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BIOLOGICAL MONITORING OF COMMERCIAL PESTICIDE APPLICATORS FOR URINE METABOLITES OF THE HERBICIDE ALACHLOR

Wayne T. Sanderson[a]
Raymond Biagini[b]
William Tolos[b]
Gerry Henningsen[c]
Barbara MacKenzie[b]

[a]Division of Surveillance, Hazard Evaluations, and Field Studies, National Institute for Occupational Safety and Health, 4676 Columbia Parkway, Cincinnati, OH 45226; [b]Division of Biomedical and Behavioral Science, National Institute for Occupational Safety and Health, 4676 Columbia Parkway, Cincinnati, OH 45226; [c]U.S. Environmental Protection Agency, 999 18th Street, Denver, CO 80202

Alachlor (2-chloro-2',6'-diethyl-N-[methoxymethyl] acetanilide), the active ingredient in several trade name herbicides, is absorbed through the skin and readily excreted in the urine as conjugated metabolites. This paper presents the results of a study to measure alachlor metabolites in the urine of commercial pesticide applicators who were applying alachlor to corn and soybean crops under normal work conditions. Three spot urine samples, collected at the beginning and end of the work shift and the morning after the exposure survey, were collected from 20 applicators, 7 hauler-mixers, and 8 controls. Each sample was analyzed using both a competitive, solid-phase, enzyme-linked immunoassay (ELISA) and a high-performance liquid chromatography (HPLC) technique. Although the urine metabolite concentrations measured by ELISA were consistently higher than the respective HPLC measurements, a high correlation (r = 0.90) was observed between the ELISA and HPLC measurements. The controls, with little exposure to alachlor, had metabolite levels below or near the lower limits of detection for each analysis technique. Similar urine metabolite concentrations were observed for the applicators and hauler-mixers, suggesting similar work exposures. The average postexposure urine concentrations were not correlated with the amount of alachlor handled and applied, suggesting that other factors, such as work practices, are greater determinants of absorbed doses of alachlor.

Alachlor (2-chloro-2',6'-diethyl-*N*-[methoxymethyl] acetanilide), the active ingredient in several trade name herbicides used for control of grasses and broadleaf weeds, acts as a herbicidal chemical by inhibiting protein synthesis in susceptible plants.[1] In animals alachlor has been found to be hepatotoxic, to cause an ocular lesion referred to as uveal degeneration syndrome (UDS), and to be a skin sensitizer.[2–5] Alachlor also has been found to cause an increased incidence of tumors at multiple sites in two species of laboratory animals. Specifically, alachlor was found to cause a statistically significant increase in lung bronchioalveolar tumors in female mice with doses of 260 mg/kg/day administered orally for 18 months.[6] In rats alachlor caused a statistically significant increase in nasal turbinate, stomach, and thyroid follicular tumors in both sexes with doses of 126 mg/kg/day administered orally for two years.[7] No epidemiologic studies of the carcinogenic potential of alachlor in humans have been conducted.

The metabolism and excretion kinetics of alachlor have been determined in studies of Rhesus monkeys.[8,9] An average of 87% of the administered dose was recovered in the urine after intravenous administration of radiolabeled alachlor; an average of 79% of the total administered dose was eliminated in the urine within the first 24 hours after dosing.[8,10] The urinary metabolites have been identified as secondary and tertiary mercapturate conjugates and as cysteine, thioacetic acid, and glucuronide conjugates of alachlor.[9] The chemical structure of alachlor and the Rhesus monkey metabolites are shown in Figure 1. No parent alachlor was detected in the urine.

In a dermal absorption study of Rhesus monkeys, an average of 8.5% of an alachlor dose topically applied in an emulsifiable concentrate formulation and 3.7% of an alachlor dose applied in a microencapsulated formulation were absorbed during a 12-hour exposure period. Approximately 75% of the absorbed doses was excreted in the urine within 24 hours after exposure.[10,11] The results of the intravenous and dermal absorption studies demonstrate that alachlor is absorbed through the skin and is rapidly eliminated as urinary metabolites within the first 24 hours

