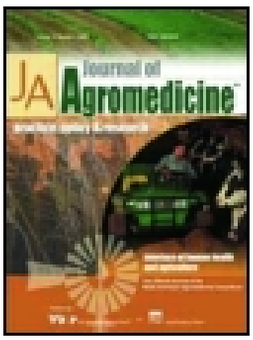

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Analysis of Pesticide Exposure and DNA Damage in Immigrant Farmworkers

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ABSTRACT. In the last decade, the Comet assay has been used increasingly in studies of workers potentially exposed to genotoxic substances in the workplace or environment. Significant increases in DNA damage measured with the Comet assay has been reported in lymphocytes of agricultural workers; however, less intrusive means of biomonitoring are needed in epidemiological investigations. This study was designed to use the Comet assay to describe the association of markers of DNA damage in oral leukocytes with biomarkers of pesticide exposure in 134 farmworkers working in berry crops in Oregon compared to control populations. The authors also examined the extent of DNA damage in young workers compared to adults and the effect of work histories, lifestyle factors, and diet on markers of DNA damage. Urinary levels of organophosphate pesticides were low at the time of sampling; however, mean levels of the Captan metabolite tetrahydrophthalimide (THPI) were found to be shifted significantly higher in the farmworkers (0.14 μg/ml) compared to controls (0.078 μg/ml) (one-sided *p* value = .01). Likewise, the combined molar equivalent of all dialkylphosphate metabolites was marginally higher in farmworkers (*p* value = .05). The mean tail intensity was significantly greater for agricultural workers compared to controls (one-sided *p* value <.001), indicating more DNA damage in the oral leukocytes. On average, the mean tail intensity was 10.9 units greater for agricultural workers (95% CI: 6–16 units greater). Tail moment was also significantly greater for agricultural workers compared to nonagricultural workers (one-sided *p* value <.001). No Comet parameter was significantly associated with years spent working in agriculture, age, sex, body mass index, diet, and alcohol or tobacco use. The results of this study demonstrate the feasibility for using the Comet assay in biomonitoring studies of farmworkers. Additional studies are needed to examine the effects of different pesticide types on DNA damage and to capture the temporal relationship between exposure to agricultural chemicals and changes in Comet parameters.

KEYWORDS. Organophospate pesticides, comet assay, farmworkers, agriculture, genotoxicity, occupational exposure

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INTRODUCTION

Farmworkers are exposed to pesticide spray, drift, and residues in the soil and on foliage; however, little scientific evidence is available to determine acceptable levels of pesticide exposure to this population. Although considerable advances have been made in estimating the amount of exposure of this population to pesticides, there remain distinct challenges in assessing the associations between these exposures and health risks. Although certain safety practices are known to protect workers from the acutely harmful health effects of exposure to agricultural chemicals, less is known regarding protection against exposures to low levels of pesticides, and the association of chronic low-level pesticide exposure and potential neurotoxicity, reproductive toxicity, endocrine disruption, and carcinogenic effects.

Within the last decade, the Comet assay has been used with increasing popularity to investigate the level of DNA damage in biomonitoring studies.[1] The Comet assay (single-cell gel electrophoresis) is a rapid and highly sensitive method to detect several types of genotoxic damage including DNA single-strand breaks, alkali-labile sites, and incomplete excision repair sites. Cells with damaged DNA resemble comets with tails due to DNA fragmentation while the DNA of undamaged cells remains intact.

Faust et al. reviewed the use of the Comet assay in 30 occupational studies.[2] The objective of the review was to determine whether human lymphocytes are relevant target cells for monitoring purposes or if non-blood cells, such as exfoliated buccal cells (i.e., epithelial cells) could provide a more precise prediction of health effects. Three agricultural studies were reviewed in this panel, all conducted on pesticide plant workers and each showing a significant relationship between Comet results and occupational exposure. Faust et al. summarized that the Comet assay was a fast and cost-effective test and that there is remarkable concordance between the Comet assay and other cytogenetic assays. They did point out that a shortcoming of the test is the low number of study participants, a limitation frequently found in studies requiring blood samples from working populations.

