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# Predictors of 2,4-dichlorophenoxyacetic acid exposure among herbicide applicators

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## Abstract

To determine the major factors affecting the urinary levels of 2,4-dichlorophenoxyacetic acid (2,4-D) among county noxious weed applicators in Kansas, we used a regression technique that accounted for multiple days of exposure. We collected 136 12-h urine samples from 31 applicators during the course of two spraying seasons (April to August of 1994 and 1995). Using mixed-effects models, we constructed exposure models that related urinary 2,4-D measurements to weighted self-reported work activities from daily diaries collected over 5 to 7 days before the collection of the urine sample. Our primary weights were based on an earlier pharmacokinetic analysis of turf applicators; however, we examined a series of alternative weighting schemes to assess the impact of the specific weights and the number of days before urine sample collection that were considered. The derived models accounting for multiple days of exposure related to a single urine measurement seemed robust with regard to the exact weights, but less to the number of days considered; albeit the determinants from the primary model could be fitted with marginal losses of fit to the data from the other weighting schemes that considered a different numbers of days. In the primary model, the total time of all activities (spraying, mixing, other activities), spraying method, month of observation, application concentration, and wet gloves were significant determinants of urinary 2,4-D concentration and explained 16% of the between-worker variance and 23% of the within-worker variance of urinary 2,4-D levels. As a large proportion of the variance remained unexplained, further studies should be conducted to try to systematically assess other exposure determinants.

### Keywords

2,4-D; biomonitoring; herbicide; exposure determinants

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## Introduction

Urinary measurements have been used to aid in exposure assessment of the commonly used herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) (Harris et al., 2002Harris et al., 2005; Alexander et al., 2007). As there are few metabolic intermediates, 2,4-D is excreted largely unmodified in the urine. It continues to be excreted in urine several days after exposure; therefore, exposure assessment of applicators with multiple days of exposure requires the collection of multiple urine samples (Alexander et al., 2007) or the use of models incorporating pharmacokinetic knowledge of 2,4-D when relating determinants of exposure to single urinary measurements (Harris et al., 2001, 2002).

We monitored exposure among 31 seasonal 2,4-D applicators employed in 1994 and 1995 by County Noxious Weed Departments in Kansas to characterize exposure and to relate exposure levels to various genetic and immunologic outcomes. This paper evaluates factors affecting the urinary concentration of 2,4-D among applicators and develops procedures to estimate urinary 2,4-D levels among applicators for application days where no biomonitoring data were available.

## Materials and methods

### Study Population

Participants were 31 seasonal 2,4-D applicators, who were employees of 17 county noxious weed offices in Kansas and who were recruited for a two-phase study examining the biological effects of exposure to 2,4-D (Figgs et al., 2000). County noxious weed offices are charged with controlling troublesome agricultural weeds (e.g., bindweed, wormwood, snakeweed, thistle, knapweed, larkspur, leafy spurge, locoweed, lupine, ironweed, skeleton weed) on public and private land. The pesticide applicators employed in these offices use only herbicides and spray on a regular basis between April and August.

Twelve participants with no history of cancer and no occupational pesticide use before March 1, 1994, were recruited in the first phase of the study (April to July 1994), which has been previously described (Figgs et al., 2000). Five of these participants and an additional 19 applicators were recruited into the second phase of the study (April to August 1995). In both phases of the study, participants were monitored for 12 weeks or until 2,4-D use was discontinued. The participants received a remuneration of $250 at the end of the study.

All participants signed informed-consent documents that were approved by the Human Subjects Review Committees of the National Cancer Institute and the University of Kansas Medical School.

### Data Collection

All applicators completed a 40-min, in-person, enrollment questionnaire to collect health and employment history and a 15-min post-study questionnaire to determine changes in health status, habits, or occupational history (Figgs et al., 2000). The participants kept daily work diaries in which they reported times spent doing 2,4-D-related job activities including mixing and loading, unloading and cleaning, maintaining and repairing equipment, and spraying using a backpack or a hand-held applicator from a truck with an open cab or a truck with a closed cab. They reported whether protective masks, suits, or gloves were used during mixing and loading or during application of 2,4-D. They also provided information on special exposure situations where 2,4-D came into contact with skin (face, hands, chest, legs, and/or arms), wet clothing from 2,4-D (gloves, shirt, pants, and/or hat), and whether they washed after applying pesticides (before lunch, at the end of work, or at the end of the day). From each of the 17

counties, we obtained information on application procedures including usual application concentration of 2,4-D and spray pressure for each of the two study years.

Overnight( approximately 12 h) urine samples were obtained from the study participants every other week after a typical day of 2,4-D application. A total of 140 urine samples were collected (45 samples were collected in 1994 and 95 samples were collected in 1995).