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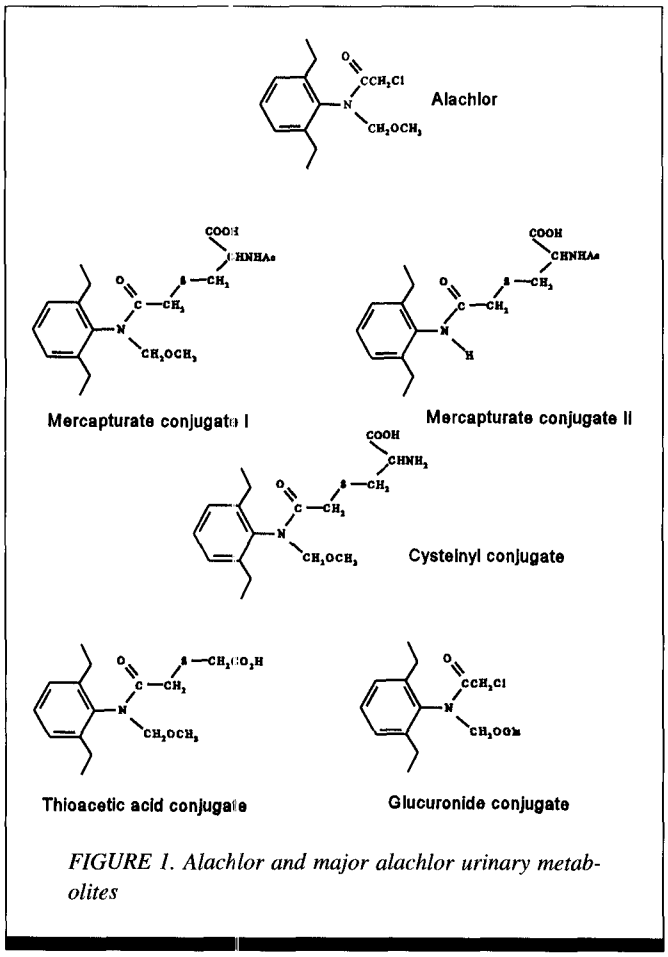

FIGURE 1. Alachlor and major alachlor urinary metabolites

after exposure. Since the major route of alachlor elimination is urinary excretion, the analysis of alachlor metabolites in the urine is an appropriate method of biological monitoring.

Presented in this paper are the results of a biomonitoring study conducted by the National Institute for Occupational Safety and Health (NIOSH) to measure alachlor metabolites in the urine of commercial pesticide applicators who apply herbicides to crop land. The objective of the study was to evaluate two methods to analyze for alachlor metabolites in human urine: (1) a competitive solid-phase, enzyme-linked immunoassay (ELISA) method and (2) a high-performance liquid chromatography (HPLC) method. These methods were used to screen for the relative alachlor metabolite concentrations and determine factors that may influence the absorbed doses of workers handling alachlor. Spot urine samples were collected concurrently with samples to evaluate inhalation and skin exposures. The results of the inhalation and skin exposure monitoring are presented separately.[12]

METHODS AND MATERIALS

Study participants were selected from commercial pesticide application companies in three counties in east central Illinois and three counties in southwestern Ohio.[12] These companies specialized in the application of fertilizer and pesticides on crop land and used alachlor-containing herbicides as some of their main pre-emergent herbicides. The amount of alachlor-containing herbicides used and the duration of the application period was recorded for each applicator, since these factors varied among study participants. The alachlor-containing herbicides were frequently mixed with other herbicides to broaden the weed control spectrum; and, from job to job, most workers switched from herbicide mixes containing alachlor to mixes that did not contain alachlor. Therefore, the amounts of all herbicides used during the workday were recorded.[12]

Urine Collection

Participants in the study were asked to provide three urine samples over a 24-hour period. One sample was collected at the work site on the morning of the exposure survey before participants began work. A second urine void was collected at the work site at the end of the workday, after the participants had washed their hands thoroughly and were prepared to leave for home. The third urine sample was collected at the worker's home or the work site before work on the morning following the exposure survey.

For comparison purposes employees of the application companies whose job duties did not involve mixing and applying pesticides and who were thought to have little exposure to alachlor-containing herbicides were also asked to submit three urine samples each for background measurements of alachlor metabolites. These individuals typically were employed as station managers, dispatchers, or grain handlers. For quality control purposes one urine sample also was collected during the course of the study from 10 laboratory workers who had no exposure to pesticides. These samples were collected to verify that urine samples containing no alachlor were being analyzed correctly.

