**Assessment of a Pesticide Exposure Intensity Algorithm in the Agricultural Health Study**

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**ABSTRACT**

The accuracy of the exposure assessment is a critical factor in epidemiological investigations of pesticide exposures and health in agricultural populations. However, few studies have been conducted to evaluate questionnaire-based exposure metrics. The Agricultural Health Study (AHS) is a prospective cohort study of pesticide applicators who provided detailed questionnaire information on their use of specific pesticides. A field study was performed for a subset of the applicators enrolled in the AHS to assess a pesticide exposure algorithm through comparison of algorithm intensity scores with measured exposures. Pre- and post-application urinary biomarker measurements were made for 2,4-D (n = 69) and chlorpyrifos (n = 17) applicators. Dermal patch, hand wipe, and personal air samples were also collected. Intensity scores were calculated using information from technician observations and an interviewer-administered questionnaire. Correlations between observer and questionnaire intensity scores were high (Spearman r = 0.92 and 0.84 for 2,4-D and chlorpyrifos, respectively). Intensity scores from questionnaires for individual applications were significantly correlated with post-application urinary concentrations for both 2,4-D (r = 0.42, $p < 0.001$) and chlorpyrifos (r = 0.53, $p = 0.035$) applicators. Significant correlations were also found between intensity scores and estimated hand loading, estimated body loading, and air concentrations for 2,4-D applicators (r-values 0.28–0.50, $p$-values<0.025). Correlations between intensity scores and dermal and air measures were generally lower for chlorpyrifos applicators using granular products. A linear regression model indicated that the algorithm factors for individual applications explained 24% of the variability in post-application urinary 2,4-D concentration, which increased to 60% when the pre-application urine concentration was included. The results of the measurements support the use of the algorithm for estimating questionnaire-based exposure intensities in the AHS for liquid pesticide products. Refinement of the algorithm may be possible using the results from this and other measurement studies.

## INTRODUCTION

Epidemiologic studies provide evidence to evaluate the risk of chronic health effects in humans associated with occupational exposure to pesticides. When interpreting epidemiologic results, the identification of an "exposure-response" relationship is considered an important factor for the identification of causal associations. However, causal inferences from studies of agricultural populations are often limited due to a lack of accurate exposure information and the use of exposure surrogates with unknown levels of exposure misclassification. Improvements in exposure assessment methods that reduce misclassification are needed to increase the power and sensitivity of epidemiologic investigations evaluating the relationship between pesticide exposures and disease (Blair et al., 1990; Zahm et al., 1997; Kromhout and Heederik, 2005).

Studies have often relied on surrogate information to assess exposures to pesticides, including ecological assessments based on crops or pesticide use in certain geographic areas (Muir et al., 2004; Schreinemachers, 2006), the development of job and crop pesticide exposure matrices (Meyer et al., 2006; Young et al., 2004; Wood et al., 2002), and crop mapping information (Ward et al., 2000; Rusiecki et al., 2006). There has been some progress in the quality of exposure assessments, including the use of questionnaires to collect individual information on uses of general pesticide classes or specific pesticides, as well as the measurement of personal exposure or biomarkers of exposure (Fleming et al., 1999; Arbuckle et al., 2002; Baldi et al., 2006). While direct measurement is the preferred approach, it is often not feasible for studies of diseases with long latency periods or for pesticides with short biological half-lives where the timing of measurements around periods of pesticide use is critical. Questionnaires have been used to obtain information about agricultural work practices in lieu of direct measurements (Alavanja et al., 2004). Research to identify specific determinants of exposure indicate that factors such as the pesticide formulations used, mixing/loading and application methods and equipment, personal protective equipment (PPE) use, and hygiene can be important predictors of exposure. Expert assessment combined with models developed from measurement studies can be used to assign an exposure

intensity rating or score based on selected determinants (Garcia et al., 2000). The intensity score may then be used to categorize individual exposures in a cohort, or may be combined with other information such as frequency or duration of pesticide use for exposure classification.

The AHS is a prospective cohort study being performed in the states of Iowa and North Carolina to study the relationship between agricultural exposures and disease (Alavanja et al., 1996 and 1999). The AHS was designed to provide improved exposure assessments as compared to previous studies by collecting information on the lifetime duration and frequency of use for over 50 specific pesticides. Another objective was to obtain information on pesticide application methods and handling procedures that can affect exposure intensity. A total of 52,395 licensed private pesticide applicators and 4,916 licensed commercial pesticide applicators were enrolled in the AHS from 1993 -1997. Self-administered questionnaires were obtained at enrollment and supplemented with a take-home questionnaire to obtain additional information on lifestyle and agricultural exposures. A computer-assisted telephone interview (CATI) was administered about 5-years after enrollment to obtain additional and updated information on pesticide use and other factors since enrollment.

An algorithm was developed for the AHS to estimate pesticide exposure intensity scores using information from the questionnaires on mixing, application, repair, and personal protective equipment (Dosemeci et al, 2002). Exposure intensity scores were multiplied by the lifetime days of use to calculate an intensity-adjusted exposure metric for use in epidemiologic analyses in the AHS. Exposure determinants and scoring weights used in the AHS algorithm were based on information derived from literature reviews and from the Pesticide Handlers Exposure Database (PHED, 1995). However, the measurement data necessary to evaluate the algorithm for pesticide applicators in the AHS cohort were not available. The AHS Pesticide Exposure Study (AHS/PES) was designed to measure urinary biomarker, dermal, and air levels for a subset of 2,4-D and chlorpyrifos applicators in the AHS cohort. Here we describe the evaluation of algorithm intensity scores using the AHS/PES measurement results.

## METHODS

*Study Design*

For this study, we recruited a subset of the AHS cohort who reported the potential use of 2,4-D or chlorpyrifos in an interview prior to the growing season. Initial telephone screening was conducted to recruit participants from the private pesticide applicators enrolled in the AHS cohort who met the following eligibility criteria: a) completed the AHS CATI questionnaire, b) reported use of 2,4-D or chlorpyrifos across a range of methods commonly used in the AHS, and c) resided in selected counties in Iowa and North Carolina. Applicators who reported that they intended to use, or might use a product containing 2,4-D or chlorpyrifos with a broadcast, banded/in-furrow, or hand spray application method on their farm during the following season were visited by study staff. Orchard, animal, and home lawn/garden uses were not eligible. Applicators who participated in either the first (Iowa and North Carolina) or second (Iowa only) year were asked to participate in another monitoring visit, either in the same or following year. Details of the AHS/PES field study design and measurement results have previously been described (Thomas et al, 2009). The study was approved by the Institutional Review Boards at the National Cancer Institute, the University of Iowa, Battelle, and RTI International.