Most of the reports to date have focused on the use of the Comet assay on lymphocytes from venous blood samples. In studies of workers in the pesticide manufacturing industry, investigators have documented significantly increased levels of DNA damage using the Comet assay.[3–7] Studies using lymphocytes isolated from the venous blood of agricultural workers have also found increased Comet assay DNA damage[8–10] and Iranian insecticide formulators,[11] whereas there have been reports of no increased damage in other agricultural worker studies.[12–14]

These studies point to the potential value of using the Comet assay as a method of monitoring pesticide-exposed populations for genotoxicity; however, the dependence on venous blood samples for lymphocytes presents logistical problems in obtaining large numbers of agricultural workers. The Comet assay can be done on buccal cells, making this technology especially attractive for epidemiological studies of large numbers of workers because buccal cells are a convenient, noninvasive tissue to collect.[15] In this report we describe the results of an investigation employing the use of the Comet assay on oral leukocyte cells in a field study of migrant farmworkers working in berry crops in Oregon. The study was designed specifically to examine the association between markers of DNA damage and biomarkers of pesticide exposure. Because a large proportion of the farmworker population consists of adolescents, we also examined the extent of DNA damage in young workers compared to adults. The study also assessed the effect of work histories, lifestyle factors, and diet on markers of DNA damage.

METHODS

Study Participants

We recruited farmworkers harvesting small fruit and berry crops in the state of Oregon during the summer of 2004. The labor force that harvests Oregon small fruit and berries is highly mobile. Most reside in the community for only the 2 months of harvesting (June to July) before moving on to other agricultural work. A comparison group of Latino individuals

who do not work in agriculture was recruited from the same geographic area. Recruitment was conducted by soliciting study participation among students of the Oregon Migrant Education Program (86% Latino). Students in this program are children whose parents work in agriculture and the student may or may not be engaged in agricultural work. In addition, we recruited farmworkers from an evening English as a Second Language (ESL) program. This recruitment site included adolescent and adult workers. We also recruited half of our sample from residents of farmworker camps. All of these individuals worked in agriculture and included both adults and adolescent workers. We also recruited Latino adults not working in agriculture from our contacts with community groups and support agencies serving the Latino population in Oregon.

Similar recruitment procedures were used in all the sites. In the school setting, preliminary information on the research study was presented by educational staff members in Spanish. The information included the purpose of the study, the amount and nature of involvement should the student choose to participate, and the monetary reimbursement for participating in the study. Students indicating an interest in the study were scheduled for an interview in which the informed consent process was explained. Study consent forms were sent home to the parents of all youth under the age of 18 years. The youth returned the signed parental consent form and also signed their consent form before they were enrolled in the project. In the farmworker camps, bilingual research staff visited the residential site in the afternoons after harvest in the fields was completed. Bilingual research staff provided information in Spanish on the purpose of the study, the amount and nature of involvement required should the individual choose to participate, and the monetary incentives for participating. Persons who indicated an interest in the study were scheduled for an interview in which the study protocol was explained and informed consent forms were signed. Informed consent was also obtained from parents of youth under the age of 18. Community controls were recruited by attending meetings of community organizations and explaining the purpose of the study, the amount and nature of involvement, and the reimbursement for participating. Interested persons were scheduled for a testing/interview session in which informed consent was obtained. All consent processes and study protocols were approved by the Institutional Review Boards of the University of Pennsylvania and Oregon Health & Sciences University.

Procedures

During the interview and test session, questionnaires on work histories and work practices associated with reducing pesticide exposure were administered. The questionnaires on work histories and work practices have been used extensively in our field studies of agricultural workers.[16–19] The questionnaire contains items on type of work activity, pesticide application, type of crop(s), hours per week, use of protective clothing, bathing, laundry, and wearing of clothing outside of fields. New sections were added to the questionnaire, including more detailed work history for the week prior to data collection, a short diet and activity history, and a brief tobacco/alcohol history. Weight and height was measured for calculation of body mass index (BMI) and a spot urine sample was obtained for analysis of organophosphate metabolites and a metabolite of the fungicide Captan.

