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Urinary biomarker, dermal, and air measurement results for 2,4-D and chlorpyrifos farm applicators in the Agricultural Health Study

KENT W. THOMAS[a], MUSTAFA DOSEMECI[b], JANE A. HOPPIN[c], LINDA S. SHELDON[a], CARRY W. CROGHAN[a], SYDNEY M. GORDON[d], MARTIN L. JONES[e,2], STEPHEN J. REYNOLDS[e], JAMES H. RAYMER[f], GERALD G. AKLAND[f], CHARLES F. LYNCH[g], CHARLES E. KNOTT[h], DALE P. SANDLER[c], AARON E. BLAIR[b] AND MICHAEL C. ALAVANJA[b]

[a]*National Exposure Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, USA*
[b]*Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH/DHHS, Rockville, Maryland, USA*
[c]*Epidemiology Branch, National Institute of Environmental Health Sciences, NIH/DHHS, Research Triangle Park, North Carolina, USA*
[d]*Battelle Memorial Institute, Columbus, Ohio, USA*
[e]*Department of Occupational and Environmental Health, University of Iowa, Iowa City, Iowa, USA*
[f]*RTI International, Research Triangle Park, North Carolina, USA*
[g]*Department of Epidemiology, University of Iowa, Iowa City, Iowa, USA*
[h]*Battelle Centers for Public Health Research and Evaluation, Durham, North Carolina, USA*

A subset of private pesticide applicators in the Agricultural Health Study (AHS) epidemiological cohort was monitored around the time of their agricultural use of 2,4-dichlorophenoxyacetic acid (2,4-D) and *O*,*O*-diethyl-*O*-3,5,6-trichloro-2-pyridyl phosphorothioate (chlorpyrifos) to assess exposure levels and potential determinants of exposure. Measurements included pre- and post-application urine samples, and patch, hand wipe, and personal air samples. Boom spray or hand spray application methods were used by applicators for 2,4-D products. Chlorpyrifos products were applied using spray applications and in-furrow application of granular products. Geometric mean (GM) values for 69 2,4-D applicators were 7.8 and 25 μg/l in pre- and post-application urine, respectively ($P<0.05$ for difference); 0.39 mg for estimated hand loading; 2.9 mg for estimated body loading; and 0.37 μg/m^3 for concentration in personal air. Significant correlations were found between all media for 2,4-D. GM values for 17 chlorpyrifos applicators were 11 μg/l in both pre- and post-application urine for the 3,5,6-trichloro-2-pyridinol metabolite, 0.28 mg for body loading, and 0.49 μg/m^3 for air concentration. Only 53% of the chlorpyrifos applicators had measurable hand loading results; their median hand loading being 0.02 mg. Factors associated with differences in 2,4-D measurements included application method and glove use, and, for hand spray applicators, use of adjuvants, equipment repair, duration of use, and contact with treated vegetation. Spray applications of liquid chlorpyrifos products were associated with higher measurements than in-furrow granular product applications. This study provides information on exposures and possible exposure determinants for several application methods commonly used by farmers in the cohort and will provide information to assess and refine exposure classification in the AHS. Results may also be of use in pesticide safety education for reducing exposures to pesticide applicators.
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Introduction

Despite low mortality and cancer incidence rates overall, farmers may experience excess risk of specific cancers and other adverse health outcomes (Cordes and Rea, 1991; Blair and Zahm, 1995; Zahm et al., 1997; Fleming et al., 1999; Alavanja et al., 2004). Farmers may be exposed to pesticides as well as a variety of other potentially hazardous substances including solvents, fuels, oils, vehicle exhaust, dust, mycotoxins, and agriculture-specific microbes. Epidemiological studies of agricultural pesticide applicators and agricultural workers have often been limited by inadequate or retrospective exposure information, leading to potential exposure misclassification (Dich et al., 1997; Sathiakumar and Delzell, 1997; Ritter et al., 2006). Exposure to pesticides used in agriculture may depend on many factors, including the amount and duration of chemical use, pesticide formulations, the physical and chemical properties of active ingredients (a.i.), mixing/loading and application methods, and the use of personal protective equipment. Although recognized as important, few epidemiological studies have been able to

1. Address all correspondence to: Kent Thomas, U.S. EPA, MD-E205-04, Research Triangle Park, NC 27711, USA. Tel.: +919 541 7939. Fax: +919 541 0905.
E-mail thomas.kent@epa.gov
[2]Current Address: Martin Jones, Veterans Affairs Medical Center, Iowa City, IA, USA and Reynolds, Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO, USA
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incorporate these factors into their exposure estimates due to the complexities involved in obtaining complete and reliable information over time as well as in estimating their impact. Thus, epidemiological analyses often assume that exposure intensities resulting from agricultural uses are the same for all pesticides and all applicators.

Obtaining measurement information to directly determine exposures for all individuals would be ideal but is costly and not feasible for large longitudinal cohorts. Thus, studies often rely on questionnaires to provide information on pesticide use as a surrogate for exposure. Relatively few studies of farmers have included direct measurements to assess questionnaire-based exposure classification systems used in epidemiological investigations (Arbuckle et al., 2002; Baldi et al., 2006). Measurement data are needed to improve the understanding of relationships between patterns of chemical use and exposures and to evaluate questionnaire-based surrogates of exposure. To address this need in the Agricultural Health Study (AHS), a longitudinal cohort study of licensed pesticide applicators and their spouses, the AHS/Pesticide Exposure Study (AHS/PES) was designed to measure exposures from the agricultural use of two pesticides, 2,4-dichlorophenoxyacetic acid (2,4-D) and *O*,*O*-diethyl-*O*-3,5,6-trichloro-2-pyridyl phosphorothioate (chlorpyrifos), with a high prevalence of use among farmers in the AHS cohort. AHS/PES objectives included measurement of farm applicator exposure through multiple routes to the selected pesticides, assessment of factors potentially associated with exposures, and the evaluation of an algorithm that uses questionnaire information to estimate relative exposure intensities. In this paper, we describe the study design; recruitment and monitoring outcomes; results for urinary biomarker, dermal, and air measurements; and assessment of some factors potentially related to exposures.

Methods

AHS Study Background

The AHS is a prospective epidemiological study to evaluate the cancer and non-cancer risks for pesticide applicators and spouses and to study the relationships between agricultural exposures and disease. The cohort includes 52,395 licensed private pesticide applicators, 4916 commercial applicators, and 32,347 spouses in the states of North Carolina (NC) and Iowa (IA) in the United States. Participants enrolled in the study from 1993 to 1997 at pesticide certification classes and provided pesticide use information through self-administered questionnaires (Alavanja et al., 1996). Questionnaires were used at enrollment to collect information on the duration (years) and frequency (days per year) of use for 50 herbicides, insecticides, fungicides, and fumigants (Alavanja et al., 1996). Information was collected to characterize personal mixing/loading, application methods, use of protective equipment, equipment repair, and personal hygiene. Additional and updated information was obtained through computer-assisted telephone interviews (CATI) conducted from 1999 to 2003. Questionnaire information is used in the AHS to stratify individual applicators with regard to their total lifetime days of use and intensity-adjusted lifetime days of use for specific pesticides using an intensity algorithm published earlier (Dosemeci et al., 2002). Relative weighting levels for algorithm factors were based on literature reviews and information from the Pesticide Handlers Exposure Database (PHED, 1995) regarding relative exposure levels for different handling and use factors.

Selection of AHS/PES Target Chemicals

Two pesticides, 2,4-D and chlorpyrifos, were selected for monitoring based on the widespread use in the cohort and available analytical methods. A wide range of application methods and practices have been reported by the full study cohort; eight categories common for crop and non-crop applications are shown in Table 1. Pharmacokinetic data were also available to guide the timing of biomarker sample collections. The urinary elimination half-life of 2,4-D ranged from 10 to 28 h after oral dose (Sauerhoff et al., 1977) and 18–68 h after dermal dose for 2,4-D and 18–87 h for 2,4-D dimethyl amine (Harris and Solomon, 1992). The half-life for 3,5,6-trichloro-2-pyridinol (TCP) in blood after oral chlorpyrifos dose ranged from 21 to 34 h (Nolan et al., 1984), and urinary half-lives, following dermal dose, of 30 h for dialkylphosphate metabolites (Griffin et al., 1999) and 41 h for TCP (Meuling et al., 2005) were reported.

AHS/PES Participant Selection and Recruitment

Private pesticide applicators enrolled in the AHS cohort and who had completed the AHS CATI before the selection for

Table 1. Common product application scenarios and reported frequencies for applicators in the AHS at enrollment during 1993–1997.