Each urine sample was collected separately in a wide-mouthed 500 mL polyethylene bottle. Participants were asked to recap the bottle and return it to the NIOSH investigators as soon as possible after voiding. Aliquots of the urine sample were then transferred to 60 mL high-density polyethylene bottles and immediately frozen for shipment to the laboratory. To assess possible contamination of the urine samples during voiding, an open 500 mL polyethylene bottle containing 50 mL of distilled water was taped to the side of the urine collection bottle. The participants were asked to recap the water bottle after voiding and return it along with the urine sample. The distilled water samples also were analyzed for the presence of alachlor.

ELISA

The concentration of alachlor metabolites in the urine samples was analyzed using a commercially available ELISA test kit (EnviroGuard®, Immunosystems, Inc., Scarborough, Maine). This kit was designed for the analysis of alachlor in water using a competitive, solid-phase, ELISA method.[13] However, preliminary studies by NIOSH, using the ELISA kit to measure alachlor metabolites in the urine of monkeys dosed with alachlor, demonstrated that mammalian urinary metabolites also could be measured (expressed as alachlor equivalents).[14] The analysis was based on inhibition of the reaction between enzyme-labeled alachlor and immobilized polyclonal antialachlor antibodies

by free alachlor in the test sample.[13] The antialachlor antibodies were produced from an antigen that is an alachlor-protein-thioether conjugate. Therefore, it was presumed that the antibodies would cross-react with alachlor mercapturic acid conjugates (thioether), which are the major alachlor metabolites present in the urine of alachlor dosed monkeys.[15]

The test kit was standardized using parent alachlor, but the metabolites may give a different response than parent alachlor. Therefore the concentration of alachlor metabolites was reported as micrograms of alachlor equivalents per milliliter of urine (μg/mL). The limit of detection (LOD) of this assay system for urine metabolites was empirically set at 0.001 μg/mL.[14] The urine samples were diluted at either 1:100 or 1:1000 with deionized water to bring their results into the range of the ELISA standard curve.

HPLC

The concentrations of alachlor metabolites in the urine samples also were analyzed using an HPLC method, which was a modification of a gas chromatography method that had been developed previously to measure urinary metabolites of alachlor.[16] Biagini et al. provide additional details regarding this HPLC analysis method, but in summary the alachlor metabolites were alkaline-hydrolyzed at 150°C in a fluidized sand bath to convert the metabolites to 2,6-diethylaniline (DEA).[14] DEA was extracted from the hydrolysis solution using a solid phase extraction cartridge and eluted with methanol.[14] The processed urine sample then was introduced into an HPLC for DEA determination.

Alachlor is reported to be excreted in human urine only as metabolites with the DEA moiety intact.[16] The metabolites are hydrolyzed to DEA during the high-temperature base hydrolysis phase of sample work up, with 1 M of DEA-yielding metabolites hydrolyzed to 1 M of DEA. The analytical results are reported in micrograms of DEA-yielding metabolites per milliliter of urine (μg/mL); the LOD for DEA-yielding metabolites was 0.051 μg/mL.[14]

Urine Creatinine Concentrations

The alachlor metabolite concentrations in the spot urine samples were adjusted for varying levels of urine dilution by using the creatinine concentrations in the urine samples.[17] Creatinine is thought to be excreted at a constant rate independent of urine flow; therefore, dividing the urine metabolite concentrations by the urine creatinine concentrations theoretically adjusts for individual differences in urine flow rates.[18] However, creatinine excretion is not constant and is subject to variability due to internal and external factors. When adjusting urine metabolite measurements by creatinine concentration, this variation in individual excretion may actually confound urine metabolite measurements. Urine concentrations referenced to creatinine are presented in this paper primarily to be consistent with the way other researchers have presented the results of spot urine measurements.

The concentrations of alachlor metabolites in the urine samples were expressed as micrograms of alachlor equivalents per milligram creatinine (μg/mg). Creatinine concentrations were determined using a Serono-Baker (Allentown, Pa.), Encore Centrifugal Analyzer®. This analyzer uses an adaptation of the Jaffe reaction, in which creatinine forms a red complex on reaction with picric acid under alkaline conditions.[19]

Statistical Analysis

The arithmetic means and standard deviations were calculated as summary statistics for the urine measurements. In calculating the summary statistics a sample below the analytical limit of detection was given a nonzero value of the limit of detection divided by the square root of two.[20] Correlation between the ELISA and HPLC urine measurements was evaluated using simple linear regression. The average of the workers' postshift urine concentration and next morning urine concentration was calculated to estimate their average postexposure urine concentration. Correlation between the average postexposure urine concentrations and the amount of alachlor used by the applicators (pounds of alachlor applied, number of acres sprayed, and length of application period) was evaluated using polynomial regression. T-tests were used to compare the average postexposure urine concentrations by worker characteristics such as job, type of clothing worn, and presence of air conditioning in their vehicles.