Subjects were given two labeled sterile 50-ml conical polypropylene centrifuge tubes each containing 20 ml sterile Hanks' balanced salt solution and were instructed to vigorously rinse their mouth twice for 60 seconds with the two 20-ml saline solutions, then spit them into a sterile 50 ml centrifuge tube. Collection was monitored and timed by study personnel. Our samples were obtained from farmworkers at least a week after the start of the berry harvesting.

Specimen Preparation

Urine and oral rinse specimens were transported on ice to our laboratory facilities. Cells in the oral rinse were processed within 8 hours after collection. Leukocytes in the oral rinses were isolated from other cells by density gradient centrifugation using Histopaque 1077. The cells were aliquoted into cryogenic vials and then

cryopreserved by placing the samples in an ice bath (45% RPMI 1640 culture medium, 50% fetal bovine serum [FBS], and 5% dimethyl sulfoxide [DMSO], a cryopreservative). The samples were cooled at a rate of −1°C/min until frozen and stored at −90°C. Frozen leukocytes were thawed quickly by submerging in a 37°C water bath and washing the cells with ice-cold wash media (40% RPMI, 50% FBS, 10% glucose). The thawed leukocytes were analyzed by the Comet assay according to the method of Singh et al.[20] with minor modifications.[10] Briefly, ~0.5×10^4 leucocytes were embedded between a layer of 1% normal melting agarose and 0.5% low melting point agarose. The slides were run in a horizontal electrophoresis apparatus, treated with an alkaline lysis solution, neutralized, stained with propidium iodide (20 μg/ml), and the extent of DNA damage determined by examining the cells for tail length and moment using a fluorescence microscope equipped with an automated digital analysis system running Comet assay III (Perceptive Instruments, UK). The Comet images were analyzed for three parameters of DNA damage: tail length, tail fluorescence intensity (percent of DNA in tail), and tail moment (the product of tail length and tail intensity).

Urine samples were analyzed for organophosphate metabolites according to previously published methods.[10,18,19,21] Five dialkylphosphates were analyzed by gas chromatography (GC) with pulsed flame photometric detection (PFPD): dimethylphosphate (DMP), diethylphosphate (DEP), dimethylthiophosphate (DMTP), dielthythiophosphate (DETP), and dimethyldithiophosphate (DMDTP). Aliquots of the sample underwent azeotropic distillation, centrifugation, and evaporation under a nitrogen stream, with reconstitution in acetonitrile and derivitization with pentafluorobenzylbromide and heating to convert phosphate acids to esters. The metabolites were confirmed with gas chromatography/mass spectrometry (GC-MS). The method has good precision, excellent recovery, and detection limits around 2 to 5 μg/L.

In addition to the organophosphate metabolites, all urine samples were analyzed for the metabolite of Captan (133-06-2; *N*-trichloromethylthio-4-cyclohexene-1, 2-dicarboximide, $C_9H_8Cl_3NO_2S$) an organochlorine fungicide of the dicarboximide chemical family that is used on both apple and berry crops in Oregon. Captan has been classified as a B2 (probable human) carcinogen.[22] Tetrahydrophthalimide (THPI), the major urinary metabolite of Captan, was analyzed in urine samples by GC/MS in a SIM mode with a detection limit of 20 ppb.[23] Urine samples were extracted with methylene chlorine, dried on a Roto-Vap evaporator, passed through a silica SepPak cartridge, taken to dryness, and each sample reconstituted with benzene prior to analysis by GC GC/MS in a SIM acquisition mode using *m/z* 151 and 278.

All urine samples were measured for creatinine concentration (mg %) using the Sigma Diagnostic Creatinine Assay Kit No. 555-A and a Spectra MAX 190 plate reader. Samples below the 5th and above the 95th percentiles (mg %) were excluded from analysis to remove very dilute and very concentrated samples.

