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Original article

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Biomonitoring of herbicides in Ontario farm applicators

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Objectives Biomonitoring of pesticide residues in urine offers the advantages of integrating exposure due to all routes of entry and accounting for individual differences in several factors such as pharmacokinetics. The study was designed to measure the body burden of 2,4-dichlorophenoxyacetic acid (2,4-D) and 4-chloro-2-methylphenoxyacetic acid (MCPA) in farm applicators and to measure compliance with label recommendations regarding the use of personal protective gear and the impact of such use on exposure.

Methods Farmers (N=126) from Ontario, Canada, collected a preexposure spot sample of urine and then two consecutive 24-hour urine samples immediately following the farmers' first use of these herbicides during 1996. Details on the pesticides used and handling practices were collected by questionnaire.

Results For the farmers who reported using 2,4-D, the mean urinary concentration was 27.6 µg/l in the day-1 sample and 40.8 µg/l in the day-2 sample. The comparable figures for MCPA were 44.4 µg/l and 58.0 µg/l, respectively. Adherence to all of the recommended personal protective gear was rare (3%). Wearing goggles or a face shield during mixing and loading was associated with the lowest exposures.

Conclusions The urinary concentrations of 2,4-D and MCPA of these farm applicators were of the same order of magnitude as those published in the past decade, but lower than earlier studies, indicating that improvements in education, equipment, and labeling have likely had an impact on the degree of exposure in occupational settings.

Key terms 2,4-dichlorophenoxyacetic acid; 4-chloro-2-methylphenoxyacetic acid; agriculture; exposure assessment; occupational exposure; pesticide; urine.

Numerous epidemiologic studies have been recently published that have examined the potential carcinogenic (1–5) and reproductive (6–10) toxicity of pesticides. What has continued to be a major limitation of these studies has been the exposure assessment. Some studies have inferred pesticide exposure based on job title [eg, greenhouse worker (11), farmer (12, 13), or job-exposure matrix (14, 15)]. Other studies have used routinely collected databases on pesticide sales or use for a geographic area (7, 16). Still others have used questionnaires to characterize exposure that has occurred either in an occupational (9, 5) or residential (17, 18) setting. In these latter studies, investigators rarely had a study population size with sufficient homogeneity of exposure patterns to identify specific pesticide products or mixtures. An additional concern is the potential for information bias in recalling the names of the pesticide products used in the past, particularly when no records are available to verify this information. Such information bias could introduce further uncertainty to an already imprecise estimate. It could be argued that farmers may be more likely to recall the names of pesticides used in the past few years, given that it is a critical item in their management of various pests, they often use the same product from year to year on specific crop types, there is considerable cost associated with using the pesticides, and they need to keep records of their expenses for tax purposes. Repeat interviews of pesticide

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applicators in the Agricultural Health Study in the United States have resulted in good agreement (70% to 90%) for questions administered 1 year apart (19).

An important downside of most exposure assessment methods is that they only represent indications of opportunities for exposure to pesticides and they do not provide information on whether measurable body burdens occur in the applicator. In contrast, biomonitoring studies measure pesticides or metabolites in body fluids or tissues as biomarkers of internal dose. They take into account the uptake of pesticide from the external environment and its dispersion to various target organs (20). For example, numerous biomonitoring studies have been published focusing on phenoxyacetic acid herbicides among farmers (21–24), forestry (25, 26) or roadside sprayers (27), lawn care applicators (28), and employees of herbicide production plants (29, 30). Each of these occupations differs in spray equipment used, application conditions, and frequency of exposure. Farmers generally use a tractor-drawn multi-nozzle boom sprayer and use herbicides intensively over a relatively short period of time. Forestry sprayers, roadside sprayers, and lawn care workers frequently use a backpack sprayer with a handheld nozzle and are exposed over a longer duration during the year. Employees in herbicide production plants are exposed throughout the workweek and probably under more-controlled conditions. Applicators using handheld backpack sprayers frequently have higher concentrations of herbicides in urine than those operating boom sprayers (27).

