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Carolina Fernandez Date

Concentrations of Urinary Dialkyl Phosphate Metabolites of Organophosphorus Pesticides in Colombian Floriculturists

By

CAROLINA FERNANDEZ
Master of Public Health

Global Environmental Health

_____

Anne M. Riederer, Sc.D.
Committee Chair

_____

Dana Boyd Barr, Ph.D.
Committee Member

_____

Paige Tolbert, Ph.D.
Committee Member

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By

CAROLINA FERNANDEZ

B.A.
Boston University
2001

Thesis Committee Chair: Anne M. Riederer, Sc.D.

An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Global Environmental Health
2011

Abstract

Concentrations of Urinary Dialkyl Phosphate Metabolites of Organophosphorus Pesticides in Colombian Floriculturists

By Carolina Fernandez

BACKGROUND: Previous studies have documented the use of organophosphorous (OP) pesticides in the Colombian floriculture industry. However, limited data are available to characterize worker exposures.

OBJECTIVES: The objective of the present study was to describe the levels of urinary dialkylphosphate (DAP) metabolites of OP pesticides in urine collected from workers in two flower-growing regions of Colombia. A secondary objective was to determine whether these levels differ significantly by sample collection period, geographic region, or worker task.

METHODS: A convenience sample of 358 floriculturists was recruited from farms in Sabana de Bogota and Antioquia. Participants provided three spot urine samples (collection periods: morning, pre- and post-shift) and answered a questionnaire collecting demographic and occupational data. Samples were analyzed for six DAP metabolites and creatinine. The two sample t-test, the Mann-Whitney U-test; the Chi-Square and the Fisher's Exact test were used to determine statistically significant differences in demographic and exposure characteristics between the two regions. A mixed-effects linear regression model was used to test whether log-transformed urinary composite dimethyl alkylphosphate (∑DMAP), composite diethyl alkylphosphate (∑DEAP) and summed DAP (∑DAP) concentrations varied by collection period, task and/or region.

RESULTS: Of the total participants, 298 (83%) worked on Sabana de Bogota farms, while 60 (17%) worked on Antioquia farms. ∑DMAP concentrations (nmol/L) of the Sabana de Bogota samples (36.6 (±2.7) morning, 15.1 (±2.9) pre-shift, 26.0 (±2.9) post-shift) were generally higher than the Antioquia samples (GMs not calculated due to low detection frequency). Conversely, geometric mean ∑DEAP concentrations (nmol/L) were higher in Antioquia (14.6 (±5.2) morning, 8.8 (±5.3) pre-shift, 12.8 (±5.6) post-shift) than the Sabana de Bogota samples (7.2 (±5.0) morning, pre-shift-- not calculated due to low detection frequency, 7.5 (±4.8) post-shift samples). ∑DMAP concentrations varied significantly by collection period ($p<0.0001$) and region ($p< 0.0147$) while ∑DEAP concentrations varied significantly by collection period ($p=0.0014$) and marginally significantly by region ($p=0.0839$). Worker task did not significantly explain variance in urinary DAP metabolite levels.

CONCLUSIONS: We detected urinary OP pesticide metabolites in the majority of farmworkers in our study, with levels varying significantly by region and collection period. These factors could be taken into consideration in the development of pesticide safety education and training programs for Colombian floriculture workers

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Acknowledgments

First and foremost I offer my sincerest gratitude to my thesis faculty advisor, Dr. Anne Riederer and to my field thesis advisor, Dr. Dana Boyd Barr who granted me unconditional support throughout the proposal and completion of this study. Their unquenchable patience and vast knowledge in the field of pesticides and exposure analysis have allowed for numerous ideas to be enlightened and turned into reality.

Completion of the thesis required extensive laboratory analyses, all of which were performed in the Pesticide laboratory at the CDC. The work with the Colombian floriculturists allowed me to see at first-hand how the work of this laboratory applies to Public Health; I now have a greater appreciation of the efforts carried out by the individuals in this laboratory, their support and encouragement throughout this academic journey was essential.

Beyond the laboratory bench, I would like to especially thank Dr. Marcela Varona and Dr. Carlos Torres for sharing their epidemiological expertise in the field. Their collaboration and that of the members the Environmental Laboratory of the Instituto Nacional de Salud in Colombia (specifically Ligia Morales, Sonia Reyes, Angélica Lancheros, Ermel Olarte, Alejandro Nivia and Andrés Monroy) made the field work a complete success. Thanks to Dr. Ivan Dario Giraldo for facilitating access to the farms and coordinating sample collection in Antioquia. I would also like to thank Dr. Jorge Tolosa for early discussions on how to promote the research and for acting as a liaison with the industry. Very special thanks to my dear friend Shannon McClintock for sharing her biostatistics expertise during the data analysis and for teaching me about Mixed Models and to Lee-Yang Wong for assisting generating NHANES weighted data using SUDAAN. I gratefully acknowledge BATTELLE for providing the funding for the travel required for the sample collection and to Atlanta's Analytical Services office staff for their administrative support and assistance.

Finally, I thank my loving husband for his unconditional support and endless encouragement throughout my studies; my parents for their love and for giving me inspiration through their efforts, hard work and resilience.

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BACKGROUND

The production and use of organophosphorus (OP) pesticides has increased significantly in recent years due to the global phase-out of the use of the persistent organochlorine pesticides in agriculture (U.S. EPA 1999). OP pesticides are among the most widely used of the non-persistent pesticides and are actively used in the floriculture industry (Blanco-Muñoz et al. 2010; Lacasaña et al. 2010) to ensure a pest and disease-free, high quality product (USDA 2010).

In the human body, OP pesticides metabolize rapidly and are excreted within a few days after exposure (Barr et al. 1999). When OP pesticides are absorbed and distributed in the human body, they act as neurotoxicants by inhibiting acetylcholinesterase (AChE), resulting in hyper-excitation of post synaptic cholinergic receptors (Kamrin 1997; Marrs and Ballantyne 2004). In the environment, OP pesticides are easily hydrolyzed in the presence of water and light therefore tend not to persist in the environment (Barr et al. 2004). However, some residues can remain on/in plants and humans can potentially be exposed via ingestion of these plants produced for consumption. Agricultural workers can have higher levels of exposure than the general population when OP pesticides are present in the workplace. For both workers and the general population, exposure to OP pesticides or their breakdown products can occur via dermal absorption, inhalation, dietary ingestion and/or non-dietary ingestion (Barr et al. 2002; Grandjean et al. 2006; Whyatt et al. 2003).

Dialkylphosphate (DAP) metabolites can be measured in human urine after exposure to OP pesticides (Bradman et al. 2005; Castorina et al. 2010). Six commonly measured DAP metabolites are dimethylphosphate (DMP), diethylphosphate (DEP),

dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP) (Bravo et al. 2004). These metabolites are common breakdown products of the majority of OP pesticides. Urinary DAP metabolites are indicators of recent exposure, given that they are typically excreted within 72 hours of exposure (Barr et al. 1999). Measurement of these six non-specific DAPs provides information about cumulative exposure to the OP pesticide class (Barr et al. 1999). Several studies have employed biomonitoring of urinary DAP metabolites to yield information on OP pesticide exposure (Arcury et al. 2009; Fenske et al. 2005; Yucra et al. 2006). However, to date, only one published study has quantified urinary DAP levels among cut-flower industry workers (Lacasaña et al. 2010). Geometric mean levels of composite DAPs measured in this male study population in Mexico ranged from 480 to 2,000 nmol/g creatinine. There are no published studies among floriculture workers describing urinary DAPs levels in a study population comprised of both sexes, including women of childbearing age. Furthermore, no studies measuring DAPs have been conducted in the floriculture regions of South America.

In Colombia, the flower industry is a major contributor to the country's economy; cut flowers are the fourth largest export after coffee, petroleum and bananas (USITC 2003). The flower industry in Colombia is a significant source of employment, generating approximately 6.3 jobs per acre, accounting for more than 120,000 employees nationwide (ASOCOLFLORES 2009; Caycedo et al. 2008). In 2008, this industry generated $1.1 billion in revenue (ASOCOLFLORES 2009). Exports of cut flowers to the United States and the European Union constitute approximately 92% of total production

(ASOCOLFLORES 2009). Two out of every three non-U.S. grown flowers sold in the United States originate in Colombia (Caycedo et al. 2008).