*Monitoring*

Monitoring was performed during growing seasons in 2000 (Iowa only) and in 2001 and 2002 (Iowa and North Carolina) in conjunction with the pesticide mixing, loading, and application (MLA) activities conducted during the course of one day. Applicators followed their usual pesticide handling and application practices. Field staff recorded information about each applicator's pesticide use and work practices during an observed MLA activity on a structured data collection form. The pesticide use component of the AHS Phase II CATI, modified for single-day use, was interviewer-administered to the applicator upon completing the monitored activity.

*Sample Collection*

Details of the sample collection have been previously described (Thomas et al., 2009). Briefly, urine, hand wipe, dermal patch, and personal air samples were collected from all participants. Three urine samples were requested from each applicator. These included a pre-application single-void urine sample collected on the morning of the monitored application; a sample combining all voids from the start of MLA activities that day through their first morning void the next morning (called the Day-1 sample); and, a first morning void collected on the third day after the monitored MLA. Applicators recorded sample collection and previous void times. Field staff recorded the total void volume for each sample. Hand wipe samples were collected at the completion of the monitored MLA activity. Twelve pre-defined locations (3 x 1 cm) on each hand were thoroughly wiped using polyurethane foam-tipped swabs wetted with isopropanol. Swabs were combined, solvent extracted, and analyzed for the applied target pesticide. Patches were applied to 10 locations on the applicator's chest, back, upper arms, forearms, thighs, and lower legs prior to the MLA activity. Chromatography paper patches were used for liquid spray applications while cotton gauze patches were used for granular product applications. 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 top of regular clothing or skin and were under any personal protective equipment worn by the applicator. The applicator wore the patches during the entire MLA activity. Patches were removed and combined for analysis. Separate compositing and analysis was performed for patches placed on skin. Results for these samples were added for estimates of total body loading. Air samples were collected in the applicator's breathing zone for the duration of their monitored MLA activities using a sampler containing a quartz fiber filter and XAD-2 resin attached to a battery-operated pump.

*Sample Extraction*

*Urine Samples:* Urine samples to be analyzed for 2,4-D were warmed to 35ºC and a 5-mL portion of a well-mixed urine sample was removed and hydrolyzed with 0.5 mL of concentrated HCl.

The sample was combined with 1 mL of 1-chlorobutane and boiling chips and heated at 80 - 90ºC for 60 min. The mixture was transferred to a 125-mL separatory funnel. The sample vial was rinsed with 2 x 5 mL portions of dichloromethane, which were added to the funnel with 1 mL of a 20% NaCl solution. After shaking, the dichloromethane layer was funneled through 5 g of $Na2SO_4$ into a tube. The sample was extracted again with 5 mL of dichloromethane which was also funneled through the $Na2SO_4$ into the tube. The $Na2SO_4$ was rinsed with 10 mL of dichloromethane which was added to the tube. The dichloromethane was concentrated to 0.5 mL and added to a clean 8-mL vial with two 0.5 mL dichloromethane rinses. The sample was then concentrated to 1 mL and 50 µL of methanol was added. The sample was derivatized with ethereal diazomethane for 30 min. Two mL of hexane was added and the volume was concentrated to 1 mL. The sample extract was transferred to a pre-conditioned Florisil SPE cartridge with two 0.5-mL hexane rinses. After draining the extract into the SPE bed the cartridge was eluted with three 6-mL portions of 1:1 diethyl ether in hexane. The eluant was concentrated to 0.5 mL and then brought to 1 mL with methyl-t-butyl ether. The procedure for extraction and derivatization of the chlorpyrifos metabolite 3,5,6-trichloro-2-pyridinol (TCP) in urine has been previously described (Morgan et al. 2005).

*Hand Wipe Samples:* For neutral analytes (2,4-D 2-ethylhexyl ester, 2,4-D butoxyethyl ester, and chlorpyrifos) 100 mL of n-hexane/acetone (50:50 v/v) was added to the polyurethane foam in a sealed container and the sample was shaken for 60 min. A second 100-mL portion of solvent was added, the sample was shaken for 15 minutes, and the second portion of solvent was combined with the first. A final 100 mL portion of solvent was added with hand-shaking for 1 min, and the solvent was combined with the other portions. The same extraction procedure was used for acidic analytes (2,4-D acid and 2,4-D dimethyl amine) except the extraction solvent was 2.0 M formic acid in methanol.

*Patch Samples:* For neutral analytes, 200 mL of n-hexane/acetone (50:50 v/v) was added to the patches in a 250-mL glass container. The sample was extracted in the following sequence:

vigorous shaking for 30 s, sonication for 15 min, shaking for 30 s, sonication for 15 min, and shaking for 30 s. The same extraction procedure was used for the acidic analytes except the extraction solvent was 2.0 M formic acid in methanol.

*Air Samples:* The Teflon retaining ring, quartz filter, front and rear XAD-2 resin beds, and the polyurethane foam separator were transferred from the sample cartridge to an 8-mL vial. For neutral analytes 4 mL of dichloromethane was added and the sealed vial was sonicated for 15 min followed by hand shaking for 30 seconds. The sonication and shaking sequence was repeated, then 1 to 2 mL of solvent was removed and filtered through a 0.45 µm PTFE syringe filter. Next, a 0.5-mL portion of methanol was added to a measured amount of sample extract and the combined solvent was concentrated to 0.5-mL in a heating block to remove the dichloromethane. The extraction procedure for the acidic analytes was similar except the extraction solvent was 2.0 M formic acid in methanol and the sample was shaken for 60 min.