Application scenario	Percent[a,b]
(A) Broadcast spray application, enclosed cab, rubber gloves	26.6
(B) Broadcast spray application, no enclosed cab, rubber gloves	18.4
(C) Broadcast spray application, enclosed cab, no rubber gloves	4.1
(D) Broadcast spray application, no enclosed cab, no rubber gloves	10.0
(E) Hand spray application, rubber gloves	40.2
(F) Hand spray application, no rubber gloves	15.7
(G) In-furrow/banded application, enclosed cab	22.8
(H) In-furrow/banded application, no enclosed cab	17.0

[a]Total exceeds 100% because applicators often reported multiple application methods.
[b]On the basis of 19,658–20,967 respondents (depending on completeness of information for specific factors) reporting any pesticide use and providing information on the AHS take-home (Q1) questionnaire.

each AHS/PES sampling year formed the sampling frame for this study. Applicators reporting the recent use of 2,4-D or chlorpyrifos in one or more of the eight application scenarios (Table 1), and who resided in selected counties in IA or NC, were selected for a telephone screening. Counties were chosen based on CATI-reported rates of target chemical use and driving proximity to field contractor facilities in Iowa City, IA, and Research Triangle Park, NC. In IA, 22 central, eastern, and southeastern counties were included in the study over 3 years (2000–2002). In NC, 22 eastern and central counties were included in the study over 2 years (2001–2002). With large numbers of Iowa applicators reporting 2,4-D hand spraying, a random sample was selected for screening in 2001 for category E in Table 1. Applicators reporting only hand spray 2,4-D applications were not selected for screening in IA in 2002.

All applicators completing the screening contact and reporting that they would or might use a product containing 2,4-D or chlorpyrifos on their farm in the coming season using broadcast, in-furrow/banded, or hand spray application methods were eligible for recruitment. Applicators reporting orchard uses were not recruited for this study, but were potentially eligible for another AHS study (Hines et al., 2008). Applicators reporting only forestry or residential lawn/garden uses were not eligible. Field study representatives met with eligible applicators and written consent was obtained. Those consenting to participate were asked to contact the field study staff before a planned application of one of the target chemicals. Applicators were asked to participate in another monitoring visit, either in the same or the following year. Participating applicators received $100, with an additional $50 for those providing four additional 24-h urine samples. This was an observational research study, as defined in 40 CFR Part 26.402. The study was reviewed and approved by the Institutional Review Boards at the National Cancer Institute, the University of Iowa, Battelle Memorial Institute, and the Research Triangle Institute.

Monitoring Design

Monitoring was performed around a pesticide mixing, loading, and application (MLA) activity on 1 day. Applicators followed their usual pesticide handling and application practices. The measurement strategy was based on using methods that could be applied within a working agricultural cohort. Urine samples were collected before, during, and after a monitored pesticide use; and patch, hand wipe, and personal air samples were collected during the monitored activity (Figure 1). Where possible, applicators were monitored during their first use of the target pesticide for the year or during a use separated by several days from other uses. Given the time requirements and high farm activity levels during the planting season, this could not always be achieved, so the timing for collecting urine samples was designed to both minimize burden and reduce the potential impact on urinary biomarker measurements from target chemical uses on the days before and after the monitored use. Dermal (patch and hand wipe) and air measurements were made to assess potential routes of exposure.

Applicators were asked to collect a first morning void urine sample before pesticide handling on the day of the monitored activity. Field staff applied the sampling patches and attached a personal air sampler before the start of pesticide handling activities. Hand wipe samples were collected and patch and air samples were retrieved at the end of the monitored activity. In several cases, two hand wipe, patch, or air samples were collected for an individual monitoring day either due to a break in work activities (for example, between

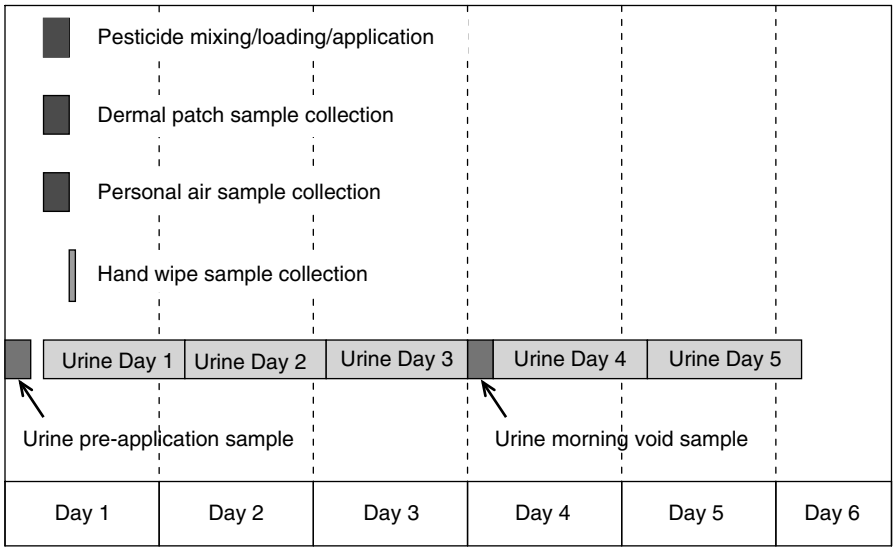

Figure 1. Timeline for the collection of samples in the AHS Pesticide Exposure Study (day 2–5 urine samples were optional).

mixing/loading and application) or when they would normally wash. In these cases, the analysis results were combined to produce a single measurement. Field staff recorded information about the MLA activity on a structured data-collection form. The pesticide-use component of the AHS Phase II CATI, modified for a single-day use, was interviewer administered to the applicator upon completing the monitored activity. Field staff made a final visit to the applicator's home to collect urine samples and to administer a questionnaire to collect additional information about the applicator as well as farm and home activities and conditions.

Sample Collection and Analysis

Urine Samples Applicators were provided 470-ml polyethylene containers for collecting a pre-application sample as a single void and a third-day post-application first morning void. Applicators were provided with larger 3-l polyethylene containers for collecting the day-1 post-application composite sample. For applicators agreeing to collect four additional 24-h urine samples, the sample collections were scheduled to start with the void after the first morning void and through the first morning void on the following day, continuing this pattern for 4 days. Applicators were provided with sample-collection instructions that included how to wash hands, the need to avoid touching the inside of the container and lid, and to place lids inside up while samples were collected. The applicator was asked to record the collection time and date, and the time of the previous void before collecting the sample. Total void volumes were recorded for each sample. Samples were stored under refrigerated conditions in the field. Aliquots were prepared and frozen after transport to the field contractor laboratories. Before analysis, samples were hydrolyzed, solvent extracted, and derivatized. Derivatized extracts were analyzed by gas chromatography/mass spectrometry (GC/MS) to determine 2,4-D or TCP concentrations. The recovery of isotopically labeled 2,4-D and TCP surrogate standards added to each urine sample was used to correct the analyte concentration. A separate aliquot of each urine sample was analyzed for creatinine. As urinary biomarker concentrations can be affected by variable urine volumes resulting from different fluid intake rates, excretion rates were also calculated for 2,4-D and TCP by multiplying the concentration by the sample volume and then dividing by the duration between the previous void time before sample collection and the final collection time.

Hand Wipe Samples Dermal wipe samples were collected from the pesticide applicator's hands at a break in work when hands were washed and/or at completion of the MLA activity. A sub-sampling approach was selected for compatibility with simultaneous biomarker measurements. In a preliminary assessment for several chlorpyrifos applicators, this method was found to correlate well ($r^2 > 0.95$) with a whole-hand wipe method (Geno et al., 1996) with loading estimates an average of 1.9 times greater than the whole-hand method (unpublished data). Twelve predefined locations (3×1 cm) on each hand were thoroughly wiped using polyurethane foam-tipped swabs wetted with isopropanol. Samples were solvent extracted and analyzed by GC/MS for the neutral analytes 2,4-D ethylhexyl ester, 2,4-D butoxyethylester, and chlorpyrifos or by liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) for 2,4-D acid and 2,4-D dimethylamine (Gardner et al., 2005). Hand loadings were estimated by multiplying the measured mg/72 cm^2 by the total area (cm^2) for both hands estimated from hand tracings.

Patch Samples Patches were applied to 10 locations on the applicator's chest, back, arms, and legs. Chromatography paper patches were used for liquid formulations, whereas cotton gauze patches were used for dry formulations. The surface area of each patch was proportional to standard surface areas of the body location the patch represented (U.S. EPA, 1996). Patches were placed on regular clothing or the skin and were placed under any personal protective equipment worn by the applicator. The applicator wore the patches during the MLA activity. Patches were removed and combined for analysis; separate compositing and analysis was performed for patches placed on the skin. Samples were solvent extracted and neutral target analytes were measured by GC/MS, whereas the 2,4-D acid or dimethylamine was measured using LC/MS/MS. An estimate of total body loading (except for the hand areas) was made based on the overall combined patch loading value (mg/cm^2) multiplied by a total standard body area (20,320 cm^2, U.S. EPA, 1996).