The Colombian floriculture industry is concentrated in two geographical regions: Sabana de Bogota and Antioquia (Figure S1). In 2009, 76% of flowers produced for export were grown in areas surrounding the capital city of Bogota (Caycedo et al. 2008). Bogota is located on the Sabana de Bogota, an Andean plateau rising 2,600 m above sea level (Ronderos 2004). Minimal seasonal fluctuations in daylight length and annual average temperatures of 14°C (IDEAM 2011), among other factors, make the Sabana de Bogota ideal for floriculture. In 2009, the province of Antioquia in the Colombian central Andes produced an additional 17% of the country's cut flowers (Caycedo et al. 2008). The average annual temperature in Antioquia is 17°C (IDEAM 2011); differing climatic conditions there allow for growth of different flower varieties than those grown on the Sabana de Bogota (Caycedo et al. 2008).

Approximately 90% of employees in the Colombian floriculture industry work in the actual production process (ASOCOLFLORES 2009). Working in the fields and handling harvested products involve tasks with potential to increase pesticide exposure via multiple routes. Two published studies describe pesticide use practices in the Colombian floriculture industry. A survey of 8,867 floriculture workers in Bogotá by Restrepo et al. (1990) reported worker exposure to pesticides including OP pesticides. More recently, Varona et al. (2005) reported that of the pesticides used by 84 surveyed Colombian cut-flower farms approximately 3% were OP pesticides.

Despite knowledge of OP pesticide use in the Colombian floriculture industry, limited data are available to characterize workers' exposure. The main objective of the

present study was to document exposure to OP pesticides in these workers by measuring concentrations of the six DAP metabolites in urine collected from floriculture workers on farms in the in Sabana de Bogota and Antioquia. A secondary objective was to determine whether or not these concentrations differ significantly by sample collection period, geographic region, and/or worker task. The study was a collaboration among researchers from the U.S. Centers for Disease Control and Prevention (CDC) (Atlanta, USA), Emory University's Rollins School of Public Health (Atlanta, USA), Universidad El Bosque (Bogota, Colombia), and the Instituto Nacional de Salud de Colombia (Bogota, Colombia). It was conducted with approval and oversight from the ethical review boards of each participating institution.

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MATERIALS AND METHODS

Study population. We used the network of Varona et al. (2005) to recruit workers from 9 and 4 farms in Sabana de Bogota and Antioquia, respectively. Figure S1 (Supplemental Material) shows a map of the study sites. Participating farms employed a total of 3,627 workers, with sizes ranging from 38-553 workers. Eligible participants were ≥18 years old, had worked on the farm for ≥6 months and had performed the same task for ≥2 days preceding sample collection.

Workers were recruited between September 2008 and February 2009. Prior to or on the day of enrollment, each farm provided an updated list of employees and their job descriptions/titles and length of employment. Following Varona et al. (2005), we stratified workers on each farm into three exposure groups by task. The 'high risk' group included workers who directly handled pesticides (i.e., spraying, mixing, storing). The 'medium risk' group was comprised of workers such as field operators and those who cut, sorted, bundled and/or packaged flowers who did not directly handle pesticides, but took part in harvesting or post-harvesting activities. Administrative personnel formed the 'low risk' exposure group (Varona et al. 2005).

Potential participants were randomly selected from the high, medium and low risk exposure groups on each farm. Random numbers, generated by a web-based program (Research Randomizer, Social Psychology Network, Middleton, CT), were used to rank and select potential participants. The percentage of participants selected from each group was proportional the group's contribution to the farm's total number of employees.

On the smaller farms (<100 workers), potential participants were approached at their work site while on the larger farms (≥100 workers) they were gathered and briefed

on the study. All potential participants were provided with Spanish oral and written information describing the research and details of participation. After the briefings, worker questions were addressed either in private or as a group.

Based on resources available for sample analysis, we targeted 300 participants for enrollment. A total of 360 workers were screened and all were determined eligible. Of these, 358 were enrolled and provided written informed consent (99.4%) while two gave verbal consent but did not show up for enrollment. Recruitment, sampling and interviewing took place during work hours. No remuneration was given for participation.

Interviews. On the day of sample collection, participants were interviewed using a standardized questionnaire designed to collect data on basic demographics and behaviors potentially related to pesticide exposure at home and at work. The questionnaire was developed in English and Spanish by a bilingual member of the research team (C. Fernandez) and the Spanish translation certified by CDC's Multilingual Services. It was pre-tested with 30 workers and revised in response to their input. Interviews were conducted in Spanish by trained field personnel from the Colombian collaborating institutions.

Sample collection, transport and storage. Participants were given written and oral instructions in Spanish following CDC guidelines for the collection of urine at home (CDC 2009b). Three spot samples were collected from each participant within a 24-hour period: 1) a first-morning void sample, collected at home after at least two consecutive days of occupational exposure, 2) a pre-shift sample, collected upon arrival at work, and 3) a post-shift sample, collected at work at the end of the shift. Samples were collected in sterile polyethylene cups. During field work and transport to the field laboratory (i.e.,

Environmental Health Laboratory, Instituto Nacional de Salud, Bogota, Colombia), samples were kept cold using frozen gel packs. Sabana de Bogota samples were transported by car, while Antioquia samples were shipped via air courier. All samples reached the field laboratory on the day of collection. Immediately upon arrival, samples were transferred to glass bottles with Teflon-lined caps and stored at -20°C until shipment overnight on dry ice to the CDC Pesticide Laboratory in Atlanta. At CDC, samples were stored at -70°C until analysis.

Sample analysis. Urine samples were analyzed for 6 DAP metabolites following Bravo et al. (2004). Briefly, a 2-mL aliquot was fortified with a solution containing isotopically labeled analogue internal standards of the six target analytes. Water was removed by overnight lyophilization. Residues were extracted with acetonitrile and ethyl ether. The metabolites were derivatized to their respective chloropropyl phosphate esters. Concentrated extracts were analyzed by gas chromatography–tandem mass spectrometry (GC–MS/MS) and quantified using isotope-dilution calibration. The limit of detection (LOD) for each analyte was calculated as $3s_0$, where s_0 is the y-intercept value of the best-fit line of the standard deviation of the lowest four calibration standards versus the known standard concentration. LODs of the target analytes were 0.6 μg/L for DMP; 0.2 μg/L for DMTP; 0.1 μg/L for DMDTP; 0.2 μg/L for DEP; 0.1 μg/L for DETP; and 0.1 μg/L for DEDTP. Creatinine concentrations in urine were determined using a Roche Hitachi Modular P Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN) following a commercially available diagnostic enzyme method (Creatinine Plus, Product Application #04903773003, Roche Diagnostics, Indianapolis, IN).

Quality control (QC). Analyses were conducted on field and laboratory QC samples. Field QC samples consisted of field blanks and field spikes. Field blanks were prepared at CDC from a base urine pool collected from anonymous volunteers. The urine pool was filtered using a sterile 0.2µm microporous polyethersulfone membrane (Whatman Inc., Florham Park, NJ), diluted (1:1) with deionized water and left to stir overnight at 4°C. The filtered and diluted urine pool was screened to ensure low or non-detectable endogenous levels of pesticide metabolites. Two sub-pools of the field blank urine were spiked with native DAPs metabolite standards to create field spikes. The sub-pools were uniformly mixed, dispensed into randomly bar-coded glass vials and stored at -70°C. Concentrations of field spikes were characterized by 5 separate analyses: field QC low (approximate concentration ranged from 5 µg/L for DMP to 15 µg/L for DEP) and field QC high (approximate concentration ranged from 18 µg/L for DMP to 50 µg/L for DEP). Prior to sample collection, field blanks (31 samples) and spikes (35 low and 34 high) were shipped to the field laboratory. In the field, field QC materials were handled, stored, and transported in an identical manner as the field samples. Analysts were blinded to sample identification codes.

One laboratory blank urine sample, previously determined to be free of DAPs, and three laboratory spikes were analyzed along with each batch of field samples. For laboratory spikes, three sub-pools of blank urine were spiked with the six native DAPs to yield an approximate concentration of 3 µg/L ('lab QCL'), 12 µg/L ('Lab QCM') and 40 µg/L ('Lab QCH'). Concentrations of spiked in-house QC urine pools were characterized by 20 separate analyses (2 analytical runs per day for 10 days). QC materials were

evaluated using standard Westgard multirule criteria (Caudill et al. 2008). 100 field and 99 laboratory QC samples were analyzed, comprising 15% of total samples analyzed.