*Sample Analysis*

Isotopically-labeled analogs of 2,4-D or TCP were added to each urine sample prior to extraction and analyte concentrations were corrected based on the labeled analog's recovery. Derivatives of 2,4-D and TCP from urine samples and neutral analytes in hand wipe and patch sample extracts were analyzed using gas chromatography/mass spectrometry (HP6890 GC and Model 5973 mass selective detector). Neutral analytes in air samples were analyzed by liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) (PE 200 series liquid chromatograph and PE-SCIEX API 3000 triple quadrupole mass spectrometer) using an atmospheric pressure chemical ionization interface. Acidic analytes in hand wipe, patch, and air sample extracts were analyzed by LC/MS/MS following a previously described procedure (Gardner et al., 2005).

*Intensity Score Calculation*

The AHS algorithm used to calculate exposure intensity scores is from Dosemeci et al. (2002) which used available literature and the Pesticide Exposure Handlers Database to develop

specific scoring weights for four algorithm variables. The algorithm and scoring weights described in Dosemeci et al. (2002) that were used in this analysis are presented below:

Intensity Score = (Mix + Apply + Repair) x PPE

where:

Mix: Applicator personally mixes/loads the pesticide (no =0 or yes =9)

Apply: Application method (banded/in-furrow = 2, broadcast spray = 3, hand spray = 9)

Repair: Applicator personally repaired equipment (no = 0 or yes = 2)

PPE: PPE categories were combined to give score weights from 0.1 to 1.0 (Table 1)

Using this algorithm, two sets of intensity scores were calculated for each AHS/PES monitoring visit. One set of scores was derived from technician observations during the MLA activity and a second set was calculated using the information from the questionnaire administered to the applicator following the MLA. Frequencies of 2,4-D and chlorpyrifos uses in the algorithm factor categories, and their corresponding scoring weights are shown in Supplemental Table S1.

There were several situations associated with the applicator's pesticide handling and use which required decisions for applying scoring weights in the algorithm that were different than the way the algorithm is applied using questionnaire data in the AHS cohort. Information about PPE use in the AHS/PES was recorded separately for the mixing/loading and application stages. The most protective PPE used in either stage was used in calculating PPE scores. Two other approaches for applying the PPE scoring weight were applied to the AHS/PES data, a) averaging mix/load and application PPE scoring weights, and b) separate distribution of PPE scoring weights across mix/load and application. These approaches resulted in slightly lower correlations between intensity scores and exposure measurements (data not shown). Separate intensity scores were calculated for urine, hand loading, body loading, and air concentration because three applicators performed mixing/loading prior to monitoring. There were also four cases where the monitored

applicator did not personally mix/load the product. One applicator used a combination of hand and broadcast spray methods during a monitored application and an application score of 6, an average of the scores for the two methods, was assigned. For 24 participants, wording in the AHS CATI questionnaire adapted for the measurement study inadvertently resulted in missing information for application method (primarily for non-crop applications). For these analyses, application methods were substituted from observation; this appeared to be a reasonable approach based on a 97% agreement rate for those application methods that were reported.

*Data Analysis*

Urinary biomarker concentrations can be affected by variable urine volumes. Creatinine adjustment is sometimes used to correct for variable urine volumes. However, there can be considerable between- and within-individual variability in creatinine excretion and declining excretion with age (Boeniger et al., 1993). Therefore, we decided to address variable urine volumes by calculating urinary biomarker excretion rates in the following manner:

$$Urinary\ Biomarker\ \ Excretion\ Rate\ (\mu g/h) = \frac{\text{Urine volume (L) x Analyte concentration } (\mu g/L)}{\text{Total time between voids (h)}}$$

This approach assumes that the entire urine void volume is collected and that the participant provides accurate information regarding their void times. Information needed to calculate excretion rates was available for 86% of the samples.

Pre-application and post-application (Day-1 composite sample) urinary biomarker concentrations and excretion rates were used in the analyses reported here and are designated PRECONC, PRERATE, PSTCONC, and PSTRATE. One chlorpyrifos Day-1 composite sample measurement was excluded from analysis because the low sample volume (0.18 L) and estimated 24-h creatinine excretion of 0.25 g (below the lower 95% confidence interval of 0.5 g/day, Boeniger et al., 1993) indicated incomplete collection. Hand loadings (designated as HAND) were estimated

using hand surface areas from hand tracings. An estimate of total body loading (designated BODY), not including the hand area, was made based on the overall combined patch loading values applied to the total standard body surface area (U.S. EPA, 1996). Analyte concentrations in air (designated AIR) were calculated based on the sampling flow rate and sample collection duration. In several cases two hand wipe, patch, or air samples were collected over the course of a monitored use; in these cases the measurement results were added together to produce a single measurement result for this analysis.

Method detection limits (MDL) were calculated for each urine, hand wipe, patch, and air sample from the standard deviation of analyte amounts measured on field blanks multiplied by the student's t-value at the 0.99 level (U.S. EPA 1986). For hand wipe, patch, and air samples with multiple 2,4-D analytes the acid equivalent of the highest MDL value obtained from field blank measurements was used for replacement. 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 which had variable endogenous levels of the target compounds in field blanks. If any amount of target analyte lower than the MDL was detected and reported, then that value was used in data analysis (Clayton et al., 2003). If the reported value was zero, then it was replaced with the value of the MDL/$\sqrt{2}$ as has been shown to be appropriate for correlation analyses when the percentage of censored data is <30% (Clayton et al., 2003). In the AHS/PES, the percentage of non-zero analysis results was ≥ 95% for urine samples and ≥ 85% for all other samples, except for chlorpyrifos hand loading samples (53%) (Thomas et al., 2009).

For 19 of the applicators in this study repeat visits were made where the same chemical and application method were used. The results for the first visit were used in this analysis to evaluate the algorithm. Two applicators had a second monitoring visit in which a different chemical was used and one applicator used a different application method. These three observations were treated as independent for data analysis. An assessment of within and between-applicator measurement results based on the subset of applicators with repeat visits using the same chemical

and application method was performed using linear regression and covariance parameter estimation.