Use of personal protective equipment (PPE) is one of the methods by which pesticide handlers can reduce their personal exposure to pesticides. Although information on PPE use is often collected in epidemiologic studies, few studies have measured PPE use and estimated its effect on internal doses under usual operating conditions (in contrast to exposure studies required for regulatory dossiers). Hence we designed a biomonitoring study of farm applicators who reported using the phenoxy herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) or 4-chloro-2-methylphenoxyacetic acid (MCPA). We focused on these herbicides because of their widespread use in agricultural and residential settings to control broadleaf weeds, their relative nonpersistence in the environment, and their known biological half-time in humans, which varies from 12 to 72 hours (23, 31–33).

Our objectives included (i) the measurement of urinary concentrations around the first application of the season to link reported use with dose measures, (ii) the determination of PPE use in association with the handling of the herbicide to assess compliance with label recommendations, and (iii) the statistical analysis of the relationships between reported PPE use and urinary concentrations to assess the impact of PPE use on body burden.

Study population and methods

Study population

An earlier retrospective cohort study of approximately 2000 families was the source of the population for the current study (34–36). In the study designed to examine the reproductive risks of exposure to pesticides, Ontario family-run farms likely to be using phenoxyacetic acid herbicides or crop insecticides or fungicides were identified from the Canadian census of agriculture. Follow-up telephone interviews identified the farms with reproductive-age couples. The subsequent Pesticide Exposure Assessment Study was a field study designed to measure the extent of and major determinants of herbicide exposure experienced by farm families during normal pesticide application procedures. Farmers (N=773) that reported current use of 2,4-D or MCPA in the cohort study were re-contacted in early 1996 to see if they were planning on using either of these herbicides in the 1996 growing season. In addition to this requirement, their house had to be on the farm property and they had to be living with their spouse. A consent form was signed by all of the applicators who agreed to participate, in keeping with a protocol reviewed by the research ethics board of the University of Guelph. In the province of Ontario, pesticide applicators must successfully complete a certification course and written examination to apply for a license to use restricted pesticides. The license is renewable every 5 years (37).

Data collection

The field study period was May through July, 1996, when the farm applicator reported using MCPA or 2,4-D for the first time during the growing season. All of the farmers participating in the study were asked to collect a preexposure spot sample of urine prior to handling and then two consecutive 24-hour urine samples after they started handling either herbicide. The applicator recorded the time that the first urine sample was collected and continued collection for 24 hours. Urine-sample containers, a cooler bag, and ice packs were provided and collected by field staff soon after the 48-hour collection period. Samples were delivered to the laboratory and placed in a walk-in refrigerator (4°C) until the analysis.

The urine samples were analyzed for residues of phenoxyacetic acid herbicide using gas chromatography–mass spectrometry (GC-MS) (38). Field spikes and laboratory quality control samples were included in the study. The detection limit for the acidic herbicides in urine was 1 µg/l (1 ppb).

The applicator also completed a questionnaire within 24 hours of first handling these herbicides. The questionnaire collected information such as date of application, pest control product number, chemical and trade names, number of acres sprayed, volume of concentrate used, number of tank loadings, size and age of tank, types of application equipment used, and PPE used during each phase of handling (mixing, loading, application, and clean up). Pesticide-active ingredients were identified by linking the pest control product number or product name to a database of pesticide products federally registered for use in Canada.

As the data on urinary pesticide residue were not normally distributed, the nonparametric Kruskal-Wallis test was used to compare distributions across groups defined by the use of PPE. In this analysis, the 24-hour urine concentrations were not adjusted for concentrations in the preexposure spot sample, if found.