## Urine Collection and Analysis

Urine collection and analysis methods have been reported earlier (Figgs et al., 2000). Briefly, the participants were instructed to urinate at 6:00 pm and collect all urine thereafter until reporting for work the next day. The participants stored overnight urine collections in a single plastic container in a refrigerator at home and transported the urine to the worksite in a cooler filled with ice packs. The study technicians at the worksite pipetted 20 ml of urine into each of two 25-ml glass vials. The vials were placed on dry ice and transported to the University of Kansas Medical Center laboratory in Kansas City, Kansas. The urine was stored in vials at −80°C and shipped on dry ice to the Centers for Disease Control and Prevention for analysis at the end of each spraying season (i.e., 1994 and 1995).

Urinary 2,4-D analysis was conducted using standard procedures (Hill et al., 1995; Norrgran et al., 2006). $^{13}C_6$-ring-labeled 2,4-D was added as an internal standard to 2ml aliquots of urine. This was followed by enzyme hydrolysis, solid phase extraction, and concentration to 100 μl of the samples. Urinary 2,4-D measurements were made using high performance liquid chromatography combined with tandem mass spectrometry with isotope dilution quantification. The detection limit for 2ml urine samples was 0.0054 μg/l, with relative standard deviations of <10%. No measurements were found to be below the limit of detection. Creatinine concentration was also measured for each urine sample (average urinary concentration=1.40 g/l). Urine analyses were repeated in 2004 as an unexpected systematic difference in mean urinary 2,4-D levels was observed between samples collected and analyzed in 1994 and samples collected and analyzed in 1995. A very high correlation was observed between the measurements from the original analyses (using Hill et al., 1995) and the re-analyses (using Norrgran et al., 2006) ($R^2$ 0.88 and 0.99 for original analyses performed in the year 1994 and 1995, respectively). However, levels originally reported for the 1995 samples were about a factor of two lower than in the re-analyses (slope estimate of linear regression analysis with intercept set to 0 was 0.53), whereas levels from the re-analyses of the 1994 samples were close to levels found in the original analyses (slope estimate of linear regression with intercept set to 0 was 1.16). We, therefore, used the results from the urinary 2,4-D re-analyses for this paper, which accounts for discrepancies with the urinary levels presented in the Figgs et al. (2000) study.

## Statistical Analysis

SAS version 8.02 (SAS Institute Inc, Cary, NC, USA) was used for all statistical analyses. On the basis of cumulative probability plots, urinary 2,4-D concentrations were found to be log normally distributed and were ln-transformed for all statistical analyses.

In the exposure models, we included a random subject effect to account for the repeated observations for each participant. All other variables were included as fixed effects (PROC MIXED in SAS). Ln-transformed creatinine concentration was included in all models to account for variable dilutions of the urine samples (Barr et al., 2005). In these models, we examined baseline subject characteristics including body mass index, alcohol consumption (yes/no), current smoker (yes/no), and sex in relation to ln-transformed urinary 2,4-D concentrations. We also examined county procedures including application concentration (qt/acre) and spray pressure (psi). Because 2,4-D was handled over multiple days before collection

of urine samples and is excreted in urine over several days after exposure, we weighted the daily diary variables for the days before urine collection according to their expected percent contribution to the measured urinary 2,4-D concentration from exposure to 2,4-D on each of those previous days. Each variable was then summed over these days and examined in the exposure models. For example, to derive a total time spent spraying variable considering 5 days before urine collection, with day 1 being the day of urine sample collection, the calculation would be as follows:

$$T_s = w_1(t_{s1}) + w_2(t_{s2}) + w_3(t_{s3}) + w_4(t_{s4}) + w_5(t_{s5})$$

where $T_s$ is the weighted total time spent spraying, $w_1$ to $w_5$ are the weights assigned to 2,4-D exposures received on days 1 to 5, and $t_{s1}$ to $t_{s5}$ are the times spent spraying 2,4-D on days 1 to 5.

Total time and time spent performing specific activities were analyzed as both ln-transformed and untransformed in our models. After they were summed, the time variables (e.g., spraying, mixing time) were ln-transformed in assuming a proportional increase in 2,4-D dose with time. We also evaluated specific activities (e.g., spraying, cleaning/maintaining equipment) as dichotomous variables (yes/no) in our models (See Supplementary Table S1 in Supplementary Materials for all variables tested).

Our primary weights (weighting scheme A) to determine the daily portion of total absorbed dose of 2,4-D excreted in urine after repeated exposure were obtained from an analysis by Harris et al. (2001) that followed six turf applicators exposed to 2,4-D over a period of 14 days (Table 1). We chose weights from the overall estimates presented for model 3 in Table 5 of Harris et al. (2001); model 3 was deemed the most biologically relevantm odel (Harris et al., 2001). Harris et al. (2001) found that by the sixth day after exposure, all absorbed 2,4-D had been excreted in the urine and that exposures on the day of urine sample collection did not contribute to urinary 2,4-D levels. To examine the impact of assigned weights and the number of days considered on the determinants selected in our final exposure models, we constructed models with uniform weights over 5 or 6 days and including or excluding the day of urine sample collection (weighting schemes B, C, D, and E) (Table 1).