Spearman correlations between the intensity scores from observations and questionnaires and between the intensity scores and measurement results were determined. Multiple linear regression analysis was performed using the natural log-transformed (ln-transformed) values of 2,4-D PSTCONC as the dependent variable and the four algorithm factors (MIX, APPLY, REPAIR, PPE) as independent variables, with and without including PRECONC as a covariate. Two categories were formed for each of the MIX, APPLY, and REPAIR algorithm variables using the score weights described earlier. However, three participants had a score factor not corresponding to the standard score factor categories. Score assignment decisions were made in order to place them into the most appropriate dichotomous category. One participant had a MIX score factor of 3 and was placed in the 0 score factor group for regression analysis because he did not mix/load on the day of application. The participant with an APPLY score factor of 6 was placed into the 9 score factor group because hand spraying could potentially result in the highest exposures. Similarly, one participant with an APPLY score factor of 2, based on a banded liquid spray application, was placed in the 3 score factor group for the regression analysis based on the similarity with liquid broadcast application.

Epidemiological analyses are often conducted based on tertiles or quartiles of exposure categorization. The 2,4-D measurement results in this study were grouped based on approximate tertiles of the intensity scores obtained from the questionnaire. Grouping was performed to avoid splitting equal intensity scores, so the resulting group sizes were slightly unequal (the number for each category was Low =22, Medium=22, and High=25). The differences between means of ln-transformed 2,4-D measurements grouped by these three intensity score tertile categories were tested for significance using two-sided t-tests. Exact categorical agreements for intensity scores and measurements were calculated along with the percentage of one-category and two-category differences. Finally, a Chi-square test for independence was performed for the three categories

based on the 2,4-D measurements and those based on the questionnaire intensity scores. All analyses were performed using SAS V9.2 (SAS Institute, Cary, NC).

**RESULTS**

Products containing 2,4-D or chlorpyrifos were used 69 and 17 times, respectively, in the first-visit applications monitored in this study. Products containing 2,4-D were applied using either broadcast spray (n=42) or hand spray (n=25) methods, with the exception of one application using a directed spray method and another using a combination of broadcast and hand spraying. Applicators used in-furrow granular (n = 12) or broadcast/directed liquid spray (n = 5) application methods for chlorpyrifos products. 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 decreasing use of chlorpyrifos on corn in Iowa during the study period and because use of chlorpyrifos products for tomato crops, previously reported with moderate frequency in North Carolina, was rescinded by EPA in 2001.

A summary of urinary biomarker, dermal, and air measurement results is provided in Table 2. Urinary pre-application concentrations (PRECONC) were higher than post-application levels (POSTCONC) for 8 of the participants who used 2,4-D and 6 participants who used chlorpyrifos. In some cases the higher pre-application levels corresponded to a reported prior use of a product containing a target chemical. Wide ranges in 2,4-D measurements were observed for all media, with lower geometric mean (GM) levels and smaller ranges measured in all media except air for chlorpyrifos. When chlorpyrifos was applied using an in-furrow method for granular products, urinary biomarker GM levels were 2.5-fold lower (8.3 vs. 21 µg/L) and estimated body loading GM levels were 6-fold lower (0.17 vs. 1.0 mg) when compared to liquid spray applications. Chlorpyrifos was not detected in the hand loading measurements for a majority of applicators who used an in-furrow granular application method but was measured for all applicators using liquid spray

application methods. More detailed monitoring and measurement results have been described in Thomas et al., 2009.

Algorithm intensity scores ranged from 1.8 to 20 for the 2,4-D applicators and from 4.4 to 14 for the chlorpyrifos applicators in this study (Table 3). The narrower intensity score range for the chlorpyrifos applicators reflected smaller differences in the APPLY algorithm scoring weight (3 vs. 2) for application methods and because all of the chlorpyrifos applicators personally performed mixing/loading. Algorithm intensity scores calculated from technician observations and from the interviewer-administered questionnaire were highly correlated for both 2,4-D and chlorpyrifos (Table 3). Among the 2,4-D applicators, Spearman correlations between the questionnaire algorithm intensity score and the corresponding measurements were 0.42 (PSTCONC), 0.50 (HAND), 0.28 (BODY), and 0.28 (AIR) with all *p*-values <0.03 (Table 4). The Spearman correlation between questionnaire algorithm intensity scores and chlorpyrifos PSTCONC measurements (0.53, $p$ = 0.046) was higher than that for PSTRATE (0.25, $p$ = 0.376) (Table 4). Across all chlorpyrifos applicators, body loading measurements were significantly correlated with intensity scores from questionnaires (r=0.50, $p$=0.039) but not from observations (r=0.18, $p$=0.482). Although the algorithm application method scoring weights were similar for chlorpyrifos applicators who used either the liquid spray or granular in-furrow method, the correlations are shown separately in Table 5 because of the differences in measurement results for these two application methods. Correlations between intensity scores and the chlorpyrifos urinary biomarker level or dermal measures for liquid spray applications ranged from 0.6 - 0.9, but the number of observations was small (4 or 5). Correlations were lower for granular in-furrow chlorpyrifos applications, ranging from 0.02 - 0.58, with the highest correlation with PSTCONC (Table 5). Dermal measures for in-furrow granular applications of chlorpyrifos were very low and not correlated with intensity scores.

Results for a multivariate linear regression model with the ln-transformed post-application urine 2,4-D concentration as the dependent variable are shown in Table 6. The model explained 24% of the variability in post-application urine concentration ($p$ = 0.0018) and the PPE scoring

weight was the only significant parameter. When the ln-transformed 2,4-D pre-application urine concentration (PRECONC) values were added, the model explained 60% of the variability in PSTCONC. In the model that included PRECONC, the APPLY and REPAIR parameter estimates increased and were marginally significant (*p*-values <0.10) while the PPE parameter remained significant. While the MIX score was not identified as an important parameter in the model, only four observations were included for which the applicator did not perform mixing, so there may not have been sufficient numbers in the two categories to fully assess this factor.

The algorithm intensity scores obtained from the questionnaires were divided into approximate tertiles. This resulted in an increasing trend in the GM of 2,4-D measurement results for all media across the three intensity score categories (Table 7). Distributions of Day-1 post-application urinary 2,4-D concentrations in the three categories are shown in Figure 1. Urinary biomarker levels and estimated hand loadings among applicators in the highest tertile were significantly higher ($p < 0.05$) than those in the lower two groups. Differences between the middle and lowest tertiles were not significant. Agreement between the tertile categories of both the intensity scores and the 2,4-D measurement results are shown in Table 8. Exact categorical agreement was found for 44 - 56% of the urinary biomarker and hand loading measurements and only 8 - 12% of these measurements had two-category differences. Body loading and air measurements had lower exact category agreement (37 - 46%) and higher frequency of two-category differences (15 - 19%). Chi-square tests show the agreement between intensity score category and urinary biomarker and hand loading measurement categories were unlikely to be due to chance ($p < 0.05$) The GM of the chlorpyrifos measurements were also higher by 1.5-fold (PSTRATE) to 5.7-fold (BODY) in high vs. low categories of intensity scores. However, the number of chlorpyrifos observations in each category was small and categorical analysis was not performed.