Data Analysis

Demographic and lifestyle characteristics (e.g., age, sex, smoking status, BMI, antioxidant intake) were summarized with either means or medians (for continuous responses) or percentages (for categorical responses) and analyzed using *t* tests or chi-squared tests of association. One half the level of detection (LOD) was substituted for pesticide metabolite levels below the LOD prior to analysis and summation of the molar equivalent concentrations. Metabolite levels and the molar sum were log-transformed to improve symmetry. General linear models were used to assess the significance of these log-transformed measurements between agricultural workers and controls while adjusting for factors such as age, smoking status, dietary intake of antioxidants, and creatinine concentration in urine. Back-transformation of these adjusted means (from the log-scale) provide an estimate of the *median* response on the original measurement scale. At the same time, additive changes observed in the log-transformed data became multiplicative effects when the data were back-transformed.[24,25] DNA damage was determined by measuring tail length, tail moment, and tail intensity of at least 10 cells/sample. The parameters tail length, tail intensity, and tail

moment were analyzed using a mixed-effect model with subject being treated as a random factor. Tail length and tail moment were log–transformed prior to analysis; tail intensity was not transformed. Analyses were done using R version 2.5.1.[26]

RESULTS

One hundred thirty-four Latino farmworkers who were working in agriculture at the time of recruitment and 55 Latino individuals who reported not currently working in agriculture participated in the study and provided biological samples (urine or buccal cells) for analyses. The majority of participants (n = 167, 88.4%) were immigrants from Mexico, primarily the state of Oaxaca. Nine participants (2 agricultural, 7 nonagricultural) were born in the United States. Sixty-seven participants (65 agricultural, 2 nonagricultural) listed an indigenous dialect as their primary language. The average age among farmworkers was 23.3 (SD = 8.6) years and was 23.1 (SD = 10.2) years for nonagricultural participants. Farmworkers reported working in agriculture an average of 6.9 years (SD = 6.3) compared to an average of 1.8 years (SD = 3.2) for nonagricultural participants. Thirty-one percent of the farmworkers were women, compared to 53% in the group of nonagricultural participants. Table 1 presents descriptive statistics for the study participants.

Twelve percent of the agricultural workers (n = 16) reported mixing/applying pesticides sometime during the past month. All of the agricultural workers reported working in fields/orchards/vineyards; 18% (n = 24) reported additional work in a nursery; 19% (n = 25) reported additional work involving packing; 8.2% (n = 11) reported some work in all three job classifications. Among the nonagricultural workers, 43.6% (n = 24) were students; 12.7% (n = 7) reported working in childcare or as a babysitter; 12.7% (n = 7) reported working in a restaurant; 12.7% (n = 7) were involved in construction/stone laying/landscaping. The remaining 10 nonagricultural participants reported various jobs, each unique to the individual.

TABLE 1. Demographic and Lifestyle Characteristics of Study Sample

	Agricultural Workers (n = 134)	Controls (n = 55)	p Value
Mean age (SD)	23.3 (8.6)	23.1 (10.2)	.87
Mean years of agricultural work (SD)	6.9 (6.3)	1.8 (3.2)	<.001
% female	30.6	52.7	.004
Mean BMI (SD)	25.4 (4.5)	26.1 (6.5)	.52
Carotenoid intake: 25th, 50th, 75th percentile	5, 6, 7	4, 6, 7	.18
Antioxidant intake: 25th, 50th, 75th percentile	10, 11, 13	8, 11, 13	.19
% smoking (≥ 1 cigarette/day)	9.0	10.1	.68
Alcohol (%≥ 1 drink/week)	21.6	25.5	.57
% reporting recent illness	35.1	36.4	.87

Means were analyzed using t tests whereas proportions were analyzed using chi-square tests. Two dietary variables (carotenoid and antioxidant intake) were analyzed using a Wilcoxon rank-sum test.

Pesticide Metabolites

One hundred eighty-six subjects (132 agricultural workers, 54 controls) provided urine samples. Subjects with creatinine levels less than the 5th percentile (31.25 mg/dl) or greater than the 95th percentile (227.5 mg/dl) were removed from the sample (n = 10 from each end) due to the possibility of dehydration or overhydration affecting the metabolite results. This left 166 subjects (121 agricultural, 45 nonagricultural) with urine samples for analysis of pesticide metabolites. Nondetectable levels of dialkylphosphate (DAP) and tetrahydrophthalimide (THPI) metabolites were replaced by one-half the minimum detectable level/limit prior to analysis or summing the molar equivalent concentrations of DAP metabolites. The Minimal Detectible Level (MDL) for THPI was 0.05 μg/ml and was 4 ng/ml (DMP), 2 ng/ml (DEP), 2.2 ng/ml (DMTP), and 1.6 ng/ml (both DMDTP and DETP) for the DAP metabolites.