Under each weighting scheme, the exposure variables were first examined individually in models that included ln-creatinine as a covariate. Only those variables with *P*-values ≤0.1 (*F*-test of fixed effects) in this analysis were considered for inclusion in the final exposure models (Supplementary Table S1). The four-spray method variables (yes/no) were considered as a single group for which statistical significance was assessed with a likelihood ratio test (4 degrees of freedom) of the models with and without the spray method variables. Under each weighting scheme, variables were added in a forward step-wise manner to the exposure models, beginning with the variable having the smallest *P*-value in the “univariate” analysis. When considering two or more similar variables, the variable producing the smaller Akaike’s Information Criterion (AIC) was chosen for inclusion. Variables were removed from the model if they lost their significance (*P*>0.1). In addition, the model identified using the Harris et al. (2001) weights (the primary model), was assessed under each of the other weighting schemes to establish whether the Harris et al. (2001) group of determinants remained significant predictors of urinary 2,4-D concentration when using different weighting schemes.

After each model was constructed, the impact of the fixed effects on the between-worker ($\sigma_b^2$) and within-worker ($\sigma_w^2$) variance components was assessed by comparing the variance estimates from the final models to the variance estimates from a model including a random worker effect and ln-transformed creatinine concentration (Peretz et al., 2002). In addition,

partial $R^2$ statistics were calculated for each variable included in the final models to determine what proportion of the total variance in urinary 2,4-D levels each variable explained (Edwards et al., 2008).

## Results

Of the 140 urine samples collected, 136 urinary 2,4-D measurements from the 31 participants were used in the analysis. Excluded were one urine sample missing a collection date and three samples lacking diary data. Over the 136 6-day periods that were considered in our analysis, the applicators reported working with 2,4-D for a total of 336 days, resulting in an average application frequency of once every 2 to 3 days. Table 2 presents distributions of various demographic factors among the study participants. Ninety-four percent of the participants were male, 13% of the participants were smokers, and 87% reported consuming alcohol. Table 3 presents descriptive statistics for various demographic and work-related factors. Over the course of an application season (April to August), applicators reported handling pesticides on 24 to 88 days (mean=48.5 days) and specifically handling 2,4-D on 7 to 46 days (mean=24.9 days). Urinary 2,4-D concentrations ranged from 0.07 to 2857.5 μg/l; the mean and geometric mean concentrations were 259.4 (standard deviation=431.7) and 63.4 (geometric standard deviation=8.1) μg/l, respectively. Over the 336 days that the applicators reported handling 2,4-D, the majority of time was spent applying 2,4-D (mean 247.9min/day). Table 4 presents the distribution of personal protective equipment use by applicators while handling 2,4-D. Applicators tended not to wear protective equipment during spraying except for gloves, which were worn for 18% of the applications. During mixing, protective equipment was worn more regularly with gloves, goggles, masks, and suits worn 46%, 11%, 19%, and 20% of the time, respectively.

Table 5 presents the models constructed under the various weighting schemes. Models A and B identified the same group of determinants, and spray method, month of observation, and application concentration were significant determinants of urinary 2,4-D concentration under all five weighting schemes; however, the point estimates for spray method varied considerably between the models. The determinants identified by models C, D, and E were quite varied, though total time mixing/loading was a significant predictor in all three models with similar point estimates. For weighting schemes A and B, a model including ln-transformed total time spraying rather than ln-transformed total time all activities had a marginally lower AIC; however, we chose to include the latter variable because it accounts for time spent conducting other activities such as mixing, cleaning, and repairing, which may result in exposures to pesticides in other circumstances.

In models A, B, C, D, and E, the reductions in $\sigma_b^2$ were 16%, 20%, 24%, 32%, and 28%, respectively. In models A through D, there was a 23% reduction in $\sigma_w^2$, and in model E, there was a 27% reduction in $\sigma_w^2$. The largest proportion of variance in urinary 2,4-D concentrations in each model was explained by the ln-transformed creatinine concentration variable ($R^2$: 22.5% to 27.3%). Month of application also explained a significant proportion of the variance ($R^2$: 5.5% to 7.9%) in each model.

Table 6 presents the results of fitting models under weighting schemes B, C, D, and E using the determinants identified in weighting scheme A (and B as they were identical). Although the point estimates varied between the models, the series of determinants were all significant predictors of urinary 2,4-D ($P \leq 0.1$) under each weighting scheme (except for wet gloves in model C), and the final models demonstrated similar $\sigma_w^2$ and $\sigma_b^2$.