RESULTS

Twenty applicators and seven hauler-mixers provided urine samples in the biomonitoring and exposure assessment study. Also, eight employees of the application companies, who were initially thought to have limited exposure to pesticides, submitted urine samples for background measurements of alachlor metabolites. All of the study participants were male.

The average duration of the workday, amount of alachlor handled by the hauler-mixers and applied by the applicators on the day of the survey, the number of acres sprayed, and duration of alachlor application are presented in Table I. The amount of alachlor applied on the day of the survey was quite variable, ranging from 65 to 897 lbs, with a mean of 436 lbs. Workers sprayed alachlor from 0.5 to 6.5 hrs, with a mean of 2.9 hrs,

TABLE I. Handling of Alachlor and Duration of the Workday for Applicators and Hauler/Mixers

	Applicators (n = 19)		*Hauler/Mixers (n = 7)*	
	Mean	*Range*	*Mean*	*Range*
Duration of workday (hrs)	11.7	3.5–15.4	11.8	6.8–14.1
Alachlor handled (lbs)	436	65–897	454	140–897
# Acres sprayed with alachlor	168	26–336	—	—
Duration of alachlor application (hrs)	2.9	0.5–6.5	—	—

during workdays that ranged from 3.5 to 15.4 hrs, with a mean of 11.7 hours.

One applicator did not provide a preshift urine sample; therefore, a total of 59 samples were collected from the 20 applicators and 21 samples from the 7 hauler-mixers. All of these samples were analyzed by ELISA (n = 80), but three samples from applicators (one preshift and two postshift) and one hauler-mixer postshift sample had insufficient volume to allow HPLC analysis (n = 76). Alachlor metabolites were above the ELISA test LOD (0.001 μg/mL) in 88% (70/80) of all the urine samples collected. Alachlor metabolites were above the HPLC test LOD (0.051 μg/mL) in 92% (70/76) of the urine samples. No applicator or hauler-mixer had metabolite levels below the LODs in all three urine samples.

Summary statistics for the alachlor metabolite concentrations in the preshift, postshift, and next-morning urine samples, as determined by both ELISA and HPLC analysis, are presented by job category in Table II. The means of the preshift and next-morning metabolite levels tend to be slightly lower than the means of the postexposure metabolite levels, but these differences are not statistically significant.

The preshift urine levels indicate that most of the applicators and hauler-mixers had substantial exposure to alachlor on the day preceding the exposure survey. In fact, only six applicators and hauler-mixers had preshift urine measurements below the ELISA test LOD, suggesting they had low exposure to alachlor on the day preceding the exposure survey. Four of these six workers also had measurements below the HPLC test LOD, and the other two workers had levels near the LOD. For the six individuals who had preshift levels below the LOD, the mean of the postexposure urine concentrations was 4.3 μg/mL by ELISA analysis and 0.49 μg/mL by HPLC analysis. For the 21 participants who had preshift levels above the LOD, the mean of their postexposure urine concentrations was significantly higher at 6.9 μg/mL by ELISA analysis and 1.1 μg/mL by HPLC analysis, using a group-comparison t-test (p = 0.006). Therefore, with the exception of these six individuals, the postshift urine measurements may partially reflect exposure from the previous day.

Summary statistics for the alachlor metabolite concentrations in the urine of eight application company employees who were initially thought to have limited exposure to pesticides also are presented in Table II. Five of these individuals actually had very limited exposure to herbicides, and the alachlor metabolite levels in all their urine samples were either near or below the LODs. The other three individuals also believed they would have limited exposure to alachlor on the day of the survey, but since the surveys usually were conducted on particularly busy days, these three workers actually mixed and loaded alachlor and other herbicides during a portion of their workday. Therefore, the urine measurements for the five workers with limited exposure to herbicides are presented separately (Table II) from the three workers with short-term exposures.