No significant increase was observed in the DMTP metabolite levels of farmworkers versus controls (one-sided p value = .63; Wilcoxon

test). The mean level of DMTP was 12.0 ng/ml among farmworkers and 14.0 ng/ml among controls. After adjusting for age, levels of DMTP among farmworkers were not significantly higher than those found in controls (one-sided p value = .64). Levels of THPI (μg/ml) were shifted significantly higher in the farmworkers compared to controls (one-sided p value = .01; Wilcoxon test). The mean level among farmworkers was 0.14 (SD = 0.15) compared to 0.078 among controls (SD = 0.10). Over 50% of both groups had nondetectable levels of THPI. Although the medians (0.025 μg/ml for both groups) did not differ in the two samples, the 60th (0.025 μg/ml for controls versus 0.127 μg/ml for agricultural workers) and 75th percentiles (0.150 μg/ml for controls versus 0.200 μg/ml for agricultural workers) were both significantly higher in the farmworker population (60th percentile, one-sided p value = .010; 75th percentile, one-sided p value = .037. The combined molar equivalent of all DAP metabolites were shifted marginally higher in farmworkers relative to controls (one-sided p value = .05; Wilcoxon test). As with THPI, the shift occurs at percentiles beyond the median. Median values were 0.098 and 0.070 μmol/L for agricultural and nonagricultural workers, respectively, and these measures did not differ (one-sided p value = .15), but the 60th percentile (respectively 0.13 and 0.091 μmol/L for agricultural and nonagricultural workers) did differ significantly (one-sided p value = .016).

Comet Assay

One hundred thirty-nine subjects (102 agricultural, 37 nonagricultural) submitted samples for Comet analysis. Eight subjects had fewer than 10 cells for analysis and were excluded, leaving 131 subjects (98 agricultural, 33 nonagricultural) with sufficient data (≥10 cells/assay).

The mean Comet assay parameters in the leukocytes of exposed farmworkers and the control subjects are summarized in Table 2. No significant difference was found between agricultural workers and controls with respect to tail length (one-sided p value = .17). The mean tail intensity was significantly greater for agricultural workers compared to controls (one-sided p value < .001).

TABLE 2. Partial Correlations Between Comet Parameters and THPI and Combined Concentration of Methyl Metabolites

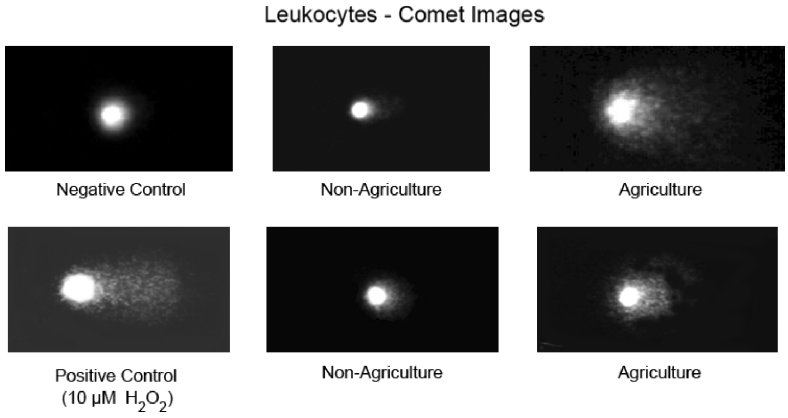
	THPI	Combined DAP
Tail length	.14 (.08)	.063 (.37)
Tail intensity	.18 (.02)	.12 (.22)
Tail moment	.21 (.01)	.13 (.17)

Analysis adjusts for creatinine concentration, cigarette consumption, and alcohol consumption. One-sided p value shown in parentheses.