Air Samples Air samples were collected in the applicator's breathing zone for the duration of the MLA activity. An OSHA Versatile Sampler (OVS SKC No. 226–58) containing a quartz filter and XAD-2 resin was clipped to the applicator's collar and connected to a battery-operated pump operated at 1.0 l/min. Flow-calibration checks were performed before and at the completion of the sampling period. Samples were solvent extracted and analyzed by LC/MS/MS for the applied target pesticide. Analyte concentrations were calculated by dividing the collected mass by the air sampling volume determined from the sampling flow rate and sample-collection duration.

Quality Control

Quality control samples and analyses were applied to assess the recovery, background, and precision. Unfortified sampling media (field blanks) and media fortified with the target analytes (field controls) were prepared, transported, stored, and analyzed with samples to assess background and

recovery for hand wipe, patch, and air samples. Pooled urine from commercial sources was obtained each year to prepare field blanks and controls, and endogenous levels of 2,4-D and TCP were subtracted to calculate recoveries. Aliquots of the spiking solutions used to prepare field controls were also spiked into vials containing solvent for analysis along with each type of sample; their recoveries were used to adjust for differences in field control recoveries resulting from spiking and spiking solution differences. Surrogate standards were added to each sample before extraction and analysis. The isotopic analogs $^{13}C_6$-2,4-D or $^{13}C_2$-^{15}N-TCP were added as surrogate recovery standards for urine samples. For hand wipe, patch, and air samples, $^{13}C_6$-2,4-D was used as the surrogate standard for acid analyses and parathion-d_{10} was used for the analyses of neutrals. Repeated analyses of sample extracts were used to assess analytical precision. Measurement results were not adjusted for field blank background or field control recovery. Each urine sample measurement value was adjusted for surrogate standard recovery.

To evaluate the completeness of urine sample collection, a separate aliquot of each urine sample was analyzed for creatinine. Estimated 24-h creatinine excretion was calculated for each sample that had complete collection time and volume information. Creatinine excretion rates were evaluated against literature values, and when multiple 24-h samples were available for the individual, excretion rates were also examined for internal consistency. The completeness and accuracy of previous void time, sample collection, and sample volume information was used to determine suitability for calculation of target analyte urinary excretion rates.

Data Analysis

2,4-D Acid Equivalents Amounts of 2,4-D 2-ethylhexyl ester and 2,4-D butoxyethyl ester measured in hand wipe, patch, and personal air samples were converted to 2,4-D acid equivalents (a.e.) using molecular weight ratio multipliers of 0.664 and 0.688, respectively. When two or more 2,4-D species were present in a sample, the a.e. for each analyte were added together. All results are reported as 2,4-D a.e.

Amount of Active Ingredient. The amount of (a.i.) handled during the MLA was estimated based on the concentration in the product and the amount of the product used. These amounts were used to calculate a.i.-adjusted measurement results for hand wipe, patch, and air sample data by dividing the measurement by the kg of a.i. used. For 55% of 2,4-D and 71% of chlorpyrifos monitoring days, the applicator used additional product later in the day after the field team departed. Estimates of the total amount of a.i. used for the entire day were based on the amount of a.i. used per acre or per hour during the monitored application and the applicator-reported total acres or time of use for the entire day. These amounts were used to calculate a.i.-adjusted day-1 post-application urine concentrations.

Method Detection Limits and Replacement of Non-Detects Method detection limits (MDLs) were calculated as analyte mass/sample from the SD of analyte amounts measured on field blanks multiplied by the Student's *t*-value at the 0.99 level. If analytes were not detected on any field blanks the MDL was calculated as one-half of the lowest analytical calibration level. This approach was also used for urine samples that had variable endogenous levels of the target compounds in field blanks. If any amount of target analyte lower than the MDL was detected in a sample the reported value was used in data analysis (Clayton et al., 2003). If no target analyte was detected it was replaced with the value of the MDL/$\sqrt{2}$. For hand wipe, patch, and air samples with multiple 2,4-D analytes, the a.e. of the highest MDL value obtained from field blank measurements was used for replacement.

Absorbed Dose Estimation Estimates of absorbed dose were calculated for the subset of 2,4-D applicators from any visit in which all standard and optional urine samples were obtained and no other uses of a product containing the target chemical in the 4 days preceding or following the monitored use were reported. The total amount of 2,4-D excreted across the 5-day post-application period (mean 118 ± 13 h) was determined from measured concentrations and urine volumes. An absorbed dose was estimated by dividing the amount excreted by a factor of 0.90, based on the 0.95 fraction of urinary excretion over 144 h (Sauerhoff et al., 1977) and the fractions of 0.85 for 2,4-D acid and 0.77 for 2,4-D dimethylamine over 96 h (Harris and Solomon, 1992). Absorbed dose estimates were not made for chlorpyrifos applicators because only two users met the conditions described above, and in both cases the pre-application urinary TCP concentrations exceeded post-application levels.

Statistical Analysis Analyses were performed using results from the first visit for applicators who had more than one monitoring visit. Results from another monitoring day were included in two cases in which the same participant used a different chemical (2,4-D *vs* chlorpyrifos) and one case in which a different application method (broadcast *vs* hand spray) was used. Univariate analyses of natural log (ln)-transformed measurement results were performed for each analyte/sample combination. The hypothesis of a normal distribution of ln-transformed data was not rejected based on the Shapiro–Wilk test (0.05 level) except for chlorpyrifos in hand wipe samples, which had a high percentage of non-detect values. Geometric means (GMs) and geometric SDs were calculated and reported for the exposure measurements,

except for chlorpyrifos hand loading estimates. Differences between pre- and post-application urine levels were evaluated using paired one-tailed *t*-tests. Spearman correlations were calculated to examine associations between measures for each chemical. Measurement results were grouped into eight categories (Table 1) based on the applicator's use during the monitoring period. Differences in GM values across the categories were assessed using an F-test of the differences in the least-square means of the ln-transformed values. Bivariate analyses were performed for selected chemical handling and application conditions, using F-tests for variables with discrete response categories and regression analysis for continuous variables. We did not adjust significance tests in the analysis for multiple comparisons because each variable was potentially an exposure determinant. However, it is possible with multiple comparisons such as these to have a significant association due to chance alone. An assessment of within- and between-applicator variability, based on a subset of applicators with repeat visits using the same chemical and application method, was performed using regression and covariance parameter estimation. Analyses were performed using SAS V9.1 (SAS Institute, Cary, NC, USA).

Table 2. Screening, recruitment, and monitoring information for the AHS/PES.

	IA 2000–2002	NC 2001–2002	Total
Screening			
Selected for screening	824	619	1443
Screening not performed	48	6	54[a]
Refusal or no contact	90	75	165
Ineligible based on screening	254	269	523
Potentially eligible	432	268	700
Recruitment			
Selected for recruitment	432	233[b]	665
Ineligible for monitoring	68	36	104
Total potentially eligible	364	197	561
Refusal or no contact	233	86	319
Total consented to participate	131	111	242
Monitoring			
Applicators monitored first time	60	24[c]	84
Repeated monitoring visits	24	0	24
Total monitoring visits	84	24	108

[a]Forty applicators in Iowa 2002 were not screened when field team capacity was reached; eight were deceased; six were not screened for other reasons.
[b]Thirty-five North Carolina applicators with early season uses were not recruited in 2001.
[c]Includes one applicator that used only atrazine during monitoring visit; not included in data analyses.

Results

Recruitment and Monitoring

Screening, recruitment, and monitoring results are shown in Table 2. Of the 242 applicators that agreed to participate in monitoring, a total of 83 actually participated in one or more monitoring visits in which a product containing 2,4-D or chlorpyrifos was used. One additional NC applicator applied only atrazine and is not included in subsequent analyses. Within the group of 83 applicators, repeat monitoring was performed 24 times, with 21 visits in which the same chemical and methods were used, and three visits in which a different chemical or application method was used. Many farmers agreed to participate in the study based on pre-planting season projections of the products they might use in the upcoming season. However, some farmers subsequently decided to use a different product or no product at all. Also, in some cases, application decisions were made in a time frame too short for a monitoring team to respond. Severe drought in NC during the spring and summer of 2002 led to decisions not to use products containing target chemicals by many of the farmers. Fewer chlorpyrifos product uses were monitored than was anticipated based on information on previous frequency of use information in the AHS cohort. This may have been a result of decreased chlorpyrifos use in general; in IA, for example, an average of 8.3% of the corn acreage was treated with chlorpyrifos from 1993 to 1999, but it dropped to 2.7% from 2000 to 2003 (NASS, 2006). Many previous uses of chlorpyrifos products were reported for tomato crops in NC but this use was rescinded in 2001.

All applicators were men and the average age at monitoring was 52 ± 11 years. A comparison of several characteristics based on information provided upon enrollment in the AHS is shown in Table 3 for those selected for screening and those monitored. Applicators participating in monitoring were younger, had more education, were less likely to be current smokers, and had a slightly higher rate of applying pesticides more than 40 days per year at enrollment. The groups had similar years of experience applying pesticides.