## Discussion

In this study of 31 2,4-D applicators, we constructed pharmacokinetically based exposure models that related urinary 2,4-D measurements to various work-related tasks assessed in detailed daily diaries up to 7 days before urine collection. The primary model (model A) incorporated pharmacokinetic weights based on a study by Harris et al. (2001) that found exposures from 6 days before urine collection contributed to the measured urinary 2,4-D levels. A model that simply related urinary 2,4-D measurements to determinants from the previous day would have given incorrect results because applicators typically worked with 2,4-D on multiple days before the urine sample was collected. Given the extended excretion time of 2,4-D, a large urinary measurement, for instance, could be due to an exposure that occurred up to 5 days previously but would be incorrectly attributed to determinants from only one day previous. A practical solution to this problem would have been to exclude any urine sample with a 2,4-D application in the previous 6 days. In many circumstances, this would result in exclusion of a large fraction of study participants. In our study, this would have left only 37 observations for the analysis. Moreover, because of the pharamacokinetics of 2,4-D, the contribution of exposure from the day of application to a subsequently collected 12-h urine sample is minimal resulting in a small biological signal.

A more appropriate set of weights for the daily determinants was not suggested by our data; a model with separate ln-total time variables for the day of urine sample collection and 5 days previous did not produce a better fit compared with a model that included a single ln-total time variable constructed using the Harris et al. (2001) weights (results not shown).

As models A and B were quite similar, it seems that the specific weights assigned to each day of exposure are not absolutely critical, although the sizes of some of the effect estimates of the exposure predictors changed substantially between the two models. However, when the numbers of days of exposure that are considered were altered, the specific determinants in the models changed. The models obtained under these weighting schemes were, however, less consistent with each other and resulted in counterintuitive results, such as lower urinary 2,4-D levels for participants reporting wet pants or shirts. It seems that the number of days considered under the Harris et al. (2001) model provided the most plausible exposure determinants. Moreover, when these determinants were tested under the other weighting schemes, most of the determinants were found to be significant and these models had only a marginal loss of fit (as indicated by the AIC) as compared with the “optimal” models actually derived under these weighting schemes. The differences in the obtained models are probably a reflection of the pitfalls of using stepwise procedures instead of expert judgment in model building. Also, because we evaluated a large number of predictors with urinary 2,4-D levels, we expect some spurious associations due to chance. Nonetheless, these observed differences may have relatively limited consequences when using the models for predicting exposures (i.e., it is the best fit to the observed data). However, for understanding exposure determinants and for estimating the impact control measures might have, dependence of the effect estimates on the chosen weighting schemes makes these models difficult to interpret.

Arbuckle et al. (2002) examined predictors of urinary 2,4-D levels among 126 farm applicators in the first 24 h after the first pesticide application of the season (Arbuckle et al., 2002). The variables pesticide formulation, protective clothing, application equipment, handling practice, and personal hygiene practice were found to explain 39% of the variability in 2,4-D dose. The mean and geometric mean urinary levels among 43 applicators reporting use of 2,4-D were 27.63 and 5.63 μg/l, respectively. The mean and geometric mean levels in our study were much higher (185.9 and 56.1 μg/l, respectively). This may be due to the longer duration and more frequent application by our participants. The strong effect of month of application may suggest

a build-up of internal 2,4-D levels over time; however, this could be a reflection of changing work habits with changes in the work environment (e.g., less clothing as the temperature rises).

In a study of turf applicators, Harris et al. (2002) used two 24-h urine measurements to calculate total doses received by applicators over 6 days of application. The calculations were based on data from the earlier pharmacokinetic study (Harris et al., 2001) and on the assumption that the ratio of the total dose to the amount of 2,4-D used did not change within the 6-day time period for an individual applicator. The total doses were subsequently linked to determinants from a questionnaire completed at the conclusion of urine sampling. Harris et al. (2002) found that volume of pesticide applied explained 20% of the variation in 2,4-D dose among 98 professional turf applicators over a 1-week period (mean and geometric mean daily dose of 2,4-D 1399 and 420 μg, respectively). Type of spray nozzle used and the use of gloves while spraying explained an additional 43% of variation in 2,4-D dose (Harris et al., 2002).

In a study of 34 farm applicators and their families with urine samples collected 1 day before through 3 days after an application, glove use, repairing equipment, and number of acres treated were found to be the most significant predictors of 2,4-D concentration among applicators (geometric mean urinary 2,4-D concentration 1, 2, and 3 days after application was 33.4, 33.3, and 16.3 μg/g creatinine, respectively) (Alexander et al., 2007).

As with our study, earlier studies found that a proxy for “amount” of 2,4-D used was an important determinant of exposure. In our study, ln-transformed total time handling 2,4-D and application concentration were found to be predictors of urinary 2,4-D levels when using the Harris et al. (2001) weights. In contrast with earlier studies, protective clothing was not found to be an important predictor in our models.