Statistical analysis. SAS version 9.2 (SAS Institute, Cary, NC) was used for statistical analysis. The two sample *t*-test was used to compare mean age between participants from the two study regions; the Mann-Whitney U-test was used to compare medians of other continuous but skewed data. The Chi-Square and the Fisher's Exact tests were used to evaluate differences between the two regions with respect to other demographic and pesticide exposure and safety behavior characteristics. Unadjusted (ug/L), dilution-adjusted (ug/g creatinine), and molar (nmol/L) urinary DAPs concentrations were calculated. Histograms of both unadjusted and dilution-adjusted concentrations of the 6 individual DAPs were right-skewed. Descriptive statistics including detection frequencies, geometric means (GM), geometric standard deviations (GSD) and percentiles were calculated for each DAP for each sample collection period. Concentrations <LOD were assigned a value equal to LOD/√2 following CDC protocol (2009). GMs and GSDs were not reported if the proportion of values <LOD exceeded 50%. Ranges of detectable values were reported for all analytes. Molar concentrations of the three dimethyl phosphates (DMP, DMTP, and DMDTP) were summed to provide a composite dimethyl alkylphosphate (∑DMAP) concentration, while concentrations of the three diethyl phosphates (DEP, DETP, and DEDTP) were summed to provide a composite diethyl alkylphosphate (∑DEAP) concentration. Concentrations of all six DAPs were summed to provide a composite ∑DAP concentration. The log-transformed composite concentrations were approximately normally distributed by the Shapiro-Wilk test (Shapiro and Wilk 1965).

We used a mixed-effects linear regression approach (Laird and Ware 1982) to test whether log-transformed urinary ∑DMAP, ∑DEAP and ∑DAP concentrations varied by collection period, task and/or region, controlling for creatinine as a measure of urinary dilution. We combined the participants' 21 tasks into 5 categories (e.g., packing/freezer rooms, green house, administrative, pesticide spraying/mixing, other). The mixed model approach was used to account for intra-participant as well as intra-farm correlations in urinary concentrations. The following is a general specification of the conditional hierarchical nested linear model fitted to the data:

$$Y_{ijkm} = ß_o + ß_1\, t_{i1} + ß_2\, t_{i2} + ß_3\, Task_{i1} + ß_4\, Task_{i2} + ß_5\, Task_{i3} + ß_6\, Task_{i4} + ß_7\, Reg_i + ß_8\, Creat_{ij} + ß_9\, Exp_i + a_{k(m)} + b_{i(km)} + e_{ijkm} \quad \textbf{[1]}$$

Y_{ijkm} denotes the log-transformed urinary DAP concentration in a sample collected from the i^{th} participant (i = 1, …, 358) at the j^{th} time point (j = 1, …, 3) in the k^{th} farm (k = 1, …, 13) from region m (m = 1, 2); $ß_o$ represents the population average concentration for reference cell categories; t_{i1} and t_{i2} represent pre- and post-shift sample collection periods; $ß_1$ and $ß_2$ are fixed effect regression parameters associated with time. The indicator variables for task and region are $Task_i$ and Reg_i respectively, their associated fixed effect regression parameters are depicted by $ß_3$ to $ß_7$. Creatinine is represented by $Creat_{ij}$ and its associated fixed effect regression parameter by $ß_8$. Pesticide exposure and safety behavior characteristics found to differ significantly between regions in the exploratory analyses were individually included in the model, represented in Equation 1 by Exp_i and its fixed effect regression parameter $ß_9$. The random effect of the k^{th} farm from region m is represented by $a_{k(m)}$, the random effect of the i^{th} subject from the k^{th} farm from region m is $b_{i(km)}$, the random error associated with the i^{th} subject from the k^{th} farm

from region m at the j^{th} time point is e_{ijkm}. It was assumed that $a_{k(m)}$, $b_{ij(km)}$ and e_{ijkm} are independent, mean zero, normally distributed random variables. We used SAS PROC MIXED to fit the mixed model and the LSMEANS statement to make pairwise comparisons among all levels of the fixed effects. Differences were considered statistically significant when $p < 0.05$ and marginally significant when $p < 0.10$, and a Tukey adjustment was used to ascertain significance or marginal significance when multiple comparisons were considered.

We evaluated model assumptions using plots of studentized residuals versus predicted values, quantile plots of residuals, and histograms of residuals. Cook's Distance was used to evaluate the influence of individual observations on fixed effects parameter estimates (Cook and Weisberg 1999). Missing data were assumed to be missing completely at random. In total, 7 morning-void and 2 post-shift samples were missing.

RESULTS

Demographic and exposure characteristics. Table 1 presents demographic characteristics of the study population. There were 298 (83%) participants from Sabana de Bogota and 60 (17%) from Antioquia. The mean age was 36.7 (±9.3) years. Age differed significantly ($p=0.007$) by region, with Antioquia participants younger on average. Other characteristics differing significantly by region included the percent of female participants ($p<0.0001$; 79% in Sabana de Bogota *vs.* 43% in Antioquia), the percent living in urban *vs.* rural areas ($p<0.0001$; 6% and 38% from Sabana de Bogota and Antioquia, respectively, reported living in rural areas), and the number of smokers ($p=0.0026$; 8% in Sabana de Bogota *vs.* 27% in Antioquia).

Table 2 presents self-reported pesticide exposure and safety behavior characteristics of the participants. The mean number of years spent working in floriculture was 11.5 (±7.7), with a significant ($p<0.0001$) difference between regions. Significantly more ($p=0.0085$) Antioquia workers (28% *vs.* 14%) reported using pesticides at home. 42% of total home users stored pesticides inside the house and 23% used these products in the five days before the interview. 98% of Sabana de Bogota participants reported eating >1 daily serving(s) of fresh fruits and vegetables, compared to 78% in Antioquia ($p<0.0001$).

19% of participants worked in packing or freezer rooms, 60% inside a greenhouse, 8% as pesticide applicators or mixers, and 6% on administrative tasks. Use of any type of personal protective equipment (PPE) was reported by 96% of participants regardless of task with a significant ($p<0.0253$) difference between regions. 90% percent of workers in Sabana de Bogota and 65% in Antioquia ($p<0.0001$) used uniforms. 95% of workers in both regions reported using gloves while 24% used masks, 12% used waterproof jackets, 11% used waterproof pants, and 82% used either short or high rubber boots. 95% of Sabana de Bogota workers reported changing their work clothes before going home *vs.* 73% in Antioquia ($p<0.0001$). Last, 87% of Sabana de Bogota *vs.* 37% of Antioquia workers ($p<0.0001$) reported washing work clothes separately.

Urinary DAPs concentrations and mixed-effects model results. A total of 351, 358, and 357, respectively, first morning void, pre-, and post-shift samples were collected. QC samples complied with quality limits ensuring valid measurements. Table 3 presents detection frequencies, geometric means, and selected percentiles of the DAPs concentrations for the Sabana de Bogota and Antioquia workers. DMTP was detected in

more Sabana de Bogota samples (88% morning, 57% pre-shift, 79% post-shift) than Antioquia samples (25% morning, 18% pre-shift, 25% post-shift). The geometric mean (geometric standard deviation) DMTP concentration (μg/L) in the Sabana de Bogota samples ranged from 0.8 (±4.9) for pre-shift to 3.1 (±3.8) for morning samples. DETP was detected in more Antioquia (53% morning, 38% pre-shift, 50% post-shift) than Sabana de Bogota samples (39% morning, 16% pre-shift, 38% post-shift). Geometric mean DETP concentrations (μg/L) of the Antioquia samples were 0.4 (±6.5) for post-shift and 0.5 (±6.4) for morning samples (pre-shift GM not calculated due to low detection frequency). ∑DMAP concentrations (nmol/L) of the Sabana de Bogota samples (36.6 (±2.7) morning, 15.1 (±2.9) pre-shift, 26.0 (±2.9) post-shift) were generally higher than the Antioquia samples (GMs not calculated due to low detection frequency). Conversely, geometric mean ∑DEAP concentrations (nmol/L) were higher in Antioquia (14.6 (±5.2) morning, 8.8 (±5.3) pre-shift, 12.8 (±5.6) post-shift) than the Sabana de Bogota samples (7.2 (±5.0) morning, pre-shift-- not calculated due to low detection frequency, 7.5 (±4.8) post-shift samples). Last, geometric mean ∑DAP concentrations (nmol/L) were higher in Sabana de Bogota (53.5 (±2.7) morning, 23.0 (±2.8) pre-shift, 41.2 (±2.9) post-shift) than Antioquia samples (34.1 (±4.0) morning, 21.9 (±3.7) pre-shift, 32.3 (±4.5) post-shift).