Product Use

Products containing 2,4-D or chlorpyrifos were used 69 and 17 times, respectively, in the first-visit applications. More than one 2,4-D compound was present in some products used by the applicators, and in several cases different products containing 2,4-D a.i. were tank mixed. Surfactants or other adjuvants were added in 24 applications. The participating applicator personally performed mixing/loading operations for all but four monitored applications; in three other cases, the mixing/loading activities were performed by the participating applicator before the arrival of the field team.

Table 3. Comparison of several demographic, lifestyle, and work characteristics for participants selected for screening and those monitored in the AHS/PES.[a]

Characteristic	Selected for screening ($n=1443$) %	Monitored ($n=84$) %
Age		
≤30 years	11.8	14.3
31–50	50.1	56.0
>50	38.0	29.8
Gender		
Male	98.8	100.0
Female	1.2	0.0
Race		
White	99.2	96.4
Other	0.1	0.0
Missing	0.8	3.6
Education		
High school or lower	53.7	45.3
Greater than high school	43.8	50.0
Missing	2.5	4.8
Smoking status		
Never smoked	60.4	64.3
Past smoker	27.4	27.4
Current smoker	10.9	4.8
Missing	1.4	3.6
Years applying pesticides		
≤5	14.0	14.3
6–20	46.4	46.5
>20	34.7	33.3
Missing	4.8	6.0
Days per year personally apply pesticides		
≤9	40.0	36.9
10–39	49.6	48.8
≥40	5.5	8.4
Missing	5.0	6.0
Number of acres planted previous year		
≤199	19.0	22.7
200–499	34.3	29.8
≥500	43.4	39.3
Missing	3.3	8.3

[a]As reported on the AHS enrollment private applicator questionnaire (1993–1997).

Broadcast spray applications of 2,4-D in IA were for soybean pre-planting burn-down, field corn, hay, and pasture. Broadcast applications of 2,4-D in NC were made for wheat, field corn, forage crops (hay and Bermuda grass), and pasture. All 2,4-D broadcast spray applications were made with tractor-mounted boom sprayers except for one truck-mounted boom sprayer and one highboy application in IA. NC 2,4-D applicators were more likely to use open-cab vehicles for broadcast spray than IA applicators (69% *vs* 26%). Hand spray uses of 2,4-D were primarily non-crop applications for weed control on fencerows and other farm areas and all but one application occurred in IA. Different combinations of tractor and all-terrain vehicle (ATV)-mounted systems were used for most hand spraying but five portable sprayer uses were also monitored. In one case, the applicator alternated between boom and hand spraying. The different 2,4-D chemicals sprayed, and their proportion of the 69 monitored uses, were 2,4-D acid (10%), 2,4-D 2-ethylhexyl ester (62%), 2,4-D butoxyethyl ester (16%), and 2,4-D dimethlyamine salt (28%); the total exceeds 100% because of product and tank mixes. All 11 chlorpyrifos applications in IA involved in-furrow planter box application of a granular product for corn. One NC chlorpyrifos use was an over-the-row cultivator application of a granular product for peanuts (this use was grouped with the 11 in-furrow granular chlorpyrifos applications for data analysis). The remaining five NC chlorpyrifos applications used liquid products for tobacco, sod, and sweet potatoes. One tobacco application was made by air-blast, the sweet potato application was made using a cultivator, and the remaining three applications were by boom spray.

Information on the amounts of a.i. mixed/loaded, time of pesticide handling and application, and the area treated is reported in Table 4 by target chemical and application method. Although the duration of 2,4-D broadcast spray applications made with enclosed-cab vehicles was about 1.3 times higher than open-cab tractors, the amount of a.i. used was about 2.2 times greater, and areas treated were about 4.1 times higher on average. For 2,4-D hand spray applicators, those applying from tractors used more a.i. on average than those using ATVs, who in turn used more than applicators not spraying from a vehicle.

Quality Control

Endogenous levels of 2,4-D and TCP in the urine purchased for field blank preparation were $0.6 \pm 0.9\ \mu g/l$ ($n=43$) and $4.1 \pm 3.4\ \mu g/l$ ($n=22$), respectively. For the four acid and neutral analytes, the mean amounts on field blanks ($n=16$–28) ranged from 0 to $0.52 \pm 2.4\ \mu g$/sample (hand wipe), 0.17 ± 0.92–$0.35 \pm 1.3\ \mu g$/sample (patch), and 0–$0.0001 \pm 0.0002\ \mu g$/sample (air). Mean recoveries of surrogate standards across all applicator urine samples were 73% ± 16% ($n=429$) for 2,4-D and 79% ± 13% ($n=102$) for TCP. Mean surrogate standard recoveries for hand wipe, patch, and air samples ranged from 93% ± 8% to 112% ± 19% ($n=23$–141). Mean surrogate-corrected recoveries of 2,4-D and TCP from urine field controls were 99% ± 34% ($n=34$) and 108% ± 28% ($n=18$), respectively. Two 2,4-D field controls with apparent recoveries >400% and two TCP field controls with apparent recoveries >250% prepared at the same time and location likely were a result of preparation

Table 4. Product use information for the monitored mixing/loading/application and estimates for the full day.

		Monitored use			Estimates for the full day		
	N	Amount of a.i. applied[a] kg	Time of use h	Area treated acres	Amount of a.i. applied kg	Time of use h	Area treated acres
		Mean (standard deviation) with range in italics					
Broadcast application—liquid products							
2,4-D							
Open tractor cab	18	3.0 (3.4)	1.5 (0.9)	11 (9.9)	7.3 (7.6)	3.9 (2.6)	21 (17)
		0.7–16	*0.6–4.1*	*2.5–46*	*1.3–32*	*1.0–8.0*	*2.5–50*
Enclosed tractor cab[b]	25	7.1 (4.9)	2.0 (0.8)	37 (25)	15.9 (11)	5.1 (3.2)	87 (71)
		1.6–22	*0.9–3.6*	*8.0–100*	*1.6–46*	*1.0–12*	*8.0–280*
Chlorpyrifos							
Open tractor cab	3	7.5 (2.6)	1.9 (1.2)	8.2 (2.8)	11 (2.6)	3.5 (0.5)	10 (5.0)
		4.5–9.2	*0.9–3.3*	*5.0–10*	*9.0–14*	*3.0–4.0*	*5.0–15*
Enclosed tractor cab[c]	2	6.1 (4.2)	1.6 (1.6)	8.5 (2.1)	8.4 (7.4)	3.1 (3.0)	11 (5.7)
		3.2–9.1	*0.5–2.7*	*7.0–10*	*3.2–14*	*1.0–5.2*	*7.0–15*
In-furrow/banded application—granular products							
Chlorpyrifos							
Open tractor cab	7	6.4 (3.2)	3.1 (1.6)	14 (9.8)	14 (5.5)	5.3 (1.9)	21 (9.7)
		1.6–12	*1.4–5.4*	*3.5–34*	*6.8–20*	*1.5–7.0*	*4.8–34*
Enclosed tractor cab	5	9.7 (2.8)	2.1 (0.7)	15 (1.7)	19 (8.5)	5.5 (2.6)	27 (11)
		6.8–13	*1.0–2.6*	*14–18*	*8.5–31*	*3.0–8.5*	*12–38*
Hand spray application—liquid products							
2,4-D							
From tractor[d]	14	2.0 (1.6)	1.5 (0.8)	NA[e]	3.3 (2.7)	2.9 (2.2)	NA
		0.08–4.9	*0.4–3.2*		*0.1–10*	*1.0–8.0*	
From ATV	7	0.49 (0.31)	1.0 (0.4)	NA	0.85 (0.85)	2.1 (2.3)	NA
		0.09–0.9	*0.6–1.7*		*0.09–2.7*	*0.2–7.0*	
No vehicle	5	0.19 (0.24)	1.1 (0.4)	NA	0.27 (0.32)	1.3 (0.9)	NA
		0.01–0.6	*0.6–1.6*		*0.01–0.7*	*0.5–2.5*	

[a]Amount of active ingredient; for 2,4-D, the amount is calculated as acid equivalents.
[b]Includes one high-boy and one truck-mounted boom sprayer.
[c]Includes one air blast sprayer.
[d]Includes one use with both hand and broadcast spray.
[e]Not applicable; includes applications for fence rows and other non-crop uses.

error and were not included in the average recoveries. Mean recoveries from field controls across all analytes for hand wipe, patch, and air samples ranged from 103% to 118% ($n = 13$–25); the associated SDs ranged from 18% to 24% for hand wipe and patch samples but were 38%–91% for air samples. Repeated extract analysis resulted in 1.1%–10% mean percent relative SDs (RSD) of paired results for most analytes and media. Exceptions were 2,4-D butoxyethyl ester in patch samples (26% RSD) that included one pair with and without a detectable result, and for neutral analytes in air samples (30%–37% RSDs).