The detailed work diaries allowed for a comprehensive examination of the impact of work tasks, personal protective equipment, and personal hygiene practices over multiple days on urinary 2,4-D concentration; however, the primary model explained only 16% of $\sigma_b^2$ and 23% of $\sigma_w^2$ in urinary 2,4-D concentration. The small amount of explained variation may be in part because we modeled 12-h urinary 2,4-D concentrations rather than doses, which the pharmacokinetic weights were based on (Harris et al., 2001). We were unable to model dose because data on the times and volumes of urine sample collection were unavailable. Unobserved behavioral or environmental factors may also account for a large portion of the unexplained variability. These factors (e.g., more detail on use of personal protective equipment and protective practices, contamination of surfaces in the home and elsewhere, meteorological conditions, quality of application equipment, skin permeability differences) may be difficult to capture in questionnaires, but studies to identify them are needed.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations

| | |
|---|---|
| 2,4-D | 2, 4-dichlorophenoxyacetic acid |

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**Table 1**

Weighting schemes used in the construction of 2,4-D exposure models among County Noxious Weed Applicators in Kansas[a].

| | Weighting scheme | | | | |
|---|---|---|---|---|---|
| | **A** | **B** | **C** | **D** | **E** |
| | **From Harris et al. (2001)** | **Uniform (days 2 to 6)** | **Uniform (days 1 to 5)** | **Uniform (days 2 to 7)** | **Uniform (days 1 to 6)** |
| Day 1[b] | — | — | 0.20 | — | 0.17 |
| Day 2 | 0.24 | 0.20 | 0.20 | 0.17 | 0.17 |
| Day 3 | 0.28 | 0.20 | 0.20 | 0.17 | 0.17 |
| Day 4 | 0.29 | 0.20 | 0.20 | 0.17 | 0.17 |
| Day 5 | 0.14 | 0.20 | 0.20 | 0.17 | 0.17 |
| Day 6 | 0.05 | 0.20 | — | 0.17 | 0.17 |
| Day 7 | — | — | — | 0.17 | |

[a]Work task and practice variables assessed on days before urine collection.

[b]Day 1 is the work day for which a 12-h urine sample was collected.

## Table 2

Distribution of demographic factors among Kansas County Noxious Weed Applicator study participants.

| Variable | *n* (*N* = 31)[a] |
|---|---|
| *Sex* | |
| Male | 29 |
| Female | 2 |
| *Current smoker (at study entry)* | |
| Yes | 4 |
| No | 27 |
| *Current alcohol consumption (at study entry)* | |
| Yes | 27 |
| No | 4 |
| *First study year* | |
| 1994 | 12 |
| 1995 | 19 |

[a] 31 study subjects.

**Table 3**

Descriptive statistics for study- and work-related factors among Kansas County Noxious Weed Applicator study participants.

| Variable | $n^a$ | Mean | Standard deviation | Median | Minimum | Maximum |
|---|---|---|---|---|---|---|
| Age at study entry | 31 | 32.0 | 13.8 | 30.0 | 17.0 | 67.0 |
| Number of urine samples collected | 31 | 4.4 | 1.8 | 3.0 | 2.0 | 8.0 |
| Number of days worked with any pesticide over spraying season | 36 | 48.5 | 16.4 | 43.5 | 24.0 | 88.0 |
| Number of days worked with 2,4-D over spraying season | 36 | 24.9 | 9.4 | 26.0 | 7.0 | 46.0 |
| Urinary 2,4-D concentration[b] (μg/l) | 136 | 259.4 | 431.7 | 94.1 | 0.07 | 2857.5 |
| Number of days worked with 2,4-D within 6-day period before urine collection | 136 | 2.5 | 1.1 | 3.0 | 1.0 | 5.0 |
| Duration of 2,4-D application (min/day) | 336 | 247.9 | 123.4 | 240.0 | 0.0 | 720.0 |
| Duration of 2,4-D mixing/loading (min/day) | 336 | 34.6 | 34.4 | 30.0 | 0.0 | 270.0 |
| Duration of 2,4-D unloading/cleaning (min/day) | 336 | 7.4 | 31.4 | 0.0 | 0.0 | 360.0 |
| Duration of 2,4-D maintaining/repairing (min/day) | 336 | 6.8 | 30.2 | 0.0 | 0.0 | 370.0 |

[a] 31 study subjects; 36 individuals observed over two application seasons (12 in 1994 and 24 in 1995); 136 urine samples corresponding to 136 6-day observation periods; 336 days reported handling (applying, mixing, unloading, etc.) 2,4-D within the 136 6-day observation periods.

[b] Geometric mean=63.4; geometric standard deviation=8.1.

## Table 4

Personal protective equipment used during handling of 2,4-D by Kansas County Noxious Weed Applicator study participants.

| Variable | Application *n* (%) | Mixing *n* (%) | Other activities[a] *n* (%) |
|---|---|---|---|
| *Boots* | | | |
| Yes | 2 (1%) | 3 (1%) | 0 (0%) |
| No | 334 (99%) | 333 (99%) | 336 (100%) |
| *Gloves* | | | |
| Yes | 60 (18%) | 154 (46%) | 1 (<1%) |
| No | 276 (82%) | 182 (54%) | 335 (100%) |
| *Goggles* | | | |
| Yes | 2 (1%) | 36 (11%) | 0 (0%) |
| No | 334 (99%) | 300 (89%) | 336 (100%) |
| *Mask* | | | |
| Yes | 12 (4%) | 64 (19%) | 0 (0%) |
| No | 324 (96%) | 272 (81%) | 336 (100%) |
| *Suit* | | | |
| Yes | 16 (5%) | 67 (20%) | 13 (4%) |
| No | 320 (95%) | 269 (80%) | 323 (96%) |

*N*=336 days reported handling 2,4-D.