Table 1. Characteristics of the farm applicators in the Ontario pesticide exposure assessment study by reported use of 2,4-dichlorophenoxyacetic acid (2,4-D) or 4-chloro-2-methylphenoxy acetic acid (MCPA) on the field application day or the previous day.[a, b, c]

Characteristic	Reported use of 2,4-D (N=43) (%)	Reported use of MCPA (N=90) (%)
Employed off-farm	14.0	8.9
Education		
Less than high school diploma	18.6	20.0
High school diploma	51.2	48.9
College or university graduate	30.2	31.1
Per capita income (CAD)		
≤5000	35.7	37.3
5001–10 000	35.7	30.1
>10 000	28.6	32.5
Age		
<40 years	34.9	30.0
40–45 years	34.9	45.6
>45 years	30.2	24.4
Smoking status		
Current	7.0	8.9
Former	32.6	22.2
Never	60.5	68.9
Time since pesticide safety certification		
0 years	21.4	28.6
1 years	19.0	30.9
2 years	47.6	27.4
>2 years	11.9	13.1

[a] Mean time used to apply herbicide: 4.6 (SD 2.83) for 2,4-D; 4.9 (SD 3.24) for MCPA.
[b] Mean number of acres (1 acre = 0.40 ha) sprayed: 43.5 (SD 29.72) for 2,4-D; 50.4 (SD 44.46) for MCPA.
[c] Mean number of tanks filled: 2.2 (SD 1.75) for 2,4-D; 2.2 (SD 1.38) for MCPA.

Results

After a telephone screening interview in early 1996 to identify potentially eligible applicators and a visit to the farm to recruit the farmer, 215 farmers signed informed consent forms to participate in the study. However, approximately 40% subsequently dropped out, many citing the weather (an abnormally cool and wet spring), which hampered the use of the herbicides 2,4-D and MCPA as the reason for doing so. A total of 126 male applicators participated. Generally there were few differences in the demographic or application-specific characteristics between the applicators applying 2,4-D and those applying MCPA (table 1). The men ranged in age from 28 to 61 (median 41) years, with a median gross per capita family income of approximately CAD 8000 and a median number of acres farmed of 400 (161.87 ha). Eleven applicators reported applying both herbicides on the first day of the field study and hence were included in both groups.

Tables 2 and 3 set out the range of concentrations of 2,4-D and MCPA measured in urine for the applicators who reported using each herbicide. Generally, the MCPA urinary levels were found to be higher than those of 2,4-D. As expected, the applicators who reported using 2,4-D or MCPA had significantly higher concentrations of the reported herbicide in their urine. However, approximately 13% to 20% of those who reported using MCPA or 2,4-D had no detectable residues of the herbicide in their urine. Some applicators (14–40%) had measurable concentrations in their supposedly preexposure spot sample, indicating either that this sample was not collected

Table 2. Urinary concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) in farm applicators by reported use of 2,4-D.[a] [LOD = limit of detection (1 µg/l), ND = not detectable]

	Reported use [b] (N=43)							No reported use (N=83)						
	Mean	SD	Geometric mean	SD	Median	Range	Percentage >LOD	Mean	SD	Geometric mean	SD	Median	Range	Percentage >LOD
Preexposure sample	2.4	6.91	1.0	2.77	ND	ND-45.0	39.5	1.5	4.05	0.7	2.37	ND	ND-33.0	19.3
Day 1, 24-hour sample	27.6	72.48	5.4	5.84	6.0	ND-410.0	79.1	2.6	7.99	0.9	2.93	ND	ND-66.0	31.3
Day 2, 24-hour sample	40.8	91.14	9.9	6.07	12.0	ND-514.0	83.7	2.7	7.12	1.0	3.17	ND	ND-58.0	31.3

[a] The mean and other univariate statistics were calculated by estimating trace samples as one-half the limit of detection (LOD).
[b] Applicator indicated in questionnaires that he had applied the herbicide on either the day before or the first field day.