One day-1 urine sample was excluded from data analysis because the sample volume for the designated 24 h period was 0.18 l (less than the single-void pre- and post-application samples), and the estimated 24-h creatinine excretion was only 0.25 g, suggesting incomplete collection. Two optional 24 h samples that had greater than a three-fold difference of creatinine excretion from the average of other 24-h samples provided by the participant were excluded. Results were not used for two samples in which the collection time for the last sample in the series of optional 24-h samples was <5 h. Previous void times and/or final collection times were not recorded by the applicator, or the information was judged to be uncertain, for about 15% of the urine samples, and excretion rate values were not used.

Measurements

A summary of pre- and post-application urine sample measurement results for the monitored applicators is shown in Table 5. GM urinary 2,4-D concentrations were 7.8 and 25 μg/l in the pre-application and the day-1 post-application samples, respectively. Post-application 2,4-D levels were significantly higher than pre-application levels at the day-1 and day-4 morning void time points for unadjusted concentrations and at several post-application times for estimated excretion rates. Eight 2,4-D applicators had

Table 5. Summary pre- and post-application urine measurement results for 2,4-D and chlorpyrifos applicators in the AHS/PES.

Urine collection period	2,4-D							Chlorpyrifos metabolite TCP[a]						
	N	% Detect[b]	% > MDL[c]	GM	GSD	Minimum	Maximum	*N*	% Detect	% > MDL	GM	GSD	Minimum	Maximum
Unadjusted concentrations (μg/l)														
Pre-application	68	96	93	7.8	4.7	ND[d]	210	15	100	100	11	2.9	2.1	63
Day 1	68	100	100	25[e]	4.1	1.6	970	16	100	100	11	2.3	2.5	80
Day 2	28	100	100	26	3.7	2.2	1000	8	100	88	13	2.5	2.8	71
Day 3	27	100	100	23	4.1	1.3	840	9	100	89	15	3.2	2.5	130
Day 4 (morning void)	66	100	100	27[e]	4.4	1.1	1700	16	100	100	16	3.1	3.1	170
Day 4	28	100	100	20	4.3	1.2	1200	10	100	90	11	2.6	2.9	110
Day 5	26	100	100	17	5.5	0.8	2500	9	100	100	12	2.6	2.4	75
Excretion Rates (μg/h)														
Pre-application	60	95	92	0.29	4.9	ND	12	14	100	100	0.52	2.4	0.10	2.0
Day 1	59	100	100	1.3[e]	4.0	0.07	22	15	100	100	0.44	1.8	0.18	1.6
Day 2	25	100	100	1.4[e]	3.2	0.08	30	7	100	86	0.62	1.9	0.18	1.5
Day 3	20	100	100	1.2	3.7	0.09	26	8	100	88	0.72	2.1	0.19	1.8
Day 4 (morning void)	56	100	100	1.3[e]	4.0	0.06	19	14	100	100	0.61	2.0	0.24	2.6
Day 4	18	100	100	1.5[e]	3.3	0.22	11	7	100	86	0.50	1.7	0.18	0.9
Day 5	24	100	100	1.0	5.0	0.06	63	7	100	100	0.81	1.6	0.39	1.3

[a]3,5,6-Trichloro-2-pyridinol.
[b]Percent of participants for which a target analyte was detected.
[c]Percent of participants for which a target analyte amount was greater than the method detection limit.
[d]Not detected.
[e]Significantly higher than pre-application concentration based on one-tailed paired *t*-test, $\alpha = 0.05$.

Table 6. Summary of hand loading, body loading, and personal air measurement results for 2,4-D and chlorpyrifos applicators in the AHS/PES.

Measurement type	2,4-D							Chlorpyrifos						
	N	% Detect[a]	% > MDL[b]	GM	GSD	Minimum	Maximum	*N*	% Detect	% > MDL	GM	GSD	Minimum	Maximum
Estimated hand loading (mg)	68	97	84	0.39	9.2	ND[c]	22	17	53	53	0.02[d]	—	ND	0.93
Estimated body loading (mg)[e]	69	100	91	2.9	12	0.02	880	17	94	59	0.28	5.1	ND	5.8
Personal air ($\mu g/m^3$)	68	85	66	0.37	5.8	ND	10	17	100	100	0.49	3.0	0.048	2.0

[a]Percent of participants for which a target analyte was detected.
[b]Percent of participants for which a target analyte amount was greater than the method detection limit.
[c]Not detected.
[d]Median value reported for chlorpyrifos hand loading due to the number of samples with no analyte detected.
[e]The estimated total body loading does not include the hands.

pre-application concentrations that were higher than post-application levels, and in four of these cases the applicator reported the use of a 2,4-D product within 2 days preceding the monitoring day. In the other four cases, it is not known if there was an unreported product use before monitoring or whether there were other contacts with potentially contaminated equipment or surfaces. Twenty-five applicators reported additional uses of products containing 2,4-D on the 2 days following the monitoring day. The mean estimate of absorbed dose calculated for 14 2,4-D broadcast and spray applications was 0.0027 ± 0.0044 mg/kg/day (GM = 0.0016; range 0.00032–0.018). Biomarker measurements and estimates of absorbed dose reflect exposures from all sources, including the agricultural use plus any exposures occurring through dietary ingestion or contact with surfaces containing 2,4-D residues in the home or around the farm.

The GM TCP concentration for chlorpyrifos applicators was the same (11 μg/l) in the pre-application and day-1 post-application measurements. Differences in pre- and post-application levels were not significant across the applicators in this study. Three applicators reported using a product containing chlorpyrifos in the 4 days preceding, and six applicators reported uses in the 2 days following the monitored application. However, applicators with the seven highest pre-application urinary TCP concentrations did not report any chlorpyrifos product use in the 4 days preceding monitoring, and pre-application levels often exceeded day-1 post-application levels. Again, it is not known if there were

unreported uses before monitoring or whether exposures could be occurring through other types of agricultural contacts.

Summaries of measurement results for estimated hand loading, estimated body loading, and air concentration are shown in Table 6 for 2,4-D and chlorpyrifos applicators. Large ranges were measured for hand and body loading estimates, with higher variability as compared with the urine measurement results. Dermal measurements were higher for 2,4-D applicators compared with chlorpyrifos applicators in this study, largely as a result of higher values for 2,4-D hand spray applicators and lower dermal loadings measured for chlorpyrifos granular product applicators. Spearman correlations between measures are shown in Table 7. Estimated 2,4-D hand loading values had the highest correlations with urine biomarker concentrations, but all 2,4-D measures were significantly correlated. Estimated body loading had the highest correlation with urine biomarker levels for chlorpyrifos, but the strongest correlations were between estimated hand and body loadings.

Distributions of day-1 post-application urinary biomarker concentrations in eight categories are shown in Figure 2 separately for 2,4-D and chlorpyrifos applicators, with the four chlorpyrifos liquid spray applicators collapsed into one category. Urine results are reported separately for 2,4-D and chlorpyrifos because of differences in absorption, metabolism, and excretion. Distributions for hand loading, body loading, and personal air measurements are shown in Figure 3, with the measurements for 2,4-D and chlorpyrifos applicators combined. Tests for overall differences across categories were significant for urine 2,4-D concentrations and excretion rates (data not shown) with F-values of 3.8–4.2 ($P<0.006$), and for the hand loading, body loading, and air measurements with a range of F-values from 2.8 to 17 ($P<0.02$). There was little difference in measurement distributions between those using open- and enclosed-cab tractors, but 2,4-D applicators using rubber gloves tended to have lower measurement levels for urine and estimated hand loading. Hand spray applicators had higher estimated body loading and air concentrations than broadcast spray applicators. No significant differences were found across the three categories for urine TCP concentrations or excretion rates but the number of observations was small for each group. Applicators had lower estimated hand and body loading measurement distributions while using in-furrow application of a granular product than for other application methods. There was overlap between many of the categorical distributions and considerable within-category variability, suggesting that other factors were often important at the individual level.

Bivariate analyses were performed for selected handling and application conditions for 2,4-D broadcast spray applicators, 2,4-D hand spray applicators, and chlorpyrifos applicators (Table 8). Several factors were found to be potentially associated with differences in measurement results. Hand, body, and air GM levels were significantly lower for broadcast spray applicators compared with hand spray applicators.