Table 4 presents results of the mixed effects model of time, task and region on log urinary DAPs (Equation 1). ∑DMAP concentrations varied significantly ($p<0.0001$) by time (*e.g.*, collection period) and region ($p< 0.0147$) while ∑DEAP concentrations varied significantly by time ($p=0.0014$) and marginally significantly by region ($p=0.0839$). ∑DAP concentrations varied significantly by time ($p<0.0001$) but not region ($p=0.3710$).

Pairwise collection period comparisons are also presented in Table 4. ∑DMAP concentrations were significantly lower in pre-shift ($p<0.0001$) and post-shift ($p=0.0004$) samples when contrasted with morning-void samples, and significantly higher in post- *vs.* pre-shift samples ($p=0.0004$). ∑DEAP concentrations were marginally significantly higher in pre-shift ($p=0.0511$) *vs.* morning-void samples and significantly higher in post-shift *vs.* pre-shift samples ($p=0.0009$). ∑DAP concentrations were significantly lower in post-shift ($p=0.0154$) and pre-shift ($p<0.0001$) samples when contrasted with morning-void samples. Post-shift ∑DAPs were significantly higher ($p<0.0001$) than pre-shift.

We found that wearing a uniform (not shown) was inversely associated with log ∑DMAP ($\beta=-6.0 \times 10^{-1}$, $p=0.0048$), log ∑DEAP ($\beta=-6.3 \times 10^{-1}$, $p=0.0495$) and log ∑DAP ($\beta=-6.7 \times 10^{-1}$, $p=0.0016$). Inclusion of uniform use in the regression model changed the effect estimate for region in the ∑DEAP model from marginally significant to not significant ($\beta =-4.8 \times 10^{-1}$, $p=0.1819$) and in the ∑DAP model from not significant to marginally significant ($\beta =4.3 \times 10^{-1}$, $p=0.0812$). No other pesticide exposure or safety behavior characteristic were significant in the mixed models.

DISCUSSION

We examined concentrations of six OP pesticide metabolites (DMP, DEP, DMTP, DMDTP, DETP and DEDTP) in urine collected from 358 floriculturists working in farms from two regions in Colombia, Sabana de Bogota and Rionegro. Measurable levels of DAP metabolites were detected in 87% morning, 67% pre-shift, 83% post-shift of samples. ∑DAP concentrations (ug/g creatinine) reported in this study (Table S2) were 7 to 22 times lower than those reported by Lacasaña et al. 2010 for Mexican floriculture workers (58- to 90 vs. 480 to 2,000) albeit the frequencies of detection were reasonably

comparable (58-90% and 90% for Colombian and Mexican floriculturist, respectively). Few factors make direct comparisons between floriculturist in our study and the study in Mexico problematic. Pesticide regulations and pest control strategies in Colombia and Mexico may be different; thus, the percentage of OP pesticides used in Mexican cut-flower farms may be higher than the 3% (Varona et al. 2005) used by Colombian farms. Further, the occupational culture (i.e. PPE provided/usage, pesticide safety training) in the industry/farms from the two different countries may affect relation between exposure and urinary metabolite concentration. Additionally, although values below LOD were imputed for the data analyses in both studies, the LODs in our study are 38 to 225 (0.1 to 0.6 *vs.* 22.5 ug/L) times lower than those reported by Lacasaña et al. 2010; direct comparison of data results generated in the two laboratories may be inaccurate.

Median urinary DMTP concentrations (ug/L) measured in Sabana de Bogota study participants (1.6 to 3.9) closely resemble the urinary concentrations (1.84 to 4.98) of Latino farm workers in the North Carolina longitudinal study by Arcury et al. 2009; while median urinary DETP concentrations (ug/L) in Antioquia study participants are moderately higher (0.5 to 1.3 in Antioquia *vs.* 0.13 to 0.52 in North Carolina). The analytical method for sample analyses and the LODs for the North Carolina study were the same as our study; thus, a direct comparison with these farm workers might be more suitable and suggests that Colombian floriculturist do not have unusually high exposures to OP pesticides.

Figure 1 compares selected percentiles of morning-void urinary unadjusted ∑DMAP, ∑DEAP and ∑DAP concentrations for the Sabana de Bogota and Antioquia workers with 20-59 year olds from NHANES 1999-2004 (CDC 2007a, CDC 2007b, CDC

2011). The 90th and 95th percentile ∑DMAP and ∑DAP concentrations of our study participants were generally lower than the corresponding NHANES percentiles, while the 75th percentile values were similar. Likewise, the upper ∑DEAP concentrations were lower or similar between the Sabana de Bogota workers and NHANES adults. In contrast, percentile ∑DEAP concentrations were generally higher in the Antioquia vs. the Sabana de Bogota or NHANES samples. It is possible that the higher urinary ∑DEAP concentrations in workers from Antioquia are attributed to seasonal pesticide use fluctuations. Generally, cut-flowers spend less than two days in the supply chain from the time they leave a farm in Colombia to the time the reach a port of entry in the U.S. (IBRD 2009); U.S. cut-flower imports from Colombia peaked at two different times during the year expanding our study's sample collection window (Figure S2); sampling in Antioquia took place during peak period, potentially during higher pesticide usage. This assumption is consistent with longitudinal studies using DAP metabolites for assessing OP pesticide exposure in occupationally exposed populations; in both Mexico (Lacasaña et al. 2010) and North Carolina (Arcury et al. 2009) significant seasonal differences were recorded.

DAP metabolite concentrations fluctuated significantly by collection period after accounting for intra-participant, intra-farm correlations, and creatinine as a measure of urinary dilution (Barr et al. 2006). These differences can be attributed, but are not limited, to the timing of OP exposure, metabolism and elimination. Urinary DAP metabolite concentrations relating to occupational exposure to OP pesticides may be confounded by dietary intake, home pesticide use and other paraocupational exposures. As suggested by a series of studies by Lu et al. (Lu et al. 2006, Lu et al. 2008) dietary intake of OP

pesticides, specifically through consumption of non-organic fresh fruits and vegetables, inflates OP exposures. Furthermore, recent findings suggest that DAP metabolites are found in fresh produce known to be treated with OPs (Zhang et al. 2008) and urinary DAP concentrations might not solely reflect exposure to OP pesticides but also to their metabolites (Bradman et al. 2005) which alone do not inhibit cholinesterase activity (Engel et al. 2011). Although pesticide use at home did not significantly influence DAP metabolites levels; the possibility of paraoccupational exposure cannot be ruled out, especially for participants who lived in rural areas were the use of agrochemicals (including OPs) for various agricultural crops is very likely.

Working in the Sabana de Bogota was associated with higher levels of ∑DMAP, while working in Antioquia was associated with higher levels ∑DEAP. Data collected from cut-flower farms in 1,999 depict a clear difference in type of pesticide used in each of the two regions sampled in our study (Varona et al. 2005), thus is possible that different classes of OP pesticides were being used during the sampling window for each region. Thought a number of the self-reported pesticide exposure and safety behavior characteristics differed by region (Table 2), the only one that influenced urinary DAP metabolite levels was uniform use. Wearing a uniform had a clear inverse association with DAP concentrations in urine corroborating observations reported in the literature (Quandt et al. 2006, Salvatore et al. 2008). Although we did not systematically record items that comprised uniforms; we observed that the majority of participants wore long-sleeved shirt, pants and closed-toe shoes, three of four items of clothing recommended by U.S. EPA (U.S. EPA 2005) examined by Salvatore et al. 2008 associated with decreased levels of ∑DMAP in urine, Salvatore 2008). Inclusion of uniform use in the regression

model changed the estimate of effect of region on urinary ∑DEAP and ∑DAP levels, indicating that the contribution of the variable region in the regression model is less stable in the presence of uniform use.