Table 3. Urinary concentrations of 4-chloro-2-methylphenoxyacetic acid (MCPA) in farm applicators by reported use of MCPA.[a] (ND = not detectable, LOD = limit of detection (1 µg/l)]

	Reported use [b] (N=90)							No reported use (N=36)						
	Mean	SD	Geometric mean	SD	Median	Range	Percentage >LOD	Mean	SD	Geometric mean	SD	Median	Range	Percentage >LOD
Preexposure sample	2.4	5.46	0.9	3.05	ND	ND-35.0	27.8	1.5	3.54	0.7	2.42	ND	ND-20.0	13.9
Day 1, 24-hour sample	44.4	109.84	8.6	6.92	11.0	ND-790.0	84.4	1.3	2.31	0.7	2.28	ND	ND-12.0	19.4
Day 2, 24-hour sample	58.0	127.82	12.9	6.69	16.0	ND-800.0	87.8	0.9	1.42	0.6	1.94	ND	ND-8.0	13.9

[a] The mean and other univariate statistics were calculated by estimating trace samples as one-half the limit of detection (LOD).
[b] Applicators indicated in questionnaires that they had applied the herbicide on either the day before or the first field day.

Table 4. Urinary concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) for farm applicators who reported using 2,4-D on the first or previous day of the pesticide exposure study by use of recommended personal protective clothing or equipment (PPE).

PPE	Day 1 urine sample				Day 2 urine sample			
	Mean	SD	Median	Maximum value	Mean	SD	Median	Maximum value
Goggles or face shield during mixing & loading (N=17)	7.0	10.96	4.0	46.0	13.4	19.06	4.0 [a]	64.0
Coveralls or chemically resistant apron during mixing & loading (N=5)	14.6	19.28	6.0	46.0	17.0	27.03	4.0	64.0
Chemical resistant gloves during mixing & loading, application and clean-up (N=16)	32.1	100.96	5.5	410.0	53.5	127.95	14.0	514.0
None of the above (N=15)	39.3	64.68	10.0	200.0	48.8	78.33	19.0	300.0

[a] Kruskal-Wallis chi-square P=0.03 in comparison with men not wearing eye protection.

Table 5. Urinary concentrations of 4-chloro-2-methylphenoxyacetic acid (MCPA) in applicators who reported using MCPA on the first or previous day of the pesticide exposure study by recommended personal protective clothing or equipment (PPE).

PPE	Day 1 urine sample				Day 2 urine sample			
	Mean	SD	Median	Maximum value	Mean	SD	Median	Maximum value
Goggles or face shield during mixing & loading (N=31)	35.0	74.97	11.0	330.0	51.8	113.77	16.0	610.0
Coveralls or chemically resistant apron during mixing & loading (N=19)	43.8	94.64	9.0	330.0	55.4	142.69	11.0	610.0
Chemical resistant gloves during mixing & loading, application and clean-up (N=25)	37.9	67.58	12.0	280.0	53.8	121.66	16.0	610.0
All of above (N=4)	77.9	134.97	15.5	280.0	174.2	292.57	43.0	610.0
None of the above (N=41)	75.3	159.11	11.0	790.0	75.4	159.11	20.0	800.0

correctly or that some contact with the herbicide had occurred prior to the start of the field study period.

All of the farmers in the study used a boom-type sprayer to apply the herbicides on their fields.

Only 4 of the 126 applicators used all of the recommended PPE as recommended on the label for these herbicides (chemical-resistant gloves, protective eye wear or face shield, coveralls or chemical resistant apron, socks and shoes, and long-sleeved shirt and long pants). We did not have information on whether the person wore long pants, long-sleeved shirts, or socks and shoes. Rubber boots were worn by about 30% of the men during activities involving the handling of pesticides. The least frequently reported protective clothing were coveralls or a chemical-resistant apron, used during mixing and loading by only 18% of the men. Chemical-resistant gloves were the most frequently reported protective clothing, worn by almost 80% of the men during mixing and loading, 46% during application (if not using a tractor with a cab), and 52% during the clean-up of equipment. However, only 31% wore these gloves during all of these activities.