The measurements for workers with short-term exposures were quite high, with the metabolite levels for one station manager among the highest recorded. As indicated by their preshift urine levels, it is evident that these three individuals also had

TABLE II. Alachlor Urine Metabolite Measurements by Job Classification

	Alachlor Metabolites Measured by ELISA[A] in μg/mL of Urine			*Alachlor Metabolite Concentrations Measured by HPLC[B] in μg/mL of Urine*		
	# Samples	Mean (Std. Dev.)[C]	Range	# Samples	Mean (Std. Dev.)	Range
Applicators						
Preshift urine concentration	19	5.1 (5.5)	<0.001–23	18	0.73 (1.05)	<0.04–4.2
Postshift urine concentration	20	6.5 (3.8)	<0.001–13	18	0.97 (0.78)	<0.04–2.87
Next morning urine concentration	20	5.2 (3.5)	<0.001–14	20	0.71 (0.71)	0.06–2.79
Hauler/mixers						
Preshift urine concentration	7	3.5 (3.3)	<0.001–9	7	0.43 (0.66)	<0.04–1.87
Postshift urine concentration	7	6.0 (3.3)	1–11	6	1.12 (0.62)	0.17–1.59
Next morning urine concentration	7	5.5 (3.0)	<0.001–9	7	0.69 (0.59)	<0.04–1.7
Workers with limited exposures to herbicides						
Preshift urine concentration	5	0.8 (0.2)	<0.001–1	5	0.14 (0.10)	<0.04–0.30
Postshift urine concentration	4	1.8 (1.3)	<0.001–3	4	0.09 (0.10)	<0.04–0.24
Next morning urine concentration	4	1.0 (0.7)	<0.001–2	4	0.10 (0.11)	<0.04–0.25
Workers with short-term exposures to herbicides						
Preshift urine concentration	3	12.0 (11.8)	2–25	3	1.10 (0.86)	0.6–2.09
Postshift urine concentration	3	8.0 (5.2)	5–14	3	0.63 (0.23)	0.39–0.84
Next morning urine concentration	3	7 (4.4)	4–12	3	0.46 (0.33)	0.20–0.83

[A] ELISA = enzyme linked immunosorbent assay.

[B] HPLC = high performance liquid chromatography.

[C] In calculating a mean and standard deviation (Std. Dev.), a sample below the analytical limit of quantification was given a value of the LOD divided by the square root of two.[20]

mixed alachlor or had been exposed to alachlor on the day preceding the survey, as they later confirmed.

The alachlor metabolite levels in all 10 control urine samples from laboratory workers not exposed to pesticides were below or very near the LODs, demonstrating that the ELISA and HPLC tests were correctly detecting samples containing little or no alachlor. Also, no alachlor was detected in the water samples attached to the urine collection bottles, indicating that the urine samples had not been contaminated during voiding.

The urine metabolite concentrations measured by ELISA were consistently higher for each urine sample than the respective concentration measured by HPLC. On average the ELISA result was 91% higher than the respective HPLC result. However, a high correlation was observed between the urine metabolite concentrations analyzed by the ELISA method and the HPLC method ($n = 98$, $r = 0.90$, $p < 0.0001$), suggesting that a systematic positive error was present in the ELISA analyses.

Summary statistics for the alachlor metabolite concentrations adjusted by the creatinine concentrations in the urine samples are presented by job category in Table III. The coefficients of variation for the creatinine adjusted urine samples are very similar to those of the unadjusted urine samples. This indicates that the creatinine adjustments do not reduce the overall variability in the urine measurements.

There was little difference between the urine metabolite concentrations of the applicators and hauler-mixers ($p > 0.05$), indicating little difference in exposure due to job (Tables II and IV). However, there was wide subject-to-subject variability, as is evident in the standard deviations and ranges of the urine concentrations. This subject-to-subject variability was not explained by the amount of alachlor used during the workday. The average postexposure urine metabolite levels were not correlated with pounds of alachlor handled and applied, numbers of acres sprayed, or amount of time spent handling alachlor ($p > 0.05$). And attempts to adjust the average postexposure urine metabolite levels by the preshift levels did not improve correlation between the metabolite concentrations and alachlor usage. The creatinine corrected urine measurements also were not correlated with alachlor usage.