The effect of worker task on urinary DAP metabolite levels was not significant. This finding suggests that OPs exposure among the study participants may be in part due to background, dietary or paraoccupational exposures not accounted for in our study methodology. Dermal exposures to OP pesticides in the agricultural setting may potentially explain the comparable measures amongst different tasks (Aprea et al. 1999, Kromhout and Heederik 2005); however, without environmental data we have no certainty that OP pesticides are ubiquitous and that participants working within the vicinity of the farm in direct contact or close proximity to foliage, plant residues and/or flowers have comparable exposures.

This study had limitations. Measurement of urinary DAP metabolites as biomarkers of exposure alone do not provide a definite assessment of OP pesticide exposure. Our study lacked to provide data on potential sources of environmental exposure to OP pesticides (i.e. house dust, dietary replicas) therefore we cannot determine with certainty potential confounders of occupational exposure to OPs. Due to our research capacity, sampling in the two different regions took place at different production cycles (low *vs.* peak season); this may have introduced regional variability unaccounted for in our statistical models. The participating farms (n=13) we conveniently chosen for participation and may not represent the floriculture industry in Colombia, all of the recruited farms were part of a Colombian association for flower exporters that only encompass 80% of exporting cut-flower farms; they also participate in socio-

environmental programs for floriculturists. Pesticide exposure and safety behavior characteristics were self-reported possibly introducing reporting bias in our findings.

CONCLUSIONS

In summary, this is the first study to document widespread exposures to OP pesticides in the floriculture industry in Colombia. We found differences in urinary concentrations of participants based upon region and time of urine collection. The differences based upon urinary collection time probably directly relate to the timing of exposure, metabolism and elimination and/or dietary or paraocupational exposures. However, the differences based upon region present specific areas where educational campaigns or promotion of use of additional personal protective equipment can be implemented to reduce overall exposures in the floriculture industry.

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Table 1. Demographic characteristics of participants and differences by region

	Total	Sabana de Bogota	Antioquia	*p*-Value for test of difference by region
Age (years)				
Mean (SD)	36.7 (9.3)	37.3 (8.9)	33.8 (10.5)	0.0070[a]
Range	19-58	19-58	19-58	
	N (%)	N (%)	N (%)	
Number of participants (n)		298 (83.2)	60 (16.8)	
Age groups				
18-20	6 (1.7)	5 (1.7)	1 (1.7)	
21-30	98 (27.7)	72 (24.2)	26 (43.3)	
31-40	132 (36.9)	115 (38.6)	17 (28.3)	
41-50	91 (25.1)	80 (26.8)	11 (18.3)	
51-60	31 (8.7)	26 (8.7)	5 (8.3)	0.0484[b]
Gender				
Female	261 (72.9)	235 (78.9)	26 (43.3)	
Male	97 (27.1)	63 (21.1)	34 (56.7)	<0.0001[b]
Residency				
Urban	317 (88.6)	280 (94.0)	37 (61.7)	
Rural	41 (11.5)	18 (6.0)	23 (38.3)	<0.0001[b]
Education: highest level completed				
Primary	158 (44.1)	133 (44.6)	25 (41.7)	
Secondary	171 (47.8)	144 (48.3)	27 (45.0)	
Technical/vocational	19 (5.3)	14 (4.7)	5 (8.3)	
College/university	9 (2.5)	6 (2.0)	3 (5.0)	
Graduate school	1 (0.3)	1 (0.3)	0 (0)	0.4039[c]
Literacy: reads a letter or newspaper				
Easily	318 (88.8)	264 (88.6)	54 (90)	
With difficulty	38 (10.6)	33 (11.1)	5 (8.3)	
Finds it impossible	2 (0.6)	1 (0.3)	1 (1.7)	0.3254[c]
Smoking status				
Current smoker	40 (11.2)	24 (8.1)	16 (26.7)	0.0026[b]

[a] Two sample t-test
[b] Chi-Square test
[c] Fisher's Exact test

Table 2. Pesticide exposure and safety behavior characteristics of participants and differences by geographic region

	Total	Sabana de Bogota	Antioquia	*p*-Value for test of difference by region
Years working in floriculture				
Mean (SD)	11.5 (7.7)	12.3 (7.8)	7.8 (6.1)	<0.0001[a]
Range	0.5-36	0.67-36	0.5-25	
Years working for current employer				
Mean (SD)	7.1 (6.4)	7.7 (6.5)	4.3 (5.0)	<0.0001[a]
Range	0.5-31	0.5-31	0.5-24	
	N (%)	N (%)	N (%)	
Residential exposure				
Uses pesticides at home	60 (16.8)	43 (14.4)	17 (28.3)	0.0085[b]
Home pesticides users:	(n=60)	(n=43)	(n=17)	
Stores inside house	25 (41.7)	20 (46.5)	5 (29.4)	0.0771[c]
Used in the last five days	14 (23.3)	11 (25.6)	3 (17.6)	0.7369[c]
No. of daily servings fresh fruits and vegetables				
0	19 (5.3)	6 (2.0)	13 (21.7)	
1-2	305 (85.2)	262 (87.9)	43 (71.7)	
3-4	28 (7.8)	24 (8.1)	4 (6.7)	
>4	6 (1.7)	6 (2.0)	0 (0.0)	<0.0001[c]
Task/job on farm				
Packing/freezer rooms	68 (19.0)	56 (18.8)	12 (20.0)	
Greenhouse	213 (59.5)	186 (62.4)	26 (43.3)	
Sprayer/pesticide preparation	28 (7.8)	21 (7.0)	7 (11.7)	
Administrative/office	20 (5.6)	16 (5.4)	4 (6.7)	
Other	29 (8.1)	18 (6.0)	11 (18.3)	0.0085[c]
Personal protective equipment				
Uses any type of PPE	343 (95.8)	289 (97.0)	54 (90.0)	0.0253[c]
Protective equipment usage by type				
Uniform	306 (85.5)	267 (89.6)	39 (65.0)	<0.0001[b]
Gloves	339 (94.7)	285 (95.6)	54 (90.0)	0.1413[b]
Mask	87 (24.3)	73 (24.5)	14 (23.3)	0.9069[b]
Waterproof jacket	44 (12.3)	34 (11.4)	10 (16.7)	0.1699[b]
Waterproof pants	38 (10.6)	28 (9.4)	10 (16.7)	0.0564[b]
Type of shoes				
High rubber boots	94 (26.3)	71 (23.8)	23 (38.3)	0.0061[b]
Short rubber boots	201 (56.1)	197 (66.1)	4 (6.7)	<0.0001[c]
Personal hygiene				
Changes work clothes after shift	328 (91.6)	284 (95.3)	44 (73.3)	<0.0001[b]
Washes work clothes separately	282 (78.8)	260 (87.2)	22 (36.7)	<0.0001[b]

[a] Mann Whitney test
[b] Chi-Square test
[c] Fisher's Exact test

Table 3. Individual (ug/L) and composite (nmol/L) urinary DAP concentrations by region and sample collection period