Among the persons who reported using 2,4-D on the day prior to or the first day of the field study, those who wore goggles or a face shield during mixing or loading had significantly lower concentrations of 2,4-D in their urine, particularly in the 24-hour collection of the second day (table 4). Interestingly, similar trends were less strong for MCPA, and, rather anomalously, the use of all PPE was associated with higher mean levels, albeit based on very small numbers (N=4) (table 5).

Discussion

Although the farm applicators in this study had the opportunity to be exposed to herbicides, as indicated by their response to the questions on agricultural herbicide use, approximately 20% of the 2,4-D users and 15% of the MCPA users had no detectable residues of these herbicides in their urine. Even among those with detectable residues (>1 µg/l), there was a wide range of concentrations (maximum values of 514 and 800 µg/l for 2,4-D and MCPA, respectively). These ranges are similar to what has been published in the literature during the past decade but considerably lower than in earlier studies (figure 1), indicating that significant improvements have been made in reducing occupational exposure to these herbicides over time. Factors that probably contributed to this reduction in exposure include improvements in spray equipment, pesticide handling education programs, pesticide-user certification programs, improved labeling on pesticide products, and the production and use of PPE. Nevertheless, the inconsistency in the associations between urinary herbicide concentrations and PPE use between 2,4-D (more with less body coverage) and MCPA (no association) is puzzling. The former would support the role of PPE in reducing farmer exposures, as would the significant reduction in detectable concentrations in urine for the use of goggles or a face shield in the case of 2,4-D. However, with this exception, we did not find that using the recommended PPE (at least those for which we had information) resulted in significant reductions in body burden as measured by detectable concentrations in the urine. In contrast, Libich et al (47) reported significant reduction in herbicide exposure after worker education and improved use of PPE among right-of-way applicators. In our study, the lack of statistical significance was at least partially due to the small number of men with higher body burdens, and this small number reduced the power of the study.

Low frequencies of compliance with PPE requirements have been reported previously in a study of 220 Wisconsin dairy farmers (48). Although certified to apply restricted-use pesticides, the vast majority of the farmers did not fully comply with PPE-use requirements. Fifty-seven of the male applicators used no required PPE when handling dicamba (a herbicide frequently used with the phenoxyacetic acid herbicides), although chemically resistant gloves, goggles, and boots are required (only 9% used all the required PPE).

Chemically resistant gloves can protect workers from hand exposure to pesticides, providing the gloves are not punctured, torn, or otherwise damaged (49). One study has shown that, at least for some pesticides, if gloves are used for more than 1 day, they will probably be contaminated after the first day (50). Opportunities for exposure will occur depending on how the gloves are put on or removed and whether they are stored separately from uncontaminated clothing between wearing.

An important source of contamination for applicators is likely to be a direct splash or contact with pesticide containers; however, pesticide residues could also be transferred from contaminated surfaces of spray equipment over extended time periods. These surfaces could be contaminated by transfer from other surfaces (eg, contaminated gloves) or by the deposition of aerosolized pesticide. Depending on the persistence of the pesticide on contaminated surfaces, tractor operators may have prolonged exposure to pesticides from pressured contact of the palm with the steering wheel, door handles, shift and control gear knobs, and arm rests. After a boom sprayer application, the surfaces with the highest contamination, as measured by a tracer substance in one study, were the instrument panel, steering wheel, outside cab window, arm rest, and outside hand rail and door handle (51). Residues may also be transported to living areas and result in postapplication exposure (52).