[a] Other activities include unloading, cleaning, repairing, and/or maintaining.

**Table 5**

Final models relating urinary 2,4-D concentration to work practice and task data from County Noxious Weed Applicators in Kansas using four different variable weighting schemes.

| Variable | Beta | 90% CI | *P*-value | AIC | Between-worker variance[a] | Within-worker variance[a] | Semipartial $R^2$ |
|---|---|---|---|---|---|---|---|
| *Model A:* Harris et al. (2001) | | | | | | | |
| Ln-creatinine concentration (ln-g/l) | 1.4 | 1.1, 1.7 | <0.0001 | 442.0 | 2.1 | 1.0 | 22.5% |
| Spraying from backpack (weighted yes/no) | 3.2 | 0.1, 6.3 | 0.0009[b] | | | | 1.4% |
| Spraying from hand-held (weighted yes/no) | 0.8 | −0.6, 2.3 | — | | | | 0.4% |
| Spraying from open cab truck (weighted yes/no) | −1.2 | −2.8, 0.3 | — | | | | 0.7% |
| Spraying from closed cab truck (weighted yes/no) | −2.0 | −3.5, −0.5 | — | | | | 2.5% |
| Month | | | | | | | |
| April | −1.9 | −3.2, −0.5 | 0.002[c] | | | | 7.9% |
| May | −2.0 | −3.0, −0.9 | — | | | | — |
| June | −1.2 | −2.2, −0.08 | — | | | | — |
| July | −0.7 | −1.8, 0.3 | — | | | | — |
| August | — | — | — | | | | — |
| Application concentration (qt/acre) | 1.4 | 0.5, 2.3 | 0.01 | | | | 1.1% |
| Wet gloves (weighted yes/no) | 2.0 | 0.4, 3.5 | 0.03 | | | | 2.0% |
| Ln-total time all activities (weighted ln-minutes) | 0.2 | 0.1, 0.3 | 0.003 | | | | 4.3% |
| *Model B* | | | | | | | |
| Ln-creatinine concentration (ln-g/l) | 1.4 | 1.1, 1.7 | <0.0001 | 442.4 | 2.0 | 1.0 | 23.8% |
| Spraying from backpack (weighted yes/no) | 3.3 | 0.6, 6.0 | 0.0009[b] | | | | 1.8% |
| Spraying from hand-held (weighted yes/no) | 1.1 | −0.2, 2.4 | — | | | | 0.8% |
| Spraying from open cab truck (weighted yes/no) | −0.7 | −2.1, 0.7 | — | | | | 0.2% |
| Spraying from closed cab truck (weighted yes/no) | −1.3 | −2.6, −0.06 | — | | | | 1.6% |
| Month | | | | | | | |
| April | −1.9 | −3.2, −0.6 | 0.002[c] | | | | 7.5% |
| May | −2.0 | −3.0, −0.9 | — | | | | — |
| June | −1.2 | −2.2, −0.1 | — | | | | — |
| July | −0.8 | −1.9, 0.2 | — | | | | — |
| August | — | — | — | | | | — |