Analyte	Detected (%)	GM	GSD	Range	Percentile 50th	75th	90th	95th
Morning-void samples								
Sabana de Bogota (n=291)								
DMP	26.1	NC	NC	<LOD–20.2	<LOD	2.4	5.7	9.0
DMTP	87.6	3.1	3.8	<LOD–77.3	3.9	7.1	11.5	17.0
DMDTP	2.1	NC	NC	<LOD–11.7	<LOD	<LOD	<LOD	<LOD
DEP	29.9	NC	NC	<LOD–41.7	<LOD	4.3	9.0	14.0
DETP (*n* =279)[a]	39.4	NC	NC	<LOD–30.1	<LOD	1.3	3.0	4.0
DEDTP	1.7	NC	NC	<LOD–6.6	<LOD	<LOD	<LOD	<LOD
∑DMAP	89.0	36.6	2.7	<LOD–702.4	37.3	69.4	116.8	175.9
∑DEAP	50.5	7.2	5.0	<LOD–325.5	5.0	35.1	75.7	99.5
∑DAP	90.4	53.5	2.7	<LOD–741.2	58.1	111.0	178.8	249.3
Antioquia (n=60)								
DMP (*n* =50)[a]	18.0	NC	NC	<LOD–13.2	<LOD	<LOD	6.2	8.6
DMTP	25.0	NC	NC	<LOD–34.5	<LOD	2.8	8.6	14.4
DMDTP	8.3	NC	NC	<LOD–5.0	<LOD	<LOD	<LOD	2.6
DEP	45.0	NC	NC	<LOD–38.4	<LOD	7.4	14.7	17.2
DETP	53.3	0.5	6.4	<LOD–10.0	1.3	2.3	4.6	6.7
DEDTP	1.7	NC	NC	<LOD–1.6	<LOD	<LOD	<LOD	<LOD
∑DMAP	31.7	NC	4.5	<LOD–379.9	<LOD	45.2	104.2	145.0
∑DEAP	68.3	14.6	5.2	<LOD–277.2	17.6	67.5	108.7	136.7
∑DAP	71.7	34.1	4.0	<LOD–410.6	47.8	109.0	191.5	252.9
Pre-shift samples								
Sabana de Bogota (n=291)								
DMP	18.1	NC	NC	<LOD–11.7	<LOD	<LOD	3.7	6.1
DMTP	57.4	0.8	4.9	<LOD–27.4	1.6	3.2	5.0	6.9
DMDTP	0.7	NC	NC	<LOD–7.5	<LOD	<LOD	<LOD	<LOD
DEP	23.2	NC	NC	<LOD–30.5	<LOD	<LOD	5.4	7.0
DETP (*n* =286)[a]	16.1	NC	NC	<LOD–13.9	<LOD	<LOD	0.9	1.7
DEDTP	1.3	NC	NC	<LOD–6.3	<LOD	<LOD	<LOD	<LOD
∑DMAP	60.1	15.1	2.9	<LOD–218.8	17.4	33.8	61.6	81.0
∑DEAP	33.6	NC	NC	<LOD–266.1	<LOD	14.5	37.2	55.5
∑DAP	68.5	23.0	2.8	<LOD–353.5	26.0	50.9	89.3	122.1
Antioquia (n=60)								
DMP (*n* =50)[a]	14.0	NC	NC	<LOD–12.1	<LOD	<LOD	4.5	5.5
DMTP	18.3	NC	NC	<LOD–15.7	<LOD	<LOD	6.1	12.5
DMDTP	1.7	NC	NC	<LOD–1.9	<LOD	<LOD	<LOD	<LOD
DEP	35.0	NC	NC	<LOD–23.7	<LOD	3.9	11.0	19.2
DETP	38.3	NC	NC	<LOD–8.0	<LOD	1.7	3.2	4.8
DEDTP	5.0	NC	NC	<LOD–1.7	<LOD	<LOD	<LOD	0.4
∑DMAP	23.3	NC	NC	<LOD–149.1	<LOD	4.8	70.2	120.0
∑DEAP	53.3	8.8	5.3	<LOD–169.0	10.9	35.6	82.0	126.3
∑DAP	58.3	21.9	3.7	<LOD–235.1	23.2	57.4	153.3	195.3
Post-shift samples								
Sabana de Bogota (n=291)								
DMP	28.6	NC	NC	<LOD–18.4	<LOD	2.4	6.1	7.5
DMTP	78.8	1.9	4.4	<LOD–52.4	2.6	5.2	8.7	12.7
DMDTP	3.4	NC	NC	<LOD–7.4	<LOD	<LOD	<LOD	<LOD
DEP (*n* =295)[a]	33.1	NC	NC	<LOD–51.0	<LOD	3.3	9.5	13.0
DETP (*n* =284)[a]	37.5	NC	NC	<LOD–30.0	<LOD	1.2	2.3	3.5
DEDTP	1.3	NC	NC	<LOD–3.5	<LOD	<LOD	<LOD	<LOD
∑DMAP	80.5	26.0	2.9	<LOD–472.4	26.9	59.9	99.7	140.5
∑DEAP	53.5	7.5	4.8	<LOD–360.6	7.1	27.9	67.5	99.2
∑DAP	85.9	41.2	2.9	<LOD–633.9	44.4	94.9	144.7	208.8
Antioquia (n=60)								
DMP (*n* =49)[a]	20.4	NC	NC	<LOD–27.6	<LOD	<LOD	8.2	13.3
DMTP	25.0	NC	NC	<LOD–59.3	<LOD	3.1	10.9	17.0
DMDTP	6.7	NC	NC	<LOD–16.9	<LOD	<LOD	<LOD	1.8
DEP	45.0	NC	NC	<LOD–39.6	<LOD	6.3	12.5	25.0
DETP	50.0	0.4	6.5	<LOD–22.5	0.5	2.0	5.8	8.2
DEDTP	3.3	NC	NC	<LOD–1.6	<LOD	<LOD	<LOD	<LOD
∑DMAP	31.7	NC	NC	<LOD–657.2	<LOD	49.6	135.0	206.0
∑DEAP	63.3	12.8	5.6	<LOD–319.1	16.3	47.8	103.7	178.2
∑DAP	68.3	32.3	4.5	<LOD–966.4	34.1	118.9	232.0	265.6

Abbreviations: GM, geometric mean; GSD, geometric standard deviation; NC, not calculated (detection frequency below 50%); <LOD, values below the limit of detection. Values below the limit of detection (LOD) = LOD/√2. LOD: DMP = 0.6 µg/L; DMTP = 0.2 µg/L; DMDTP = 0.1 µg/L; DEP = 0.2 µg/L; DETP = 0.1 µg/L; DEDTP = 0.1 µg/L. †Some measurements were excluded because the laboratory QC values were out of the established range for that analytical batch.

Table 4. Results from linear mixed-effects model of time, task, region, and urinary creatinine on log urinary DAPs concentrations

	Effect†	Contrast	Paramenter estimates (β)	SE	*p*- Value
∑DMAP	Intercept		1.6	3.3×10^{-1}	0.0004
	Creatinine		9.6×10^{-3}	4.8×10^{-4}	<0.0001
	Region		9.8×10^{-1}	3.4×10^{-1}	0.0147
	Time				<0.0001
		Pre-shift vs. morning-void	-3.9×10^{-1}	5.4×10^{-2}	<0.0001‡
		Post-shift vs. morning-void	-1.9×10^{-1}	5.0×10^{-2}	0.0004‡
		Post- vs. pre-shift	2.0×10^{-1}	5.2×10^{-2}	0.0004‡
	Task				0.3760
∑DEAP	Intercept		1.8	3.7×10^{-1}	0.0006
	Creatinine		8.6×10^{-3}	7.1×10^{-4}	<0.0001
	Region		-6.3×10^{-1}	3.3×10^{-1}	0.0839
	Time				0.0014
		Pre-shift vs. morning-void	-1.9×10^{-1}	8.0×10^{-2}	0.0511‡
		Post-shift vs. morning-void	9.3×10^{-2}	7.4×10^{-2}	0.4238‡
		Post- vs. pre-shift	2.8×10^{-1}	7.7×10^{-2}	0.0009‡
	Task				0.8581
∑DAP	Intercept		2.7	2.8×10^{-1}	<0.0001
	Creatinine		1.0×10^{-2}	4.7×10^{-4}	<0.0001
	Region		2.6×10^{-1}	2.7×10^{-1}	0.3710
	Time				<0.0001
		Pre-shift vs. morning-void	-3.6×10^{-1}	5.2×10^{-2}	<0.0001‡
		Post-shift vs. morning-void	-1.4×10^{-1}	4.9×10^{-2}	0.0154‡
		Post- vs. pre-shift	2.2×10^{-1}	5.1×10^{-2}	<0.0001‡
	Task				0.6879

Abbreviations: SE, standard error

†Region, farm nested within region, and subject nested within farm were included as random effects. Time was included as a continuous variable (unadjusted metabolite concentration) broken down into collection periods: morning-void, pre- and post-shift. Region was included as a categorical variable with two groups: Sabana de Bogota and Antioquia. Creatinine was included in as a continuous variable.