The average postexposure urine metabolite concentrations also were compared by types of clothing worn by the applicators and hauler-mixers and the presence of air conditioning in their work vehicles (Table IV). None of the comparisons achieved statistical significance (t-test, $p > 0.05$), and comparisons using either the ELISA or the HPLC measurements yielded similar conclusions. Likewise, comparisons using creatinine corrected urine measurements yielded similar results.

Statistical comparisons of the urine measurements by categories such as job and types of clothing worn, and correlation of the urine measurements with amount of alachlor used, tended to yield the same conclusions regardless of whether ELISA or HPLC measurements were used. These statistical comparisons led to similar conclusions, because the ELISA and HPLC measurements were so highly correlated ($r = 0.90$).

TABLE III. Alachlor Urine Metabolite Measurements by Job Classification Adjusted by Urine Creatinine Concentration

	Alachlor Metabolites Measured by ELISA[A] in µg/mg Creatinine			*Alachlor Metabolites Measured by HPLC[B] in µg/mg Creatinine*		
	# Samples	*Mean (Std. Dev.)[C]*	*Range*	*# Samples*	*Mean (Std. Dev.)*	*Range*
Applicators						
Preshift urine concentration	19	1.9 (1.9)	<0.2–7.9	18	0.27 (0.36)	<0.01–1.45
Postshift urine concentration	20	2.7 (2.1)	<0.3–7.7	18	0.40 (0.40)	<0.02–1.43
Next morning urine concentration	20	2.1 (1.3)	<0.4–5.2	20	0.26 (0.23)	0.04–0.83
Hauler/mixers						
Preshift urine concentration	7	1.4 (1.0)	<0.3–2.9	7	0.16 (0.21)	<0.03–0.61
Postshift urine concentration	7	2.9 (2.4)	0.5–7.1	6	0.54 (0.51)	0.07–1.54
Next morning urine concentration	7	2.4 (1.2)	<0.4–4.0	7	0.29 (0.23)	<0.02–0.60
Workers with limited exposures to herbicides						
Preshift urine concentration	5	0.5 (0.2)	<0.4–0.5	5	0.07 (0.05)	<0.03–0.15
Postshift urine concentration	4	1.0 (0.8)	<0.2–1.7	4	0.05 (0.05)	<0.01–0.12
Next morning urine concentration	4	1.0 (0.7)	<0.4–1.5	4	0.13 (0.11)	<0.02–0.19
Workers with short-term exposures to herbicides						
Preshift urine concentration	3	15.3 (12.3)	1.1–22.5	3	1.24 (0.82)	0.31–1.87
Postshift urine concentration	3	3.1 (2.3)	1.3–5.7	3	0.25 (0.13)	0.10–0.34
Next morning urine concentration	3	2.5 (0.4)	2.1–3.0	3	0.16 (0.04)	0.12–0.21

[A] ELISA = enzyme linked immunosorbent assay.

[B] HPLC = high performance liquid chromatography.

[C] In calculating a mean and standard deviation (Std. Dev.), a sample below the analytical limit of quantification was given a value of the LOD divided by the square root of two.[20]

TABLE IV. Average Postexposure Urine Metabolite Concentrations by Worker Characteristics

Characteristic	# Subjects	Average Postexposure Urine Metabolite Concentration (μg/mL) Measured by ELISA[A]	# Subjects	Average Postexposure Urine Metabolite Concentration (μg/mL) Measured by HPLC[B]
Applicators	20	5.9	18	0.85
Hauler/mixers	7	5.8	6	0.90
Not wearing cap	11	5.7	10	0.87
Wearing cap	16	5.9	14	0.87
Not wearing long-sleeved shirt	21	5.6	18	0.79
Wearing long-sleeved shirt	6	6.8	6	1.10
Not wearing undershirt	20	6.5	18	1.01
Wearing undershirt	7	4.0	6	0.45
Not wearing protective gloves	3	4.5	3	0.68
Wearing protective gloves	24	6.0	21	0.90
No air conditioning in vehicle	13	5.3	12	0.76
Air conditioning in vehicle	13	6.3	11	1.00

[A] ELISA = enzyme linked immunosorbent assay
[B] HPLC = high performance liquid chromatography

DISCUSSION AND CONCLUSIONS

Both the ELISA and HPLC analysis methods developed by NIOSH detected alachlor metabolites in the urine of exposed workers. Both tests may be used for biomonitoring of alachlor exposed individuals, but the ELISA test consistently yields higher results than the HPLC test.