On average, the mean tail intensity was 10.9 units greater for agricultural workers (95% CI: 6–16 units greater). Tail moment was also significantly greater for agricultural workers compared to nonagricultural workers (one-sided p value < .001). The median tail moment for agricultural subjects was estimated to be 43% greater than for nonagricultural subjects (95% CI: 17–75% greater). Figure 1 shows representative examples of the leukocytes of agricultural and nonagricultural workers in addition to examples of leukocytes from a negative and positive control. Because our control group contained individuals who had previously, but were not currently doing farm work, we examined the relationship between total years spent in agriculture and DNA damage. No Comet parameter was significantly associated with years spent working in agriculture (two-sided p values = .40, .93, .46 for tail length, tail intensity, and tail moment, respectively). Adolescent farmworkers did not appear more susceptible to genetic damage, with our Comet parameters showing no significant association with age (two-sided p values = .37, .10, and .17 for tail length, tail intensity, and tail moment, respectively) or sex (two-sided p values = .93, .97, and .84 for tail length, tail intensity, and tail moment, respectively).

One hundred thirty-six subjects (100 agricultural workers, 36 controls) submitted both a urine sample and an oral rinse sample. Of these, 118 samples (90 agricultural workers and 28 controls) simultaneously satisfied the restrictions placed on creatinine (≥31.25 mg/dl, ≤227.5 mg/dl) levels and on the number of cells needed for reliable Comet analysis (≥10). The partial correlation was computed between each of the three aforementioned

FIGURE 1. Representative samples of comet assays of oral leukocytes of agricultural workers, nonagricultural workers, and positive/negative controls.



Comet parameters and levels of THPI, and the molar concentration of the summed DAP metabolites while controlling for creatinine concentration and cigarette and alcohol consumption. Significant positive associations were found between THPI and both tail intensity (r = .18, one-sided p = .02) and tail moment (r = .21, one-sided p = .01). No associations were found between Comet parameters and the combined molar concentration of DAP metabolites (Table 2).

We examined the extent in which alcohol, smoking, diet, or BMI influenced the Comet analysis findings. Alcohol and cigarette use were dichotomized according to any use. Twenty-six of the subjects reported some alcohol use, two subjects reported some tobacco use, and seven reported using both. Vegetable carotenoids and antioxidant consumption were treated as continuous variables. BMI was log-transformed prior to inclusion in the model. These five variables (plus the interaction between alcohol and cigarette use) were not significantly associated with average tail length (F(6, 124) = 1.92, p = .08), tail intensity (F(6, 124) = 0.98, p = .44), or tail moment (F(6, 124) = 1.84, p = .10).

DISCUSSION

The results of this study point to the usefulness of the Comet assay in biomonitoring occupational populations exposed to pesticides. In this report we addressed two limitations noted by Faust et al.[2] in their review of the usefulness of the Comet assay by including both genders in our study sample and also a quantitative exposure measure. We also included information on physical activity and diet between control and exposed groups.

Cigarette smoking is assumed to be a potential confounder of studies of pesticide exposure and DNA damage due to the large number of genotoxic substances in tobacco smoke. We did not find a significant relationship in our results. This is particularly surprising because Faust suggests that exfoliated oral or nasal cells might be more susceptible to damage given the direct contact of the cells in these locations with tobacco agents. The amount of smoking could be a major determinant of DNA damage. Likewise, we did not find a significant relationship between age of controls or farmworkers and levels of DNA damage. As Faust has suggested, although it seems reasonable to speculate that DNA damage and Comet parameters may increase in older subjects as compared to younger ones, most studies do not support this assumption. Our sample also consisted of a large proportion of younger workers who could be having rapid growth, thereby increasing the risk of DNA damage. However, we found very similar levels of DNA damage in both our adult and adolescent farmworkers.

A strength of our study was the large number of subjects and the ability to control for

confounding variables such as diet and ethnicity. Although it has been speculated that dietary antioxidants, exercise, sunlight, and air pollution can influence Comet results, Moller and colleagues report that they are virtually never important determinants in cross-sectional studies.[1,27] Nonetheless, the major difference between our agricultural workers and the controls in this study could be sunlight exposure related to working in agriculture. Agricultural workers routinely wear long sleeve shirts, long pants, and caps; however, specific sun exposure was not assessed in this study. It is possible that the differences that we observed result from a combination of chemical exposures, sunlight exposure, and strenuous work in agriculture. Future studies will investigate the relationship between DNA damage in occupational groups with comparable work outside, but with varying levels of pesticide exposure.