For 2,4-D broadcast spray applicators, minor spills, splashes, drips, or leaks were associated with higher GM hand and body loadings, and equipment repair was associated with a higher body loading (Table 8). Broadcast spray applicators using an adjuvant had higher GM levels for all media but the differences were not significant. Lower GM urinary biomarker and hand loading levels were associated with the use of rubber gloves. Lower urinary biomarker levels were associated with use of long sleeves, but the use of long sleeves was also related to glove use, and separate measures of forearm patches did not correlate as strongly with urinary biomarker levels as did those for hand loading or overall body loading. Only after adjustment for the amount of a.i. used did 2,4-D broadcast applicators using enclosed-cab tractors have lower GM values than those using open-cab tractors, but the differences were not significant at the $P=0.05$ level. Broadcast spray applicators in NC used open cab tractors more often than applicators in IA, but had lower day-1 GM urine levels (16 *vs* 23 μg/l) and estimated hand loading values (0.11 *vs* 0.33 mg) than IA applicators. The differences could be related to the lower amounts of a.i. used in NC compared with IA (mean 5.8 *vs* 16 kg).

Use of an adjuvant by 2,4-D hand spray applicators was associated with higher day-1 urine GM levels; equipment repair was associated with higher GM levels for all media except air (Table 8). GM levels were higher across all media for those with minor spills, splashes, drips, or, leaks or contact with sprayed vegetation, but in most cases, the differences were not significant at the $P=0.05$ level. Lower urine and hand loading GM levels were found for the use of rubber gloves, but the result was significant at the $P=0.05$ level only for hand loading. Day-1 urine and air levels showed large but not significant differences in the order of no vehicle < ATV < tractor. Body loadings were lowest for ATV users.

Table 7. Spearman correlations between measurements.

Measures (all urine values are day 1)	2,4-D			Chlorpyrifos/TCP		
	N	*r*	*P*-value	*N*	*r*	*P*-value
Urine (μg/l) and urine (μg/h)	59	0.93	<0.001	15	0.59	0.020
Urine (μg/l) and hand loading	67	0.74	<0.001	16	0.19[a]	0.471
Urine (μg/l) and body loading	68	0.43	<0.001	16	0.41	0.110
Urine (μg/l) and air concentration	67	0.40	<0.001	16	0.02	0.940
Hand loading and body loading	68	0.61	<0.001	17	0.70[a]	0.002
Hand loading and air concentration	67	0.34	0.006	17	0.13[a]	0.619
Body loading and air concentration	68	0.62	<0.001	17	0.04	0.874

[a]Only 53% of chlorpyrifos hand loading samples had detectable levels.

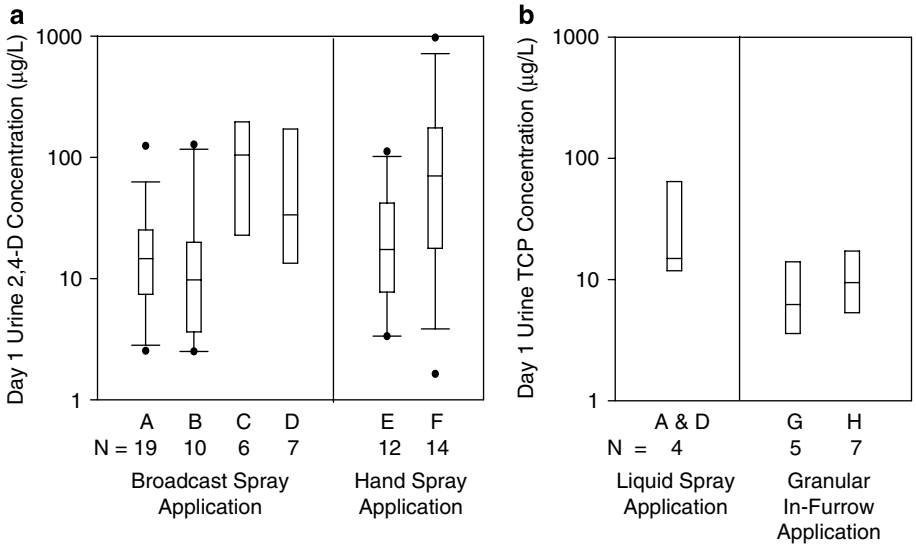

Figure 2. Distributions of day-1 urine biomarker concentrations for 2,4-D applicators (a) and chlorpyrifos applicators (b) for eight pesticide use categories described in Table 1. Boxes show the median, 25th, and 75th percentile intervals; whiskers show the 10th and 90th percentile intervals for those with sufficient numbers of observations, and dots show high and low values outside the percentile ranges.

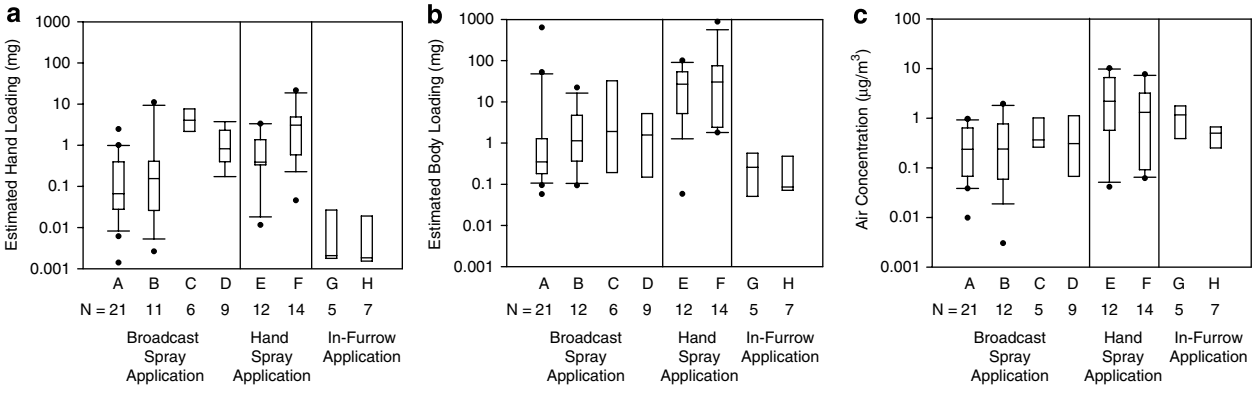

Figure 3. Distributions of estimated hand loading (a), estimated body loading (b), and personal air concentrations (c) for eight pesticide use categories described in Table 1. Results for both 2,4-D and chlorpyrifos are included. Boxes show the median, 25th, and 75th percentile intervals; whiskers show the 10th and 90th percentile intervals for those with sufficient numbers of observations, and dots show high and low values outside the percentile ranges.

The amount of a.i. used by 2,4-D broadcast spray applicators was not associated with urine concentrations or excretion rates but was weakly associated with hand and body loadings, with r^2 values of 0.09 ($P = 0.060$) and 0.15 ($P = 0.010$), respectively. For 2,4-D hand spray applicators, the day-1 urine concentrations increased with increasing duration of use ($r^2 = 0.30$, $P = 0.004$), but were not significantly associated with the amount of a.i. used ($r^2 = 0.07$, $P = 0.189$). We examined meteorological variables including temperature and wind speed. Increasing wind speed was modestly associated with post-application urine levels ($r^2 = 0.11$, $P = 0.033$) and body loading ($r^2 = 0.18$, $P = 0.005$) only for 2,4-D broadcast spray applicators; but wind speed was also associated with the amount of product used. Temperature was modestly associated with post-application urine levels only for 2,4-D broadcast spray applicators ($r^2 = 0.10$, $P = 0.042$). Although we did see some 2,4-D measurement differences based on nozzle type (in particular, higher body loading and air levels for those using flat fan *vs* hollow cone or flooding type nozzles), the numbers for some categories were small and none of the differences were significant. Likewise, applicators that handled the nozzles were more likely to have higher measurements, but these differences were not significant.

Chlorpyrifos applicators using a granular product and in-furrow or over-the-row application had significantly lower GM day-1 urine and body loading levels than those performing spray applications of liquid mixtures (Table 8). Hand loading measurements were also much lower for those applying a granular chlorpyrifos product. This application

Table 8. Measurement result comparisons for selected handling and use conditions.

