assumed that sprayers represented random effects (20). We applied the same methodology to characterize within- and between-city variability for the same pesticides. Dot plots were created to depict differences in the ranges of ICC values for each of the pesticides across the spraying seasons.

PROC Mixed also was used to fit a hierarchical model to apportion sources of variance for the pesticide measures. Specifically, we estimated the percent of variability accounted for by city, individual, season of spraying, and day of sampling. In doing so, we applied models that took into account the nested design of the study. In particular, the sprayers who volunteered for this study were nested within cities, and urine samples obtained from these applicators also were nested within individual and spray season. All factors were treated as random effects.

RESULTS

A total of 135 sprayers provided urine samples, and nearly all ($n = 132$) were male. Participants ranged from 19 to 59 years of age, with a mean age of 32.6. In total, there were 1028 urine samples, and of these, approximately half were collected during the summer spraying season. The number of worker days that urine samples were available in each branch and spray season is presented in Table 1. For the summer and fall spray seasons, the number of worker days in Richmond accounted for approximately half of all worker days (301/580). For Plainfield, no sampling was done in the fall spray season, whereas no sampling was done in Richmond in the spring spray season.

In total, there were 515 12-h urine samples collected during the summer spray season of the study (Table 2). There were a nearly equal number of 24-h samples collected ($n = 513$). For each pesticide or metabolite, the LOD (ppb), LOQ (ppb), and number of samples with trace amounts detected are reported in Table 2. The metabolite 6-CNA had concentrations that exceeded the LOD in only 5 of 513 24-h samples and 4 of 515 12-h samples (Table 2). Similarly, for MPA, 90.7 and 84.3% of the 24- and 12-h urine samples, respectively, were below the LOD. In contrast, levels of 2,4-D, MCPA, and MCPP were above detection in at least half of the sprayers. The median and range of values above the LOD observed in both the 12- and 24-h samples, and as well as those adjusted for urinary creatinine concentration, are provided in Table 2.

Box plots created for the different pesticides revealed considerable differences in the 24-h concentrations of pesticides across spray seasons (Figures 1–4). 2,4-D and dicamba urinary levels were highest in the spring spraying season, whereas variations in metabolite levels across season were less marked for MCPA. This reflects the recommended use patterns for MCPA. For MCP, concentrations were highest in the 2004 summer (median = 21.9 μg). It is important to note that in the interpretation of these graphs, 2003 sampling results are based exclusively on samples taken from sprayers who were employed in Richmond, VA. These applicators were not using 2,4-D at their branch and were sampled in only the summer and fall of 2003.

To explore variations in pesticide levels within sprayers, we calculated both the median exposure value based on all samples and the median intraindividual range for each spray season among those sprayers who provided more than one sample (Table 3). A median intraindividual range that exceeds the median...
exposure across all samples provides an indication that the variability of exposure within individuals is an important source of the total variability. Ignoring 6-CNA and MPA, which were virtually all null values, this pattern held true for the remaining five pesticides for the fall spray season and for four of the five pesticides during the summer spray season. In contrast, during the spring spray season, the median exposure was modestly higher than the median intrasubject range for the remaining five pesticides.

Intraclass correlations provide an even better indicator of variations in pesticide levels within and between sprayers. The ICCs calculated for each pesticide on the basis of all collected samples ranged from 0.0 to 0.31. However, ICCs calculated within each season were generally much higher (Figure 5). Large ICCs (> 0.7) were observed for dicamba, MCPP, and 2,4-D in the summer and fall spray seasons. The ICCs for between- and within-branch variability were relatively small with the exception of herbicide 2,4-D in the fall spray season (Figure 6). Greater variability in 2,4-D exposures was expected between branches in the fall spray seasons because the Richmond branch was not using 2,4-D in the fall of 2003. Use of 2,4-D was reinstated in 2004 during the national study, and all participating branches applied it in the fall. Overall, this suggests that branch was not an important determinant of urinary pesticide levels.

The apportionment of variances by city, subject, cycle (or season), and sample are presented in Table 4. For 2,4-D, seasonal variability contributed approximately 84% of the total variability. Results for 2,4-D did not change substantially when Richmond data for 2003 (2,4-D not used at that time) were excluded from the analysis. For MCPA and dicamba, seasonal variability accounted for the largest percentage of overall variability (approximately 55%). For all pesticides, the proportion of the overall variance explained across samples (nested within subject, city, and season) was larger than the between-subject variability.