‡Tukey-corrected level of significance

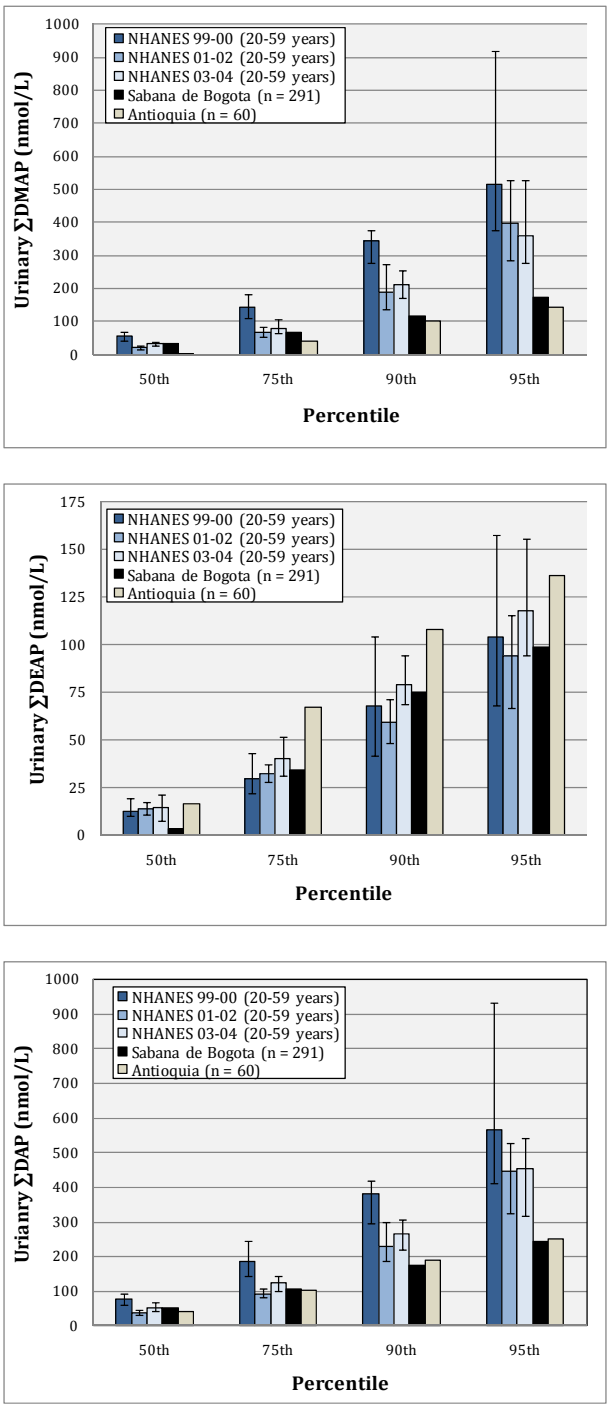


Figure 1. Selected percentiles of morning-void urinary (A) dimethyl alkylphosphate (∑DMAP), (B) composite diethyl alkylphosphate (∑DEAP) and (C) summed DAP (∑DAP) concentrations (nmol/L) for Sabana de Bogota and Antioquia participants *versus* 20-59 year olds in NHANES 1999-2004(CDC 2007a, CDC 2007b, CDC 2011). Error bars represent NHANES 95% weighted confidence intervals. Values below the limit of detection (LOD) = LOD/√2 for this study and NHANES. Our samples were analyzed in the CDC laboratory that analyzes NHANES samples thus our LODs are similar to those reported by NHANES.

Table S1. Additional pesticide exposure and safety behavior characteristics of participants by region

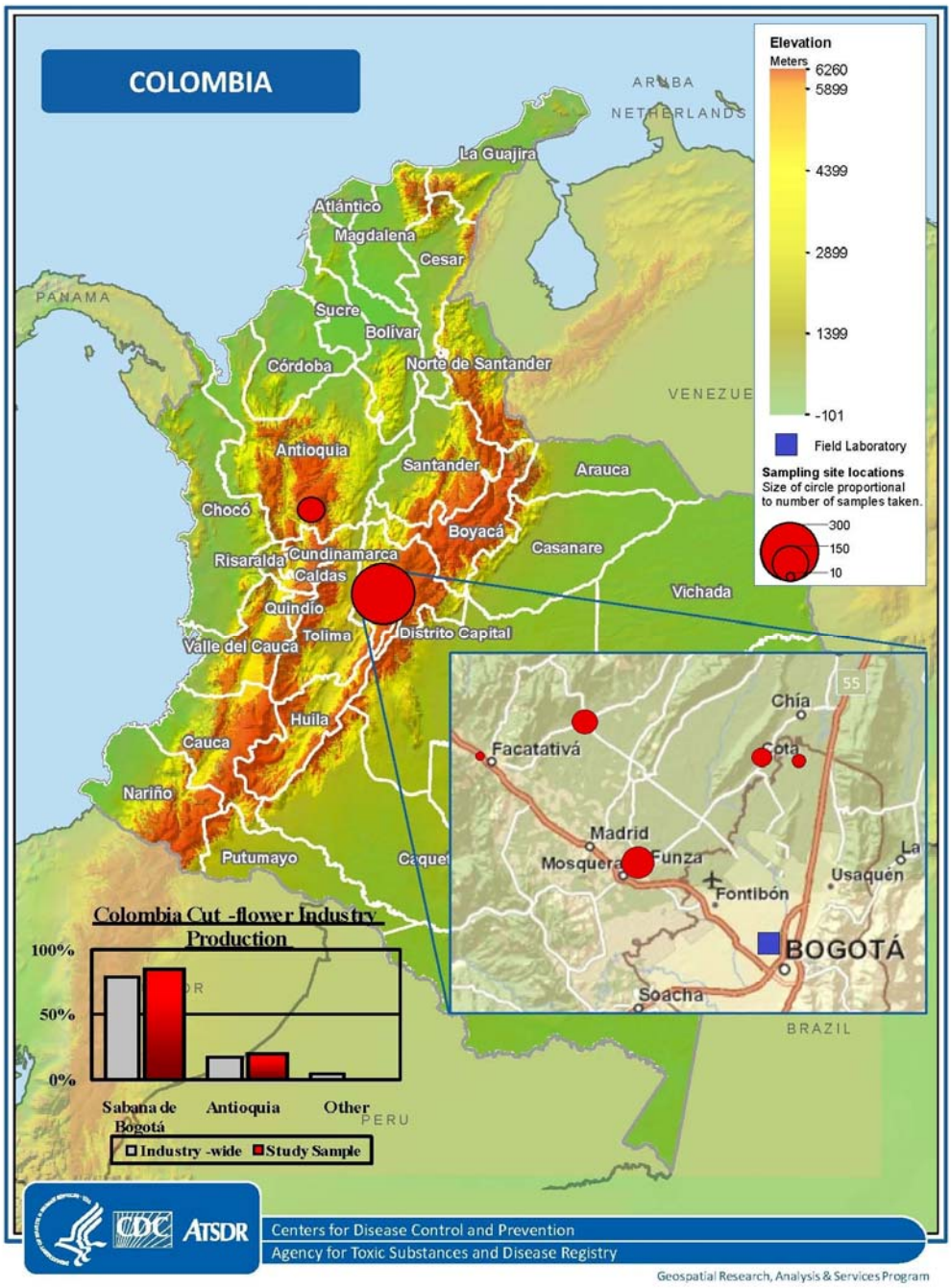
	Total	Sabana de Bogota	Antioquia	p-Value for test of difference by region
Pesticides at work				
Directly handles pesticides	40 (11.2)	32 (10.7)	8 (13.3)	0.5605[b]
Pesticide Handlers:	(n=40)	(n=32)	(n=8)	
Received pesticide training	35 (87.5)	29 (90.6)	6 (75.0)	0.2568[c]
Used in the last five days	29 (72.5)	21 (65.6)	8 (100.0)	0.0803[c]
Pesticide Sprayers	23 (57.5)	17 (53.1)	6 (75.0)	0.2449[c]
Personal hygiene				
Place where work clothes are washed				0.7389[c]
Home	309 (86.3)	268 (89.9)	41 (68.3)	
Work place	20 (5.6)	17 (5.7)	3 (5.0)	
Work clothes storage				<0.0001[c]
Work place	6 (1.7)	2 (0.7)	4 (6.7)	
At home inside	281 (78.5)	260 (87.2)	21 (35.0)	
At home outside	42 (11.7)	23 (7.7)	19 (31.7)	
Area where food eaten at work				0.0145[c]
Inside greenhouse	1 (0.3)	0 (0.0)	1 (1.7)	
Assigned resting area	9 (2.5)	5 (1.7)	4 (6.7)	
In kiosk/cafeteria	348 (97.2)	293 (98.3)	55 (91.7)	
Washes hands before eating at workplace				0.1057[c]
Never	13 (3.6)	11 (3.7)	2 (3.3)	
Sometimes	104 (29.1)	93 (31.2)	11 (18.3)	
Always	240 (67.0)	194 (65.1)	47 (78.3)	