The 19 applicators with repeat monitoring visits included 2,4-D boom-spray applications (n=4), 2,4-D hand spray applications (n=14), and in-furrow granular chlorpyrifos product application

(n=1). The intervals between visits ranged from one week to 14 months. The within-person variability in intensity scores from questionnaires was smaller than the between-person variability ($\sigma^2_w$ = 4.7 and $\sigma^2_b$ = 23.4 for questionnaire-derived scores). Similar ratios of within-person to between-person variances were found for ln-transformed PSTCONC and PSTRATE. A regression of first-visit questionnaire intensity scores with second-visit scores across the 19 applicators resulted in an $r^2$ = 0.70, while $r^2$ values for ln-transformed PSTCONC and ln-transformed PSTRATE were 0.66 and 0.57, respectively.

**DISCUSSION**

Epidemiological studies examining relationships between pesticide use and health in agricultural populations often rely on questionnaires to determine which pesticides or classes of pesticides were used, and to collect some information about when they were used and duration of use. The accuracy of self-reported information on specific pesticides used and their duration of use have been demonstrated among applicators in the AHS (Hoppin et al., 2002; Blair et al. 2002). However, it may be possible to improve exposure classifications based only on duration of use by using questionnaire information regarding work practices that affect exposure intensities (Hoppin, 2005). Questionnaires used to collect pesticide handling and work practice information in the AHS have included both written respondent-completed forms (used at enrollment) and telephone interviews (administered in a 5-year follow-up). In the AHS/PES, a similar questionnaire was interviewer-administered to the applicator following a monitored pesticide application. Algorithm intensity scores from observations and the interviewer-administered questionnaires were highly correlated with each other indicating that, at least within short time frames, an interviewer-administered questionnaire can produce reliable information about important work factors in this population.

The AHS/PES made measurements for a subset of 2,4-D and chlorpyrifos applicators in the AHS cohort and assessed the relationships between measurements and scores from an exposure

intensity algorithm. The assessment was made using urinary biomarkers as well as measures of external exposures (dermal and air measures). Urinary biomarker measurements can provide information that is considered to be more directly related to absorbed dose. However, interpreting biomarker measurements with regard to a specific application event can be problematic due to a number of factors, including the influence of pesticide use before or following the monitored event. Dermal and air measurements relate more directly to algorithm scores because they measure only the exposures that occur during a specific monitored event on which the score is based, and they provide information on exposure pathways. The AHS exposure intensity algorithm was developed using information primarily based on dermal measurements (Dosemeci et al., 2002). Assessment of correlations between exposure intensity scores and body loading estimates in this study may be impacted by the choice to minimize participant burden by applying dermal patches over clothing for most body locations, so that the measurement may not directly represent external dermal exposure.

Urine measurements for applicators applying the same pesticide for a single day varied more than two orders of magnitude for 2,4-D and more than one order of magnitude for chlorpyrifos, reinforcing the fact that exposure for a single day of pesticide application can be highly variable. This variability in exposure is dependent on multiple determinants, including but not limited to the type of application method, amount of pesticide applied, duration of use, PPE use, as well as other hygiene-related factors. Mixing, application, repair, and PPE factors are included in the AHS exposure intensity algorithm. Algorithm intensity scores calculated from both observations and questionnaires were significantly correlated with urinary biomarker, hand, body, and air measurements for 2,4-D in this study. Significant correlations were also measured between algorithm intensity scores and urinary biomarker concentrations (but not excretion rates) across all chlorpyrifos applications. Urinary biomarker and dermal measurements for in-furrow applications of granular chlorpyrifos products were lower than those measured for liquid spray applications. The physical state of the product used (liquid vs. granular) was not included in the algorithm, and the measures for applicators who used a granular product with an in-furrow method were not as well

correlated with the algorithm scores when compared to applicators who used liquid spray, although the number of participants in the latter category was small.

Correlations and categorical evaluations between urinary biomarker concentrations and algorithm intensity scores have been previously assessed among orchard fungicide applicators in the AHS (Hines et al., 2008) and in two other farming populations, the Pesticide Exposure Assessment Survey (PEAS) in Ontario, Canada (Coble et al., 2005), and the Farm Family Exposure Study (FFES) in Minnesota and South Carolina (Acquavella at al., 2006). In the PEAS, Spearman correlations between urine concentrations of 2,4-D and intensity scores based on questionnaires from individual applications were 0.39 and 0.49, respectively, for 24-h urine samples collected one and two days after an agricultural application. In the FFES, correlations of 0.45 and 0.25 were reported between 24-h urinary 2,4-D concentrations and intensity scores derived from observation and farmer-completed questionnaires from individual applications, respectively. PEAS and FFES correlations between urinary 2,4-D biomarker concentrations and intensity scores were similar to correlations determined here for applicators from the AHS cohort. Categorical analysis in the PEAS, FFES, and AHS/PES showed increasing GM 2,4-D levels with increasing tertiles or quartiles of intensity scores. Spearman correlations between intensity scores from observations and urinary TCP concentrations were lower in the FFES (0.12) than in this study (0.64). However, the correlation between intensity scores and the TCP urinary excretion rate in the AHS/PES was 0.36 and it is possible that urine volume differences affected the concentration-based correlation for chlorpyrifos applicators. Among AHS orchard captan applicators in the AHS, exposure intensity algorithm scores were associated with some dermal patch measures, but were not significantly associated with air, hand rinse, or urinary biomarker levels; possibly as a result of assignment of equal scoring weights for air blast and hand spray application methods in the algorithm (Hines et al., 2008).