Minimally invasive methods of biomonitoring are needed in epidemiological studies of farmworker populations.[28] The results of this study using oral leukocytes in the Comet assay demonstrate the feasibility for use with large population studies and the sensitivity to detect differences in exposures to pesticides. The Comet assay was conducted on leukocytes retrieved from buccal samples. The limitations that we encountered were fewer cells per sample compared to studies using venous blood lymphocytes and less hardiness to survive the cryofreezing procedure needed to store samples in epidemiological investigations. The predominate type of white cell found in saliva as been reported to be polymorphonuclear neutrophils and only 1% of the white cells in saliva are lymphocytes.[29] There is marked difference in the life span of these two types of cells, with lymphocytes living much longer. This variation could affect the interpretation of the exposure and DNA damage time frame. Future papers will describe the differences in the Comet assay results conducted on fresh versus frozen leukocytes and methods to improve the viability of oral leukocytes once frozen. Studies are also needed to simultaneously examine DNA damage in oral leukocytes and venous lymphocytes.

We did not find a strong correlation between the levels of urinary pesticide metabolites and Comet parameters. This was not surprising, because the urinary metabolite levels are a short-term marker of exposure,[30] and the damage observed the leukocyte samples may not reflect recent exposures. It is also very difficult to fully characterize the amount and types of pesticide exposures that occur among farmworker populations. In a recent publication of a pesticide guide for Oregon and Washington berry crops, more than 40 insecticides are listed as potentially effective on berry crops, including organophosphates such as diazinon, phosmet, chlorphyrifos, and malathion.[31] Our previous studies of this population have revealed low level of organophosphate metabolites in the urine at the time of harvest compared to populations harvesting other crops such as orchard fruit.[18,19] Based on our previous work, we did predict that fungicides would be the major exposure among our agricultural workers at the time they were harvesting berry crops. However, we did not find a strong correlation between levels of the Captan metabolite and Comet results. The increased genetic damage that we observed could be a factor of exposures that occurred prior to harvesting the berry crops in Oregon. We have previously shown that families migrating from different agricultural regions demonstrate changes in their urinary pesticide metabolites as quickly as 2 weeks.[18]

Zeljezic and Garaj-Vrhovac studied Croatian pesticide production workers and found that after a period of high exposure to a mixture of pesticides, there were statistically significantly increased levels of DNA damage in the Comet assay (i.e., tail length and tail moment).[4] After the workers were removed from production for 8 months, both Comet parameters decreased significantly compared with the first sampling point, but they remained increased compared with the control. Additional studies are needed to demonstrate the sensitivity of the Comet assay in detecting changes in exposure to agricultural pesticides and factors that might decrease the amount of DNA damage that occurs, including the type of pesticides used and personal protective clothing.

The results of this study are limited to the work characteristics and pesticide exposures of agricultural workers in berry crops in Oregon and cannot be generalized to other workers who

may be exposed to different classes of pesticides and other agricultural chemicals. Our previous studies have demonstrated that farmworkers recruited from ESL classes have higher knowledge of pesticide safety than workers recruited from farmworker camps.[32,33] The extent to which pesticide safety knowledge effects exposures and DNA damage is unknown.

CONCLUSION

The need for noninvasive measures of biological markers of pesticide exposure and genotoxicity are needed to characterize potential health risks associated with exposure to agricultural chemicals. This study demonstrates the ability of the Comet assay to detect subtle differences in oral leukocytes from working populations exposed to low levels of agricultural chemicals compared to control populations. The results point to the need for additional studies to determine optimal methods of preserving oral leukocytes for Comet analyses, and the need to incorporate measures of factors that might confound an observed association between pesticide exposure and DNA damage. Studies to capture the temporal relationship between exposure to agricultural chemicals and changes in DNA damage are needed, along with intervention studies to detect measures that decrease the amount of DNA that occurs.

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