The consistently higher ELISA results may be caused partially by a greater affinity of the antialachlor antibodies used in the ELISA test for the metabolites of alachlor over parent alachlor.[14] Since alachlor metabolites were not available to standardize the ELISA analyses, the analyses were standardized using parent alachlor. If the alachlor metabolites were in fact more reactive with the antibody substrate than parent alachlor, then the ELISA concentrations would be inflated. Also, the ELISA test may not be entirely specific to alachlor metabolites. Metabolites of other herbicides such as metolachlor, which are chemically similar to alachlor, also may react competitively with the antibodies in the ELISA. Since most of the participants did use other herbicides during the surveys, including metolachlor, nonspecificity of the technique also may have led to apparently elevated results. Comparison of these two analysis techniques is discussed further in a paper by Biagini.[14]

This study characterizes the concentrations of alachlor metabolites in three spot urine samples from 27 commercial applicators and hauler-mixers during the spraying of corn and soybean crops. Since most of the pesticide application workers had used alachlor on days before the exposure survey, the metabolite concentrations in the spot urine samples may partially represent exposure from previous days. This would lead to a higher estimate of alachlor metabolite excretion than would be represented by the amount of alachlor handled on the day of the survey. On the other hand, since only two spot urine samples were collected after the exposure survey, most of the alachlor metabolites excreted in the urine due to exposure on the day of the survey probably went undetected. Although the spot urine samples do not fully represent alachlor metabolite excretion from exposure specifically acquired on the day of the survey, they do indicate the relative metabolite concentrations that may be detected using ELISA or HPLC analysis in the urine of workers employed in the commercial use of alachlor.

The urine measurements also showed that application company employees who do not routinely work with herbicides had low levels of alachlor metabolites in their urine. But three individuals who mixed and loaded alachlor for relatively short periods of time had comparatively high levels of alachlor metabolites in the urine. Thus workers who believe their exposures to herbicides are short-term and minimal should be aware that even short-term exposures may result in substantial absorption of alachlor. Workers also should be aware that they may be exposed to pesticides from previously soiled clothing even after the clothing has been laundered.[21]

Creatinine adjustments made no difference in the interpretation of the urine data and did not reduce the overall variability in the urine measurements. Creatinine excretion is subject to wide fluctuations due to various internal and external factors and will not necessarily improve the correlation between absorbed dose and exposure estimates.[18]

The lack of correlation between the average postexposure urine levels and the amount of alachlor used during the survey may be due to the poor representation of the total absorbed dose of alachlor by the spot urine samples. Or it could mean that other factors, such as work practices, have a greater effect on subject-to-subject variability than amount of alachlor usage.

There was little difference between the urine metabolite concentrations of the applicators and hauler-mixers, indicating that their exposures were probably very similar. This conclusion is consistent with the results of the exposure assessment survey.[12] Both the applicators and hauler-mixers are involved in handling and loading alachlor into the application tanks, but only the

applicators spray alachlor on the cropland. The similarity in their excretion levels may indicate that exposure primarily occurs during the mixing and loading of alachlor. All of the applicators in this study used enclosed cab vehicles, which have been shown to reduce the exposure of applicators greatly when spraying crop land.[22]

All the applicators and hauler-mixers in this study used very similar types of mixing and application equipment, but there were individual differences in types of clothing worn. The lack of significant differences in the workers' average postexposure urine concentrations by types of clothing worn does not support the conclusion that clothing has an effect on absorbed dose. However, this is a small cross-sectional study with few observations in each comparison group, and the power to detect differences in urine concentrations by clothing groups is low.

It also may be important to examine simultaneously the joint effects and interactions of multiple factors, such as various combinations of clothing worn and amount of alachlor usage. But as already mentioned, the sample size in this study was too small. Conclusions about the workers' absorbed doses could be made with more confidence if the study size were larger, the contributions from the previous days' exposures were known, and all urine voids had been collected until the absorbed alachlor was completely excreted.

No objective, systematic measures of individual work practices were collected during the study; therefore, the effect of work practices on absorbed doses could not be evaluated. However, several workers were observed not properly following label requirements.[12] Recommendations for reducing worker exposures, and in turn absorbed doses, are offered in the exposure assessment paper.[12]

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