A factor that could potentially affect the association observed between the algorithm intensity scores and the urinary biomarker measurements are pre-application urine levels that, in

some cases, exceeded post-application levels. This occurred for eight 2,4-D applicators and six chlorpyrifos applicators. In some cases, higher pre-application biomarker levels were associated with reported uses of a product containing the target pesticide in the days prior to monitoring. In other cases, there were no reported uses, and prior exposures may have resulted from unreported product use or contact with contaminated equipment or clothing. In the case of chlorpyrifos, most of the U.S. population has measurable urinary levels of TCP that may result from dietary and residential exposures to chlorpyrifos or the metabolite itself (CDC, 2005; Morgan et al., 2005). Inclusion of pre-application urinary concentrations in a regression model with the algorithm scoring factors substantially increased the amount of variability accounted for in post-application 2,4-D biomarker levels (from 24 to 60%) with higher parameter estimates and lower p-values for application and repair algorithm factors. Previous exposures and elevated pre-application urinary biomarker background levels may affect correlation and categorical assessments between measurements and algorithm scores in this or other studies. Accurate adjustment of post-application urinary biomarker concentrations or excretion rates for exposures prior to the monitoring day would require detailed information on the timing, duration, and amount of previous exposures and accurate models of uptake and elimination kinetics.

The algorithm was designed to estimate exposure intensity based on information available from the AHS enrollment questionnaire. However, measurement variability in the AHS/PES was not fully explained by differences in algorithm intensity scores. Factors other than those included in the algorithm can also affect exposures, and may explain why several relatively high or low measurements for applicators were not consistent with the algorithm scores (i.e., those with two-category differences). Several potential factors not included in the algorithm were directly assessed for applicators in this study (Thomas et al. 2009). Based on these measurements, chemical- or application method-specific differences were found in one or more of the measured media for the use of an adjuvant, minor spills/splashes/leaks, contact with sprayed vegetation, physical state (liquid vs. granular), amount or duration of a.i. use, and wind or temperature conditions. While it

was feasible to collect detailed information on many potential exposure factors for a small number of applicators over a short time period in the AHS/PES, it would be difficult to obtain usable information for many of these factors using a questionnaire that provides information regarding general practices across long time periods in an epidemiological cohort.

There are some limitations in applying information from this study to the overall AHS. The study sample was not selected to be representative of the full AHS cohort and some selection bias was possible. However, while participating applicators were somewhat younger, had somewhat more education, and were less likely to be current smokers than those that did not participate in the AHS/PES, the groups had similar levels of experience applying pesticides (Thomas et al., 2009). Measurements were made for only two chemicals, 2,4-D and chlorpyrifos, and the number of monitored chlorpyrifos applications was relatively small. Multiple repeated measurements for individual applicators would provide a more accurate estimate of the individual applicator's actual exposure for categorization of exposure in the cohort. However, the multiple measures needed for this type of assessment would be difficult to perform given the participant burden, limited number of independent applications within a year for many products, and year-to-year decisions on product use. The limited set of repeat measurements in this study suggests smaller intra-individual vs. inter-individual variances in intensity scores and urinary biomarker measurements over time periods ranging from weeks to 14 months. However, additional repeated measures are needed to confirm these findings for more applicators and over longer time frames. Finally, the assessment made in this study is based on current uses and does not directly assess relationships between retrospective questionnaire information and algorithm scores for past pesticide uses.

This study provides a direct assessment of the intensity score algorithm within the AHS cohort for several application methods. Both objective observer-based and questionnaire-based methods for capturing algorithm factor information were used and provided similar results with regard to agreement between measurements and factors. The results from this study indicate that algorithm exposure intensity scores based on self-reported data are significantly related to

measured levels. The Spearman correlations used to assess the relative ranking of subjects based on the algorithm scores compared with measurements showed moderate associations. This analysis demonstrates that applicators with lower algorithm scores do have, on average, lower exposure measurements. Comparison of intensity scores with the measurements supports the use of the algorithm for estimating subject-specific exposure intensities in the AHS. It is important to recognize that correlations between algorithm intensity scores and measurements reported in this study are based on individual application days, while the algorithm is applied across cumulative lifetime days of pesticide use in the epidemiological analyses. In a prospective study such as the Agricultural Health Study, participants report exposure prior to the onset of disease, and exposure misclassification is expected to be non-differential and would tend to bias estimates of relative risks toward the null (Checkoway et al., 2004). While further refinement of the algorithm scoring may be warranted based on information from this and other studies, the use of measurement data to evaluate the exposure algorithm is a unique strength of the AHS exposure assessment.

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Table 1. Algorithm score factors for personal protective equipment (PPE)

| PPE Categories[a] | | | | |
|---|---|---|---|---|
| PPE-3 | PPE-2 | PPE-1 | PPE-0 | Overall PPE Score |
| Yes | Yes | Yes | No | 0.1 |
| Yes | Yes | No | No | 0.3 |
| Yes | No | Yes | No | 0.4 |
| No | Yes | Yes | No | 0.5 |
| Yes | No | No | No | 0.6 |
| No | Yes | No | No | 0.7 |
| No | No | Yes | No | 0.8 |
| No | No | No | Yes | 1.0 |

[a]PPE-3: Chemically resistant rubber gloves
PPE-2: Cartridge respirator or gas mask
Disposable outer clothing
PPE-1: Face shields or goggles
Fabric/leather gloves
Other protective clothing, such as boot
PPE-0: PPE not used

Table 2. Summary of 2,4-D and chlorpyrifos measurement results from the AHS Pesticide Exposure Study in Iowa and North Carolina

| | 2,4-D | | | Chlorpyrifos | | |
|---|---|---|---|---|---|---|
| Measurement[a] | N | GM (GSD) | Range | N | GM (GSD) | Range |
| PRECONC (µg/L) | 68 | 7.8 (4.7) | ND[b] - 210 | 15 | 11 (2.9)[c] | 2.1 - 63 |
| PSTCONC (µg/L) | 68 | 25 (4.1) | 1.6 - 970 | 16 | 11 (2.3)[c] | 2.5 - 80 |
| PRERATE (µg/h) | 60 | 0.29 (4.9) | ND - 12 | 14 | 0.52 (2.4)[c] | 0.10 – 2.0 |
| PSTRATE (µg/h) | 59 | 1.3 (4.0) | 0.07 - 22 | 15 | 0.44 (1.8)[c] | 0.18 – 1.6 |
| HAND (mg) | 68 | 0.39 (9.2) | ND - 22 | 17 | 0.02[d] | ND – 0.93 |
| BODY (mg) | 69 | 2.9 (12) | 0.02 - 880 | 17 | 0.28 (5.1) | ND – 5.8 |
| AIR (µg/m$^3$) | 68 | 0.37 (5.8) | ND - 10 | 17 | 0.49 (3.0) | 0.05 – 2.0 |