**DISCUSSION**

In this study, we provide detailed data about pesticide exposure patterns in a workforce known to experience exposure at much higher levels than background and for which epidemiologic studies are suggestive of increased risk of cancer. Our analyses demonstrate that for most pesticides, worker exposure cannot be determined using pesticide levels estimated from a single urine sample.
Moreover, they highlight important differences in urinary metabolite levels that exist across spraying seasons.

In comparison to previously published studies in professional turf applicators in Canada (3–5), we found on average lower amounts of herbicides, specifically 2,4-D, MCPP, MCPA, and dicamba, in the urine samples. This is likely not reflective of differences in geography, spraying practices, or hygiene, but rather reflects our current study design. In past studies, samples were collected when applicators were at the height of the spraying season and would spend 7–10 h a day spraying only herbicides. Because we measured urinary metabolites over an entire work season, and during times when only insecticides were being sprayed, we expect greater variation in the levels. This variation is reflected in the significant number of samples with nondetectable or trace values. No studies on the exposures of professional applicators have been published for the two insecticides studied, but in ≥88% of the samples, no residues were detected.

It is important to have a better understanding of this variation in urinary metabolites for both the design and interpretation of epidemiologic studies and for human health risk assessment. Over all spraying seasons, proportion of variances for 2,4-D indicates that only a small proportion was explained by “between-subject” variability. However, our stratified analyses revealed the opposite to be true when analyses were conducted separately by season. Hines and colleagues (21) also examined between- and within-worker variability among corn and soybean field applicators for alachlor, atrazine, metolachlor, and 2,4-D 2-ethylhexyl ester (2,4-D EH) as measured using air, patch, and handwash samples. They found that 89% of the 2,4-D variability was explained by within-worker variability. Within-worker variability also was larger for the other applicators. Therefore, both dermal and urine measures of pesticides would seem to indicate that within-subject variability is greater than between-subject variability. MacIntosh found among nonoccupationally exposed individuals that single measures of 3,5,6-trichloro-2-pyridinol (TCPY) and 1-naphthol (1NAP) was not sufficient to characterize the relative magnitude of a person’s typical acute or chronic exposure (22). The result is also consistent with other studies that have reported greater within-worker variability (than between-worker variability) for occupations where the work is outdoors, the process is intermittent, or workers are highly mobile (23, 24). The finding of greater within- to between-worker variability implies that other factors that vary on a day-to-day basis influence total variability.

Figure 1. Distributional properties of 2,4-D mass (in μg) in 24-h urine samples, by sampling cycle. The dashes indicate the median, and the values are provided above. The whiskers extend to the 10th and 90th percentiles, whereas the box extends from the 25th to the 75th percentile. 2,4-D was not used in the summer or fall of 2003. Use was reinstated in the spring of 2004.

Figure 2. Distributional properties of dicamba (in μg) in 24-h urine samples, by sampling cycle. Dashes indicate the median, and the values are provided above. The whiskers extend to the 10th and 90th percentiles, whereas the box extends from the 25th to the 75th percentile. 2,4-D was not used in the summer or fall of 2003. Use was reinstated in the spring of 2004.
more than individual work practices that may be stable over time. In fact, larger within- than between-subject variability for pesticides has also been observed in children (25).

Large variability observed with season (cycle) for 2,4-D suggests that exposure measurement error could be reduced by performing sampling across seasons for this herbicide. Large variability

Table 3. Median Intraindividual Ranges in 24-h Pesticide Levelsa (Micrograms), by Pesticide and Spraying Season