		Day 1 post-application urine		Estimated hand loading		Estimated body loading		Air concentration	
	N	GM (GSD)	*P*-value	GM (GSD)	*P*-value	GM (GSD)	*P*-value	GM (GSD)	*P*-value
		μg/l		*mg*		*mg*		*μg/m³*	
All 2,4-D applicators									
Broadcast spray application	42–43[a]	21 (1.2)	0.181	0.22 (1.4)	0.007	0.99 (1.4)	<0.001	0.21 (1.3)	<0.001
Hand spray application[b]	26	33 (1.3)		0.97 (1.5)		17 (1.5)		0.97 (1.4)	
2,4-D broadcast spray applicators									
Used adjuvant	14–15	26 (1.4)	0.392	0.36 (1.8)	0.317	1.8 (1.7)	0.191	0.32 (1.5)	0.165
No adjuvant used	27–28	18 (1.3)		0.17 (1.6)		0.72 (1.5)		0.16 (1.3)	
One tank load	34–35	—[c]		0.19 (1.5)	0.453	0.99 (1.5)	0.991	0.19 (1.3)	0.601
Two tank loads	8			0.39 (2.3)		0.98 (2.2)		0.26 (1.7)	
Used rubber gloves	29–30	13 (1.2)	<0.001	0.090 (1.4)	<0.001	0.95 (1.5)	0.834	0.18 (1.3)	0.304
Did not use rubber gloves	12–13	57 (1.4)		1.6 (1.7)		1.1 (1.8)		0.30 (1.5)	
Repaired equipment	17	19 (1.4)	0.739	0.25 (1.8)	0.900	2.6 (1.6)	0.066	0.25 (1.5)	0.640
No equipment repair	21–22	22 (1.3)		0.23 (1.7)		0.77 (1.5)		0.20 (1.4)	
Minor splashes/leaks/drips	11–12	25 (1.5)	0.543	0.73 (1.9)	0.042	2.9 (1.8)	0.046	0.14 (1.5)	0.272
No minor splashes/leaks/drips	30–31	19 (1.3)		0.14 (1.5)		0.66 (1.5)		0.24 (1.3)	
Enclosed tractor cab	23–24	24 (1.3)	0.495	0.20 (1.6)	0.660	1.1 (1.6)	0.717	0.24 (1.4)	0.460
Open tractor cab	17–18	18 (1.4)		0.28 (1.8)		0.87 (1.7)		0.17 (1.4)	
Adjusted for amount of a.i. mixed		*μg/l/kg a.i.*		*mg/kg a.i.*		*mg/kg a.i.*		*μg/m³/kg a.i.*	
Enclosed tractor cab	23–24	2.0 (1.4)	0.153	0.033 (1.6)	0.068	0.18 (1.5)	0.297	0.04 (1.4)	0.302
Open tractor cab	17–18	4.1 (1.4)		0.12 (1.7)		0.37 (1.6)		0.07 (1.5)	
2,4-D hand spray applicators		*μg/l*		*mg*		*mg*		*μg/m³*	
Used adjuvant	8	87 (1.7)	0.031	2.1 (1.8)	0.142	27 (2.0)	0.473	1.1 (1.9)	0.832
No adjuvant used	18	21 (1.4)		0.69 (1.5)		14 (1.6)		0.92 (1.5)	
One-tank load	17	—[c]		1.1 (1.6)	0.577	24 (1.6)	0.218	1.3 (1.5)	0.290
Two-tank loads	9			0.74 (1.8)		9.0 (1.9)		0.58 (1.8)	
Used rubber gloves	12	18 (1.5)	0.059	0.43 (1.6)	0.026	14 (1.8)	0.610	1.4 (1.7)	0.378
Did-not-use rubber gloves	14	56 (1.5)		2.0 (1.6)		21 (1.7)		0.72 (1.6)	
Repaired equipment	6	130 (1.8)	0.011	4.5 (1.9)	0.013	120 (2.0)	0.004	1.9 (2.1)	0.304
No equipment repair	20	22 (1.4)		0.61 (1.4)		9.9 (1.5)		0.79 (1.5)	
Minor splashes/leaks/drips	16	41 (1.5)	0.397	1.1 (1.6)	0.595	27 (1.6)	0.157	2.5 (1.4)	<0.001
No minor splashes/leaks/drips	10	24 (1.6)		0.76 (1.8)		8.7 (1.8)		0.21 (1.5)	
Contact sprayed vegetation	5	100 (1.9)	0.070	2.2 (2.2)	0.263	61 (2.3)	0.115	2.4 (2.2)	0.207
No contact sprayed vegetation	21	25 (1.4)		0.80 (1.5)		13 (1.5)		0.78 (1.5)	
No vehicle used	5	18 (2.0)	0.385	0.76 (2.3)	0.703	18 (2.1)	0.008	0.38 (2.2)	0.240
ATV used	7	24 (1.8)		0.66 (2.0)		2.9 (1.9)		0.66 (1.9)	
Tractor used	14	49 (1.5)		1.3 (1.6)		42 (1.6)		1.6 (1.6)	
Adjusted for the amount of a.i. mixed		*μg/l/kg a.i.*		*mg/kg a.i.*		*mg/kg a.i.*		*μg/m³/kg a.i.*	
No vehicle used	5	200 (2.1)	0.051	11 (2.3)	0.042	250 (2.1)	0.005	5.4 (2.2)	0.199
ATV used	7	42 (1.9)		1.7 (2.1)		7.4 (1.9)		1.6 (2.0)	
Tractor used	14	21 (1.6)		0.73 (1.7)		24 (1.6)		0.94 (1.6)	

Table 8. *Continued*

		Day 1 post-application urine		Estimated hand loading		Estimated body loading		Air concentration	
	N	GM (GSD)	*P*-value	GM (GSD)	*P*-value	GM (GSD)	*P*-value	GM (GSD)	*P*-value
		μg/l		*mg*		*mg*		*μg/m³*	
Chlorpyrifos applicators		*μg/l*		*mg*[d]		*mg*		*μg/m³*	
In-furrow, granular product	12	8.3 (1.2)	0.045	0.002	—	0.17 (1.5)	0.033	0.52 (1.4)	0.736
Spray, liquid product	4–5	21 (1.4)		0.27		1.0 (1.9)		0.42 (1.7)	
Adjusted for the amount of a.i. mixed		*μg/l/kg a.i.*		*mg/kg a.i.*		*mg/kg a.i.*		*μg/m³/kg a.i.*	
In-furrow, granular product	12	0.57 (1.2)	0.002	0.0003	—	0.015 (1.5)	0.004	0.05 (1.4)	0.604
Spray, liquid product	4–5	2.5 (1.4)		0.042		0.16 (1.8)		0.07 (1.6)	

[a]Number of measurements may vary by sample type because of missing data.
[b]Includes one applicator that used both broadcast and hand spray.
[c]Number of tank loads was counted only for period with dermal and air measurements.
[d]Median values reported for chlorpyrifos hand loading, no tests of differences due to number of non-detects.

Table 9. Correlation and variance for the subset of applicators with repeat measurements.[a]

Measurement		Regression[b]	Variance[c]	
	N	r^2	σ^2_W	σ^2_B
Urine day-1 concentration	19	0.66	0.37	1.79
Urine day-1 excretion rate	16	0.57	0.44	1.49
Estimated hand loading	18	0.21	1.61	1.27
Estimated body loading	19	0.43	1.63	3.32
Personal air	19	0.09	2.10	0.81

[a]For a subset of Iowa applicators with repeat visits in the same (48%) or different (52%) year.
[b]Regression of visit 1 and visit 2 natural log of measurement results across applicators with repeat visits.
[c]Within (σ^2_W)- and between (σ^2_B)-person variance, based on natural log of measurement values.

method/formulation combination was the predominant predictor of measurement difference for chlorpyrifos applicators in this study. Although this may reflect different exposure potential based on formulation type, the differences between crops, application methods, or other factors in the two states may also contribute to this result as all of the liquid spray applications were performed in NC. Across the three application methods for 2,4-D and chlorpyrifos, the GM day-1 urine, hand loading, and body loading levels were in the order of in-furrow < broadcast spray < hand spray. GM air concentrations fell in the order of broadcast spray < in-furrow < hand spray.

Approximately half of the repeat measurement visits were within the same year and half in the following year. All but one set of repeat visits involved 2,4-D and included one in-furrow, 12 hand spray, four boom spray, and two hand and boom spray applications. Results are shown in Table 9 for regressions between first and second visits across applicators and for within- and between-person variances. Regressions for urine measures were higher than those for hand, body, and air measures. Within-person variance was lower than between-person variance for urine and estimated body loading measures, whereas the opposite was true for estimated hand loading and personal air concentrations. In two cases, the adjustment for amount of a.i. used decreased within-person differences to less than two-fold, but in two other cases, this adjustment increased differences to greater than two-fold. Five of the 13 applicators who had a two-fold or greater difference in estimated hand loading measurements at different visits also had differences in glove use. Other factors that differed between visits such as performing repairs, contact with sprayed vegetation or equipment, and equipment cleanup may have contributed to within-person variability for some individuals.

Discussion

Challenges were encountered in identifying, recruiting, and monitoring members of a cohort with a wide geographic dispersion and multiple crop-protection product options. Although many farmers agreed to participate in the study, many were not subsequently monitored because of changes in product use plans and scheduling issues. These challenges may be addressed in future studies through decisions on which chemicals to monitor or through different measurement approaches. For example, expanding the number of pesticides that could be monitored would make it more likely that more farmers in a region would be both eligible and able to participate, although the sample analysis might be more difficult and costly. If future studies are designed to make only urinary biomarker measurements, it may be feasible to develop an approach in which participants can collect and return samples without the need for field teams to visit the farm.

We found a wide range in 2,4-D urinary biomarker, estimated hand loading, estimated body loading, and air concentrations for the applicators participating in this study.