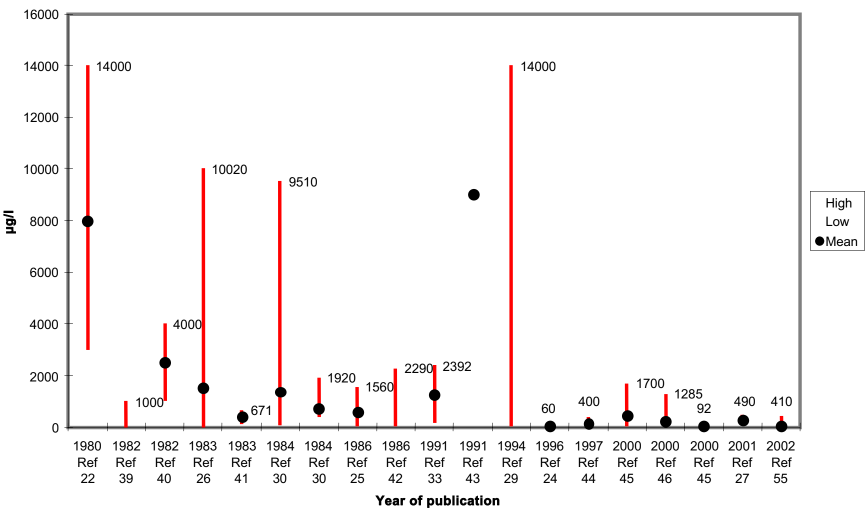

Figure 1. Urinary 2,4-dichlorophenoxyacetic acid (2,4-D) concentrations in occupationally exposed applicators as reported in the literature.

Various environmental (eg, temperature, humidity, wind speed, and direction), product (eg, use of surfactants or adjuvants with the pesticide product), and personal factors (area and condition of body part exposed) may have an impact on the amount of pesticide-active ingredient that is absorbed into the body. The type of pesticide (lipid and water solubility), nature of the formulation, physical form, concentration, and use of any carriers, solvents or adjuvants will all affect absorption. The percutaneous absorption of 2,4-D, when applied to the ventral forearm, has been estimated to be 5.8%, in contrast to the insecticide carbaryl, for which 73.9% of the percutaneous dose is absorbed (53). Some factors have been identified as influencing absorption after a pesticide comes into contact with the skin (ie, applied amount, frequency of application, surface area, skin site of application, occlusion, skin condition, and skin contact time) (53). Higher total absorption has been found for head, neck, and genital regions (54). Occlusion (eg, covering of the applied dose with clothing) increases the temperature and hydration of the skin, which under optimal conditions can create a pesticide reservoir and greatly increase absorption (53). Damaged skin can increase the absorbed dose of 2,4-D by sixfold (54). Furthermore, the rate of elimination of phenoxy herbicides is dose-dependent, with lower half-times for higher doses (21). Loss of volume for some urine specimens, on which we did not have complete information, may also have accounted for some of the variability in the measured concentrations. Hence numerous and varied sources of variation may account for the wide ranges of internal dose measured in this study.

Biomonitoring studies such as ours are useful for confirming exposure or estimated internal dose through all routes, for different occupations and under different use conditions. They can also track changes in pesticide exposure over time. However, they provide little information regarding the potential health risk unless people are followed prospectively to determine health outcomes. Given the relative rarity of most health conditions of interest (eg, cancer) and the expense and response burden (and accompanying low participation rates) associated with biomonitoring studies, the use for all members of a cohort is likely not feasible.

Biomonitoring studies are also useful for validating sets of questions for estimating pesticide exposure. Considerable research is still required to identify the major determinants of pesticide exposure among applicators so that valid and reliable questionnaires can be developed. Collecting information on PPE alone is not sufficient for individual exposure estimation. As our research suggests (55), exposure estimation is a complex process involving the documentation of a variety of interacting factors (ie, a person's pharmacokinetics, area of body exposed, handling practices, and the frequency and duration of pesticide use; the physicochemical properties of the pesticide product or mixture; and weather conditions).

In the meantime, a focus on new practices, including perhaps the washing of equipment surfaces or methods to reduce the likelihood of equipment contamination, and new innovations, such as less cumbersome PPE, are needed to achieve maximum feasible reductions in applicator pesticide exposure.

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