| Variable | Beta | 90% CI | *P*-value | AIC | Between-worker variance[a] | Within-worker variance[a] | Semipartial $R^2$ |
|---|---|---|---|---|---|---|---|
| Application concentration (qt/acre) | 1.3 | 0.4, 2.2 | 0.02 | | | | 1.0% |
| Ln-total time all activities (weighted ln-minutes) | 0.2 | 0.07, 0.3 | 0.01 | | | | 3.0% |
| Wet gloves (weighted yes/no) | 1.5 | 0.2, 2.9 | 0.06 | | | | 1.5% |
| *Model C* | | | | | | | |
| Ln-creatinine concentration (ln-g/l) | 1.5 | 1.2, 1.8 | <0.0001 | 439.3 | 1.9 | 1.0 | 25.3% |
| Spraying from backpack (weighted yes/no) | 1.8 | −1.1, 4.8 | 0.002[b] | | | | 0.2% |
| Spraying from hand-held (weighted yes/no) | 2.3 | 1.0, 3.7 | — | | | | 3.5% |
| Spraying from open cab truck (weighted yes/no) | 0.4 | −0.9, 1.7 | — | | | | 0% |
| Spraying from closed cab truck (weighted yes/no) | 0.07 | −1.1, 1.2 | — | | | | 0% |
| Month | | | | | | | |
| April | −1.9 | −3.2, −0.6 | 0.009[c] | | | | 5.5% |
| May | −1.7 | −2.8, −0.6 | — | | | | — |
| June | −0.9 | −2.0, 0.2 | — | | | | — |
| July | −0.9 | −1.9, 0.3 | — | | | | — |
| August | — | — | | | | | — |
| Arm skin contact (weighted yes/no) | 2.2 | 0.8, 3.6 | 0.01 | | | | 2.8% |
| Total time mixing/loading (weighted minutes) | 0.03 | 0.01, 0.04 | 0.01 | | | | 2.8% |
| Application concentration (qt/acre) | 1.3 | 0.4, 2.2 | 0.02 | | | | 1.4% |
| Wet pants (weighted yes/no) | −2.4 | −4.6, −0.08 | 0.09 | | | | 0.9% |
| *Model D* | | | | | | | |
| Ln-creatinine concentration (ln-g/l) | 1.5 | 1.2, 1.8 | <0.0001 | 440.7 | 1.7 | 1.0 | 23.1% |
| Spraying from backpack (weighted yes/no) | 4.3 | 1.3, 7.3 | 0.0005[b] | | | | 2.5% |
| Spraying from hand-held (weighted yes/no) | 1.8 | 0.5, 3.0 | — | | | | 2.1% |
| Spraying from open cab truck (weighted yes/no) | −0.9 | −2.4, 0.6 | — | | | | 0.4% |
| Spraying from closed cab truck (weighted yes/no) | −0.4 | −1.6, 0.8 | — | | | | 0.2% |
| Month | | | | | | | |
| April | −2.1 | −3.4, −0.8 | 0.002[c] | | | | 7.1% |
| May | −1.9 | −3.0, −0.8 | — | | | | — |
| June | −1.0 | −2.1, 0.09 | — | | | | — |
| July | −0.9 | −2.0, 0.2 | — | | | | — |
| August | — | — | | | | | — |

| Variable | Beta | 90% CI | *P*-value | AIC | Between-worker variance[a] | Within-worker variance[a] | Semipartial $R^2$ |
|---|---|---|---|---|---|---|---|
| Facial skin contact (weighted yes/no) | 3.3 | 1.4, 5.2 | 0.004 | | | | 3.4% |
| Application concentration (qt/acre) | 1.2 | 0.3, 2.0 | 0.03 | | | | 1.7% |
| Total time mixing/loading (weighted minutes) | 0.03 | 0.007, 0.05 | 0.03 | | | | 1.9% |
| Wet shirt (weighted yes/no) | −2.4 | −4.7, −0.2 | 0.08 | | | | 1.3% |
| *Model E* | | | | | | | |
| Ln-creatinine concentration (ln-g/l) | 1.6 | 1.3, 1.9 | <0.0001 | 437.4 | 1.8 | 0.9 | 27.3% |
| Total time mixing/loading (weighted minutes) | 0.04 | 0.02, 0.05 | 0.0002 | | | | 5.3% |
| Spraying from backpack (weighted yes/no) | 2.6 | −0.06, 5.2 | 0.0001[b] | | | | 0.9% |
| Spraying from hand-held (weighted yes/no) | 2.3 | 1.1, 3.5 | — | | | | 4.0% |
| Spraying from open cab truck (weighted yes/no) | −3.3 | −5.5, 1.0 | — | | | | 2.1% |
| Spraying from closed cab truck (weighted yes/no) | −0.4 | −1.5, 0.7 | — | | | | 0.2% |
| Ln-total time open cab spraying (weighted ln-minutes) | 0.3 | 0.1, 0.5 | 0.009 | | | | 2.7% |
| Month | | | | | | | |
| April | −1.9 | −3.2, −0.7 | 0.009[c] | | | | 5.5% |
| May | −1.8 | −2.9, −0.8 | — | | | | — |
| June | −1.1 | −2.2, −0.08 | — | | | | — |
| July | −1.0 | −2.0, 0.08 | — | | | | — |
| August | — | — | — | | | | — |
| Application concentration (qt/acre) | 1.1 | 0.2, 2.0 | 0.04 | | | | 0.8% |
| Facial skin contact (weighted yes/no) | 1.7 | 0.3, 3.0 | 0.05 | | | | 1.3% |

[a] In model with ln-creatinine and random subject effects, $\sigma_b^2=2.5$ and $\sigma_w^2=1.3$.

[b] Four-spray method variables assessed as single group in models with four degrees of freedom likelihood ratio test.

[c] *F*-test for group effect of month variables.