Agricultural and forestry worker exposure to 2,4-D has been measured in earlier studies (Draper and Street, 1982; Grover et al., 1986; Abbot et al., 1987; Lavy et al., 1987; Knopp and Glass, 1991) but changes in products, equipment, and practices may have occurred as these studies were conducted. Our results can be compared with more recent measurements made for other applicators. GM urinary 2,4-D levels for broadcast spray applicators in this study (GM 21 μg/l, range 2.5–270 μg/l for day-1 urine samples) were lower than those measured by Acquavella et al. (2006) (GM 64 μg/l, range 2–1856 μg/l). GM results from our study were higher than measurements reported by Arbuckle et al. (2002) (GM 5.4 μg/l, range 0.5–410 μg/l) for 43 Ontario farm applicators. Few recent comparable data are available for 2,4-D dermal and air levels. Hines et al. (2001) reported an adjusted GM hand loading for 2,4-D ethylhexyl ester of 0.16 mg/hand (0.21 mg for two hands calculated as 2,4-D a.e. with an assumption of equal loading on both hands), and air concentrations of 0.36 μg/m^3 (0.24 μg/m^3 as 2,4-D a.e.) across 12 spray days, similar to the GM values of 0.22 mg (both hands) and 0.21 μg/m^3 for broadcast spray applicators in this study. No urinary 2,4-D biomarker data from studies of farmer non-crop hand spray applications were found for comparison. Day-1 urinary levels for hand spray applicators in this study (GM 33 μg/l, range 1.6–1040 μg/l) were lower than those reported by Garry et al. (2001) (GM of 185 µg/l, range 28–1700 μg/l) for seven forestry workers using backpack sprayers. These professional forestry applicators likely sprayed more a.i. on a daily basis in their jobs than the farmers in this study. We found a more limited range of chlorpyrifos measurement results in this study, based on a smaller number of participants and a different mix of application methods. The GM day-1 urine TCP levels in this study were 21 μg/l (range 11–80 μg/l) for spray applicators and 8.3 μg/l (range 2.5–29 μg/l) for in-furrow applications of granular products. Alexander et al. (2006) reported a GM of 31 μg/l following liquid spray applications and 11 μg/l following granular applications based on the maximum urine TCP concentrations in post-application 24 h samples.

Differences in exposure between and within different agricultural applicator populations are likely to be explained by a combination of factors. These may include differences in the amount and duration of pesticide use, handling and application methods and equipment, crop differences, and use of personal protective equipment. Differences in measurement methods and strategies may also lead to different results. For example, urine samples may be collected at different time points and spot urine samples may yield different results than 24-h composite samples. A categorical presentation of results in Figures 2 and 3 reveals some important differences associated with application method and glove use in this study, but the wide range and overlap in many of the distributions also suggests that multiple factors were affecting exposures at an individual level. In general, we found a pattern of increasing exposure from granular in-furrow, to broadcast liquid, to hand spray liquid applications. The use of rubber gloves was associated with lower GM urinary 2,4-D levels in this study, but distributions overlapped and glove use alone did not distinguish between urinary biomarker levels for many applicators. The use of enclosed tractor cabs was associated with lower 2,4-D exposures only when the measurements were adjusted for the amount of a.i. used. Broadcast spray applicator exposures may occur primarily during the mixing/loading operation and may not necessarily depend on the amount of chemical used. We found other factors to be associated with increased levels in some sample types and for some 2,4-D application methods, including equipment repair, use of adjuvants, and minor spills, splashes, or leaks. Each of these factors can affect the amount of contact with the chemical or, in the case of an adjuvant, may affect contact time or dermal absorption. Combinations of these factors likely contribute to the overall range and variability in measured exposures.

Exposure determinant assessments have been reported for broadcast spray applicators for glyphosate (Acquavella et al., 2004), chlorpyrifos (Alexander et al., 2006), atrazine (Perry et al., 2006) and for dithiocarbamates for vineyard workers (Baldi et al., 2006). A multivariate analysis approach has been taken for broadcast spray 2,4-D applicators (Hines et al., 2001, 2003; Arbuckle et al., 2002) or professional turf 2,4-D applicators (Harris et al., 2002). Associations were seen in some studies between exposure and a measure of chemical use, such as the number of tank loads, that may be related to the number of mixing/loading operations or the amount of chemical applied. Many studies report associations between exposure and skin or body contact with the chemical or potentially contaminated surfaces and many, but not all, studies reported reduced exposures with use of protective gloves. Lower chlorpyrifos biomarker concentrations were found by Alexander et al. (2006) and in this study for both biomarker and dermal measures for applicators using granular products compared with those performing liquid spray applications. It is not clear whether this is due to product formulation, the in-furrow application method, a combination of the two, or other crop and method factors that differ between mid-western and southern states. Interpreting chlorpyrifos exposure using urinary biomarkers is also complicated because of the other potential sources of exposure to the pesticide or its metabolite, especially through dietary ingestion (Morgan et al., 2005).

Estimates of absorbed dose may be the most relevant measure for assessing exposures related to agricultural chemical use. However, measuring and interpreting biomarker data can be difficult in an active farm population when products containing the a.i. are likely to be used on the days before and after a monitored use. In this study, there were 14 monitored applications for which there were no reported uses

of products containing 2,4-D on the 4 days preceding and following the monitored application, and the applicator provided complete sets of urine samples over 5 days. Estimated doses were compared with the 15 mg/kg per day intermediate-term dermal/inhalation toxicological end point for occupational risk assessment in the U.S. EPA reregistration eligibility decision (U.S. EPA, 2005), giving an estimated average margin of exposure of 5600 (range 830–47,000). The long-term toxicological significance of these dose levels is less clear when considering that agricultural uses in this cohort typically occur only several days per year but may continue across multiple years. These dose estimates have some uncertainty due to individual differences in 2,4-D absorption, metabolism, and excretion rates and variability in sample-collection durations when compared with the assumption of 90% excretion in 120 h. The measurements may overestimate the dose ascribed to the monitored agricultural application because of existing body burden at the start of the collections and the possibility of additional occupational, residential, or dietary exposure during the measurement period.

Each measurement approach has strengths and limitations. In general, the air concentration did not explain much of the variability in urine concentrations. Dermal measurements were helpful in understanding components of the exposure route, but it is difficult to make dermal measurements in large studies, and they represent only what is present at a boundary, not necessarily what is absorbed. Urinary biomarker measurements have the greatest relevance with regard to internal exposure, but it can be difficult to interpret biomarker measurements given the uncertainties in exposure timing, individual differences in absorption, distribution, metabolism and excretion (ADME), and exposures from other routes and pathways. In future studies in epidemiologic cohorts, it might be recommended to focus on biomarker measurements (when the biomarkers and ADME parameters are reasonably well characterized) and collection of adequate information to improve interpretation and dose estimation. Limited repeat measure results in this study suggest that urinary biomarker measurements could be a reasonable epidemiological tool for exposure classification over longer periods, but more data are needed over longer time periods and for additional pesticides. Improved approaches for data collection and biomarker interpretation that better account for exposure timing uncertainties and individual differences in absorption, metabolism, distribution, and excretion would result in a more accurate dose estimation for risk assessment.

Other limitations apply to this study. Measurements were made for only two chemicals and, although these chemicals and their application methods were selected because of their wide use in the cohort, chemicals with different formulations or physical/chemical properties may result in different internal exposures. Although we have no objective data to evaluate this, it is possible that participants behaved differently during the study with regard to their normal product handling and use practices. Residential and dietary contributions to exposure were not assessed, which complicates the interpretation of urinary biomarkers with regard to understanding important factors and sources that contribute to total exposures, particularly for chlorpyrifos. Measurements in this study were made only once or twice across 1 or 2 years and may not represent product uses in prior years and long-term exposures in the AHS cohort. The study sample was not selected to be representative of the full AHS cohort and extrapolation of measurement distribution parameters beyond the study sample is not recommended.

In summary, we made urinary biomarker, dermal, and air measurements for a subset of farmers in the AHS epidemiological cohort who used several application methods, including agricultural non-crop hand spraying, which has not been widely reported on elsewhere. There was a wide range and considerable variability in 2,4-D measurements. Several factors were associated with differences in 2,4-D measurements, including application method, glove use, and other handling and contact variables. Chlorpyrifos measurements were lower, on average, than those for 2,4-D in this study. Chlorpyrifos measurement results were higher for liquid spray application compared with in-furrow/banded application of granular products. We found significant correlations between the different types of 2,4-D measures, but the smaller sample size and lower rates of detection for hand wipe samples limited the assessment for chlorpyrifos measures. Study results will inform the development of questionnaires for use in agricultural populations and will improve exposure classification in the AHS epidemiological study by providing information for assessment and refinement of the AHS exposure intensity algorithm. Results may also be of use in pesticide safety education for reducing exposure to pesticides.

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