[a] PRECONC = pre-application concentration in urine, PSTCONC = post-application concentration in urine, PRERATE = pre-application urinary excretion rate, PSTRATE = post-application urinary excretion rate, HAND = estimated hand loading, BODY = estimated body loading not including hands, AIR = personal air concentration
[b] ND = target analyte was not detected
[c] For chlorpyrifos, the urinary biomarker 3,5,6-trichloro-2-pyridinol (TCP) was measured
[d] Median value; 47% of measurements were non-detects

Table 3. Algorithm intensity scores from observations and an interviewer-administered questionnaire and correlations between scores[a]

| | | Observation | | Questionnaire | | Spearman | |
|---|---|---|---|---|---|---|---|
| | N | Mean ± SD | Range | Mean ± SD | Range | r | *p* |
| 2,4-D | 69 | 9.9 ± 4.5 | 1.8 - 20 | 10.3 ± 4.6 | 3.0 - 20 | 0.92 | <0.001 |
| Chlorpyrifos | 17 | 9.2 ± 2.4 | 4.4 - 14 | 9.4 ± 2.6 | 6.6 - 14 | 0.84 | <0.001 |

[a] Based on scores calculated for post-application urine samples

Table 4. Spearman correlations between measurements and algorithm intensity scores from observation and from an interviewer-administered questionnaire

| | | Observation | | Questionnaire | |
|---|---|---|---|---|---|
| Measurement | N | r | *p* | r | *p* |
| *2,4-D* | | | | | |
| PSTCONC | 68 | 0.39 | 0.001 | 0.42 | <0.001 |
| PSTRATE | 59 | 0.33 | 0.011 | 0.40 | 0.002 |
| HAND | 68 | 0.48 | <0.001 | 0.50 | <0.001 |
| BODY | 69 | 0.31 | 0.008 | 0.28 | 0.022 |
| AIR | 68 | 0.26 | 0.030 | 0.28 | 0.023 |
| *Chlorpyrifos* | | | | | |
| PSTCONC | 16 | 0.64[a] | 0.008 | 0.53[a] | 0.035 |
| PSTRATE | 15 | 0.36[a] | 0.194 | 0.25[a] | 0.376 |
| HAND | 17 | 0.17 | 0.512 | 0.37 | 0.140 |
| BODY | 17 | 0.18 | 0.482 | 0.50 | 0.039 |
| AIR | 17 | 0.47 | 0.057 | 0.28 | 0.269 |

[a] The urinary biomarker 3,5,6-trichloro-2-pyridinol (TCP) was measured

Table 5. Spearman correlations between chlorpyrifos measurements for liquid and granular applications and algorithm intensity scores from an interviewer-administered questionnaire

| | Liquid Spray | | | Granular In-Furrow | | |
|---|---|---|---|---|---|---|
| Measurement | N | r | *p* | N | r | *p* |
| PSTCONC[a] | 4 | 0.80 | 0.200 | 12 | 0.58 | 0.046 |
| PSTRATE[a] | 4 | 0.80 | 0.200 | 11 | 0.20 | 0.56 |
| HAND | 5 | 0.90 | 0.037 | 12 | 0.13 | 0.69 |
| BODY | 5 | 0.60 | 0.285 | 12 | 0.39 | 0.21 |
| AIR | 5 | 0.80 | 0.104 | 12 | 0.02 | 0.95 |

[a] The urinary biomarker 3,5,6-trichloro-2-pyridinol (TCP) was measured

Table 6. Multivariate regression results for algorithm score factors from an interviewer-administered questionnaire and urinary 2,4-D concentrations

| | Not Including Pre-Application Urine Concentration | | Including Pre-Application Urine Concentration | |
|---|---|---|---|---|
| Dependent Variable | lnPSTCONC[a] | | lnPSTCONC | |
| Overall Model Results | | | | |
| N[b] | 67 | | 66 | |
| F-Value | 4.85 | | 18.1 | |
| $r^2$ | 0.24 | | 0.60 | |
| *p* | 0.0018 | | <0.0001 | |
| Independent Variables[c] | PE[d] | *p* | PE | *p* |
| Intercept | 0.68 | 0.421 | 0.34 | 0.579 |
| MIX Score (0 or 9) | 0.39 | 0.553 | -0.09 | 0.850 |
| APPLY Score (3 or 9) | 0.20 | 0.536 | 0.45 | 0.067 |
| REPAIR Score (0 or 2) | 0.29 | 0.363 | 0.41 | 0.085 |
| PPE Score (0.3 – 1.0) | 2.78 | 0.0001 | 2.06 | 0.0001 |
| lnPRECONC[e] | - | - | 0.56 | <0.0001 |

[a] lnPSTCONC is the ln-transformed post-application urinary 2,4-D concentration
[b] The N values are lower than 69 due to one or more missing variable values.
[c] For Mix, Apply, and Repair variables the lower score is the reference value.
[d] Parameter estimate
[e] lnPRECONC is the ln-transformed pre-application urinary 2,4-D concentration