**Table 6**

Models using Harris et al. (2001) determinants relating urinary 2,4-D concentration to work practice and task data from County Noxious Weed Applicators in Kansas under weighting schemes C, D, and E.

| Variable | Beta | 90% CI | *P*-value | AIC | Between-worker variance[a] | Within-worker variance[a] | Semipartial $R^2$ |
|---|---|---|---|---|---|---|---|
| *Model C* | | | | | | | |
| Ln-creatinine concentration (ln-g/l) | 1.5 | 1.1, 1.8 | <0.0001 | 445.3 | 1.9 | 1.0 | 22.7% |
| Spraying from backpack (weighted yes/no) | 2.4 | −0.6, 5.5 | 0.002[b] | | | | 0.6% |
| Spraying from hand-held (weighted yes/no) | 1.9 | 0.7, 3.2 | — | | | | 2.9% |
| Spraying from open cab truck (weighted yes/no) | −0.4 | −1.8, 1.1 | — | | | | 0% |
| Spraying from closed cab truck (weighted yes/no) | −0.3 | −1.6, 1.1 | — | | | | 0% |
| Month | | | | | | | |
| April | −1.7 | −3.0, −0.3 | 0.02[c] | | | | 4.9% |
| May | −1.6 | −2.8, −0.4 | — | | | | — |
| June | −0.8 | −1.9, 0.2 | — | | | | — |
| July | −0.7 | −1.8, 0.4 | — | | | | — |
| August | — | — | — | | | | — |
| Application concentration (qt/acre) | 1.3 | 0.4, 2.1 | 0.02 | | | | 1.8% |
| Wet gloves (weighted yes/no) | 0.9 | −0.3, 2.1 | 0.2 | | | | 0.4% |
| Ln-total time all activities (weighted ln-minutes) | 0.5 | 0.04, 0.9 | 0.07 | | | | 1.3% |
| *Model D* | | | | | | | |
| Ln-creatinine concentration (ln-g/l) | 1.5 | 1.2, 1.8 | <0.0001 | 444.5 | 2.1 | 1.0 | 24.8% |
| Spraying from backpack (weighted yes/no) | 3.8 | 0.8, 6.9 | 0.002[b] | | | | 2.0% |
| Spraying from hand-held (weighted yes/no) | 1.3 | −0.04, 2.6 | — | | | | 1.1% |
| Spraying from open cab truck (weighted yes/no) | −0.4 | −1.8, 1.0 | — | | | | 0.1% |
| Spraying from closed cab truck (weighted yes/no) | −0.8 | −2.1, 0.6 | — | | | | 0.5% |
| Month | | | | | | | |
| April | −2.0 | −3.3, −0.6 | 0.006[c] | | | | 6.3% |
| May | −1.9 | −3.0, −0.8 | — | | | | — |
| June | −1.2 | −2.3, −0.1 | — | | | | — |
| July | −1.0 | −2.0, 0.1 | — | | | | — |
| August | — | — | — | | | | — |

| Variable | Beta | 90% CI | *P*-value | AIC | Between-worker variance[a] | Within-worker variance[a] | Semipartial $R^2$ |
|---|---|---|---|---|---|---|---|
| Application concentration (qt/acre) | 1.3 | 0.4, 2.2 | 0.02 | | | | 1.2% |
| Wet gloves (weighted yes/no) | 1.6 | 0.3, 2.9 | 0.05 | | | | 1.4% |
| Ln-total time all activities (weighted ln-minutes) | 0.1 | 0.01, 0.3 | 0.08 | | | | 1.4% |
| *Model E* | | | | | | | |
| Ln-creatinine concentration (ln-g/l) | 1.5 | 1.2, 1.8 | <0.0001 | 441.2 | 1.9 | 1.0 | 24.3% |
| Spraying from backpack (weighted yes/no) | 2.8 | 0.1, 5.6 | 0.002[b] | | | | 1.3% |
| Spraying from hand-held (weighted yes/no) | 1.5 | 0.2, 2.8 | — | | | | 1.7% |
| Spraying from open cab truck (weighted yes/no) | −0.6 | −2.0, 0.8 | — | | | | 0.1% |
| Spraying from closed cab truck (weighted yes/no) | −0.8 | −2.0, 0.5 | — | | | | 0.4% |
| Month | | | | | | | |
| April | −1.8 | −3.1, −0.4 | 0.01[c] | | | | 5.1% |
| May | −1.8 | −2.9, −0.7 | — | | | | — |
| June | −1.0 | −2.1, 0.07 | — | | | | — |
| July | −0.8 | −1.9, 0.2 | — | | | | — |
| August | — | — | — | | | | — |
| Application concentration (qt/acre) | 1.3 | 0.4, 2.2 | 0.02 | | | | 1.8% |
| Wet gloves (weighted yes/no) | 1.2 | −0.03, 2.4 | 0.1 | | | | 0.8% |
| Ln-total time all activities (weighted ln-minutes) | 0.6 | 0.2, 1.0 | 0.01 | | | | 2.5% |

[a] In model with ln-creatinine and random subject effects, $\sigma^2_b = 2.5$ and $\sigma^2_w = 1.3$.

[b] Four-spray method variables assessed as single group in models with four degrees of freedom likelihood ratio test.

[c] *F*-test for group effect of month variables.