<table>
<thead>
<tr>
<th>spraying season</th>
<th>median exposure</th>
<th>median intraindividual range</th>
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<tbody>
<tr>
<td></td>
<td>samples</td>
<td>median</td>
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<tr>
<td>pesticide</td>
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<td></td>
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<tr>
<td>6-CNA</td>
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<tr>
<td>imidacloprid</td>
<td>193</td>
<td>0</td>
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<tr>
<td>dicamba</td>
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<td>MPA</td>
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<td>3.09</td>
</tr>
<tr>
<td>MCPP</td>
<td>193</td>
<td>14.29</td>
</tr>
</tbody>
</table>

a Restricted to workers who had at least two 24-h urine measures in each season.
between days in the same season for imidacloprid and MCPP and, to a lesser extent, MCPA and dicamba suggests the need to take multiple samples from an individual within a season. A strength of the present study was that these analyses were conducted on pesticide residue levels (i.e., mass) estimated from 12- or 24-h samples, and thus the results on which the statistical analyses were conducted were not corrected for creatinine concentrations. However, to allow for comparison with previously published exposure studies, the descriptive statistics for the adjusted values are reported. Furthermore, it should be recognized that in large cohorts it is very difficult and expensive to collect 12- or 24-h samples. Most often, spot samples will be collected or morning urine. Because of difference in hydration over a day and especially over a work season, spot samples will likely show even greater variation in pesticide concentrations. Thus, given the variation we have observed in subjects over time, for large cohorts, it is likely

Figure 5. Intraclass correlation coefficients for between- and within-subject variability, by spray season.

Figure 6. Intraclass correlation coefficients for between- and within-branch, by spray season.
that single spot urine samples taken within a given spray season are inadequate to characterize workers’ long-term exposures. Additional factors may explain within-individual variability (i.e., season, pesticides being applied, amount applied, time spent applying, PPE use, etc.). In addition, with the exception of the 2,4-D data collected in the fall, assigning exposure according to geographic area, even when accounting for season, would also likely lead to a large amount of exposure error/misclassification (Figure 6).

Additional analyses of these data are planned to evaluate factors such as the amount of pesticide or area sprayed, formulation, protective clothing worn, glove use, smoking behavior, etc., associated with the urinary levels and to develop models to predict absorbed dose. In large-scale studies it may be most cost-effective to predict absorbed dose on the basis of a combination of questionnaire information and employer records. However, the models must be developed using data obtained from studies that appropriately sample the cohort and capture the true seasonal variation.

There are some limitations in our study, most notably, the subjects were volunteers who came forward from within each of the six branches and were not selected at random. Participation of eligible subjects was close to 100% at five of the six branches (2) however, we should be somewhat cautious in generalizing these results to all applicators employed by TruGreen. Furthermore, the locations were not selected randomly but rather to represent the geographic and climatic variation and different use patterns in the United States.

In conclusion, in a large study using a repeated-measures design, we observed significant variability in the urinary excretion of pesticides in a cohort of professional turf applicators, and this variation was dependent on the pesticide measured. Furthermore, we observed many nondetectable or trace background levels, indicating a large range in exposures, which could be expected in a workplace over time. Recommendations regarding the best methods to validate or measure exposures in these cohorts for large-scale epidemiologic studies will need to be individually tailored.

**ABBREVIATIONS USED**

1NAP, 1 naphthol; 2,4-D, 2,4-dichlorophenoxyacetic acid; 6-CNA, 6-chloronicotinic acid; CI, 95% confidence interval; ICC, intraclass correlation coefficient; LOQ, level of detection; LOD, level of quantification; MCPA, 4-chloro-2-methylphenoxyacetic acid; MCPP, mecoprop, 2,4-chloro-2-methylphenoxypropionic acid; NHL, non-Hodgkin’s lymphoma; SE, standard error; SMR, standardized mortality ratio; TCPY, 3,5,6-trichloro-2-pyridinol.

**SAFETY**

The study, associated questionnaires, and consent forms were reviewed and approved by the Virginia Commonwealth University Institutional Review Board in 2003.

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**LITERATURE CITED**


**Table 4. Variance Apportionment in 24-h Urinary Pesticide Mass Levels (in Micrograms)**

<table>
<thead>
<tr>
<th>pesticide</th>
<th>variance component</th>
<th>variance</th>
<th>SE</th>
<th>% of total variance</th>
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<tr>
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<td>1111.1</td>
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</tr>
<tr>
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<sup>a</sup> Between-city variance.  
<sup>b</sup> Between-worker variance after accounting for nesting within city.  
<sup>c</sup> Between-cycle (spraying season) variance after accounting for nesting within worker and city.  
<sup>d</sup> Between-sample variance after accounting for nesting within season, worker, and city.


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