Table 7. Low, medium, and high algorithm intensity score categories from interviewer-administered questionnaires and corresponding 2,4-D measurement levels

| | | | Exposure Intensity Score | |
|---|---|---|---|---|
| | Category | N | Mean ± SD | Range |
| Intensity Score | Low | 22 | 5.5 ± 1.7 | 3.0 - 7.2 |
| | Medium | 22 | 9.4 ± 1.2 | 8.4 - 11.2 |
| | High | 25 | 15.2 ± 3.2 | 12.0 - 20.0 |
| | | | 2,4-D Measurements | |
| | Category | N | GM (gsd) | Range |
| PSTCONC (µg/L) | Low | 21 | 13** (3.5) | 2.5 - 170 |
| | Medium | 22 | 19* (3.0) | 2.5 - 180 |
| | High | 25 | 52 (4.3) | 1.6 - 970 |
| PSTRATE[b] (µg/h) | Low | 16 | 0.79** (2.8) | 0.14 - 7.7 |
| | Medium | 21 | 0.83** (3.3) | 0.10 - 6.7 |
| | High | 22 | 2.8 (4.5) | 0.07 - 22 |
| HAND (mg) | Low | 21 | 0.13** (9.7) | 0.003 - 11 |
| | Medium | 22 | 0.25* (5.4) | 0.012 - 6.5 |
| | High | 25 | 1.4 (7.2) | 0.001 - 22 |
| BODY (mg) | Low | 22 | 1.4 (11) | 0.058 - 100 |
| | Medium | 22 | 3.6 (10) | 0.058 - 640 |
| | High | 25 | 4.8 (15) | 0.020 - 880 |
| AIR (µg/m$^3$) | Low | 22 | 0.24 (4.0) | 0.039 - 8.8 |
| | Medium | 21 | 0.38 (7.4) | 0.003 - 10 |
| | High | 25 | 0.54 (6.2) | 0.010 - 7.7 |

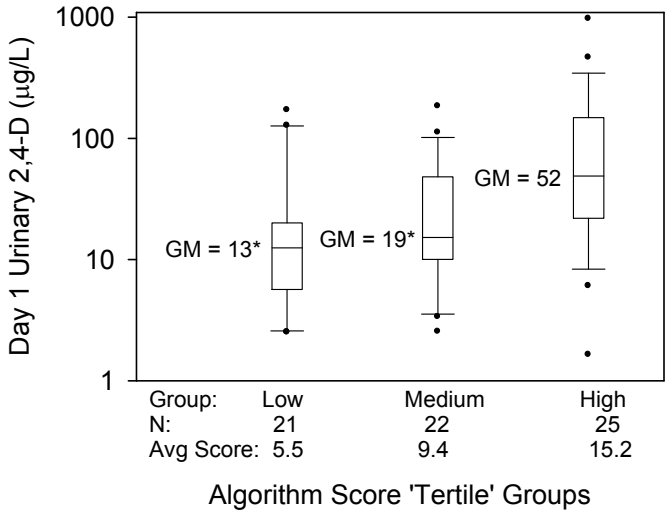
**p-value <0.005 or *p-value <0.05 for t-test of difference between this group and the high group using ln-transformed measurement results.

Table 8. Categorical agreement between interviewer-administered questionnaire algorithm intensity scores and 2,4-D measurements for low, medium, and high groups

| | Categorical Difference (%)[a] | | | Chi-Square | |
|---|---|---|---|---|---|
| Measurement | 0 | 1 | 2 | Statistic | p-value |
| PSTCONC | 50 | 38 | 12 | 10.4 | 0.034 |
| PSTRATE | 44 | 48 | 8 | 9.3 | 0.053 |
| HAND | 56 | 35 | 9 | 19.7 | <0.001 |
| BODY | 46 | 35 | 19 | 6.5 | 0.162 |
| AIR | 37 | 48 | 15 | 7.8 | 0.099 |

[a] 0 = exact agreement, 1 = one-category difference, and 2 = two-category difference

Figure 1. Distributions of Day-1 post-application urinary 2,4-D concentrations across three “tertiles” of algorithm intensity scores (GM values for low and medium groups are significantly different from the GM in the high group).



Supplemental Table for "Assessment of a Pesticide Exposure Intensity Algorithm in the Agricultural Health Study" K. Thomas et al.

Table S1. Intensity algorithm factors, scoring weights, and frequencies in the AHS Pesticide Exposure Study.

| | Measurement Media and Scoring Weights | | | | Frequency in the AHS/PES | |
|---|---|---|---|---|---|---|
| AHS Exposure Intensity Algorithm Factors | Urine | Air | Body | Hand | 2,4-D | CHL |
| **MIX** | | | | | | |
| Personally mixed/loaded | 9 | 9 | 9 | 9 | 63 | 16 |
| Personally mixed/loaded prior to field team arrival | 9 | 0 | 0 | 9 | 1 | 0 |
| Personally mixed/loaded one day prior to monitoring | 6 | 0 | 0 | 0 | 0 | 1 |
| Personally mixed/loaded two days prior to monitoring | 3 | 0 | 0 | 0 | 1 | 0 |
| Did not personally mix/load for the monitored use | 0 | 0 | 0 | 0 | 4 | 0 |
| **APPLY** | | | | | | |
| Banded, directed, or in-furrow liquid spray | 2 | 2 | 2 | 2 | 1 | 2 |
| In-furrow granular application | 2 | 2 | 2 | 2 | 0 | 12 |
| Broadcast spray from tractor or vehicle | 3 | 3 | 3 | 3 | 42 | 2 |
| Hand spray and broadcast spray from vehicle | 6 | 6 | 6 | 6 | 1 | 0 |
| Hand held sprayer, from tractor or vehicle | 9 | 9 | 9 | 9 | 20 | 0 |
| Hand held sprayer, not from a vehicle | 9 | 9 | 9 | 9 | 5 | 0 |
| Fogger/airblast spray | 9 | 9 | 9 | 9 | 0 | 1 |
| **REPAIR** | | | | | | |
| Yes | 2 | 2 | 2 | 2 | 23 | 8 |
| No | 0 | 0 | 0 | 0 | 46 | 9 |
| **PPE Category** | | | | | | |
| PPE-1 & PPE-2 & PPE-3 | 0.1 | 0.1 | 0.1 | 0.1 | 0 | 0 |
| PPE-2 & PPE-3 | 0.3 | 0.3 | 0.3 | 0.3 | 9 | 0 |
| PPE-1 & PPE-3 | 0.4 | 0.4 | 0.4 | 0.4 | 0 | 1 |
| PPE-1 & PPE-2 | 0.5 | 0.5 | 0.5 | 0.5 | 0 | 0 |
| PPE-3 | 0.6 | 0.6 | 0.6 | 0.6 | 33 | 8 |
| PPE-2 | 0.7 | 0.7 | 0.7 | 0.7 | 4 | 0 |
| PPE-1 | 0.8 | 0.8 | 0.8 | 0.8 | 3 | 2 |
| PPE-0 | 1.0 | 1.0 | 1.0 | 1.0 | 20 | 6 |