[a] Mann Whitney test
[b] Chi-Square test
[c] Fisher's Exact test

Table S2. Dilution-adjusted individual (ug/g creatinine) and composite (nmol/g creatinine) DAP metabolite concentrations in Sabana de Bogota and Antioquia study participants' morning void, pre- and post-shift urine samples

Analyte	Detected (%)	GM	GSD	Range	Percentile 50th	75th	90th	95th
Morning-void samples								
Sabana de Bogota (n=291)								
DMP	26.1	NC	NC	<LOD–15.3	<LOD	2.4	5.9	8.3
DMTP	87.6	3.0	3.3	<LOD–60.4	3.7	5.8	9.5	14.9
DMDTP	2.1	NC	NC	<LOD–7.7	<LOD	<LOD	<LOD	<LOD
DEP	29.9	NC	NC	<LOD–39.2	<LOD	3.7	8.7	14.4
DETP (n=279)[a]	39.4	NC	NC	<LOD–42.3	<LOD	1.0	2.3	3.2
DEDTP	1.7	NC	NC	<LOD–3.6	<LOD	<LOD	<LOD	<LOD
∑DMAP	89.0	35.5	2.4	<LOD–425.6	36.9	62.3	101.8	148.6
∑DEAP	50.5	7.0	4.9	<LOD–262.2	5.1	32.2	67.8	99.5
∑DAP	90.4	51.9	2.5	<LOD–591.4	54.2	103.7	153.6	197.8
Antioquia (n=60)								
DMP (n=50)[a]	18.0	NC	NC	<LOD–14.3	<LOD	<LOD	5.7	9.0
DMTP	25.0	NC	NC	<LOD–23.4	<LOD	1.4	7.8	14.6
DMDTP	8.3	NC	NC	<LOD–10.6	<LOD	<LOD	<LOD	2.7
DEP	45.0	NC	NC	<LOD–36.4	<LOD	6.8	11.2	17.8
DETP	53.3	0.5	5.6	<LOD–10.6	0.7	2.1	4.4	6.5
DEDTP	1.7	NC	NC	<LOD–1.5	<LOD	<LOD	<LOD	<LOD
∑DMAP	31.7	NC	NC	<LOD–257.3	<LOD	26.4	99.5	157.1
∑DEAP	68.3	15.5	4.5	<LOD–261.8	17.9	53.0	94.8	149.0
∑DAP	71.7	36.5	3.5	<LOD–503.8	47.7	91.4	171.0	200.0
Pre-shift samples								
Sabana de Bogota (n=291)								
DMP	17.1	NC	NC	<LOD–16.8	<LOD	<LOD	4.8	10.3
DMTP	57.4	1.0	3.8	<LOD–44.8	1.7	3.9	8.2	12.9
DMDTP	0.7	NC	NC	<LOD–18.8	<LOD	<LOD	<LOD	<LOD
DEP	23.2	NC	NC	<LOD–25.3	<LOD	<LOD	8.4	13.2
DETP (n=286)[a]	16.1	NC	NC	<LOD–40.9	<LOD	<LOD	1.2	2.2
DEDTP	1.3	NC	NC	<LOD–43.7	<LOD	<LOD	<LOD	<LOD
∑DMAP	60.1	27.0	2.5	<LOD–315.7	25.2	47.4	100.6	138.9
∑DEAP	33.6	NC	NC	<LOD–405.3	<LOD	20.6	62.9	98.2
∑DAP	68.5	41.1	2.5	<LOD–667.0	38.0	84.8	136.6	179.4
Antioquia (n=60)								
DMP (n=50)[a]	14.0	NC	NC	<LOD–11.5	<LOD	<LOD	3.3	5.3
DMTP	18.3	NC	NC	<LOD–21.2	<LOD	<LOD	6.5	8.8
DMDTP	1.7	NC	NC	<LOD–1.1	<LOD	<LOD	<LOD	<LOD
DEP	35.0	NC	NC	<LOD–23.2	<LOD	6.1	11.7	17.1
DETP	38.3	NC	NC	<LOD–7.4	<LOD	2.1	3.2	5.5
DEDTP	5.0	NC	NC	<LOD–1.4	<LOD	<LOD	<LOD	0.5
∑DMAP	23.3	NC	NC	<LOD–149.5	7.4	25.7	78.5	94.8
∑DEAP	53.3	11.2	5.0	<LOD–194.2	8.9	47.0	93.3	119.8
∑DAP	58.3	27.7	3.7	<LOD–282.8	28.1	99.6	135.7	155.0
Post-shift samples								
Sabana de Bogota (n=291)								
DMP	28.7	NC	NC	<LOD–14.8	<LOD	2.8	5.9	8.3
DMTP	78.7	2.1	4.5	<LOD–58.8	2.6	4.8	8.6	13.3
DMDTP	3.4	NC	NC	<LOD–10.5	<LOD	<LOD	<LOD	<LOD
DEP (n=295)[a]	33.2	NC	NC	<LOD–25.6	<LOD	4.4	10.2	15.5
DETP (n=284)[a]	37.7	NC	NC	<LOD–19.8	<LOD	1.1	2.0	2.5
DEDTP	1.4	NC	NC	<LOD–18.0	<LOD	<LOD	<LOD	<LOD
∑DMAP	80.4	29.6	2.6	<LOD–586.3	27.8	61.2	100.8	132.7
∑DEAP	53.7	8.6	4.5	<LOD–175.2	7.2	34.5	69.8	115.6
∑DAP	85.8	47.0	2.6	<LOD–586.3	51.0	92.9	156.2	220.6
Antioquia (n=60)								
DMP (n=49)[a]	20.4	NC	NC	<LOD–32.7	<LOD	<LOD	7.7	10.5
DMTP	25.0	NC	NC	<LOD–37.3	<LOD	1.6	7.6	12.5
DMDTP	6.7	NC	NC	<LOD–10.6	<LOD	<LOD	<LOD	1.2
DEP	45.0	NC	NC	<LOD–25.0	<LOD	4.8	12.5	17.3
DETP	50.0	0.3	6.5	<LOD–14.9	0.3	1.6	4.4	6.3
DEDTP	3.3	NC	NC	<LOD–1.3	<LOD	<LOD	<LOD	<LOD
∑DMAP	31.7	NC	NC	<LOD–413.7	4.5	35.7	85.1	181.7
∑DEAP	63.3	10.3	5.5	<LOD–212.1	11.1	39.9	97.9	141.2
∑DAP	68.3	26.1	4.4	<LOD–608.5	26.1	91.2	176.9	211.2

Abbreviations: GM, geometric mean; GSD, geometric standard deviation; NC, not calculated (detection frequency below 50%); <LOD, values below the limit of detection. Values below the limit of detection (LOD) for the urine levels not corrected for creatinine = LOD/√2. LOD: DMP = 0.6 µg/L; DMTP = 0.2 µg/L; DMDTP = 0.1 µg/L; DEP = 0.2 µg/L; DETP = 0.1 µg/L; DEDTP = 0.1 µg/L. †Some measurements were excluded because the laboratory QC values were out of the established range for that analytical batch.



Map author: S. Graham, GIS Specialist, Public Health Geospatial Research, Analysis, and Services Program (GRASP)

Figure S1. Topographic map of Colombia (CDC/ATSDR/GRASP, 2011) depicting Study sampling sites (red dots) and field laboratory (blue dot). *Bottom* Comparison between production percentages by region of the cut-flower industry in Colombia (grey bars, source ASOCOLFLORES 2009) and Study sample percentage by region in (red bars, source Caycedo et al. 2008)

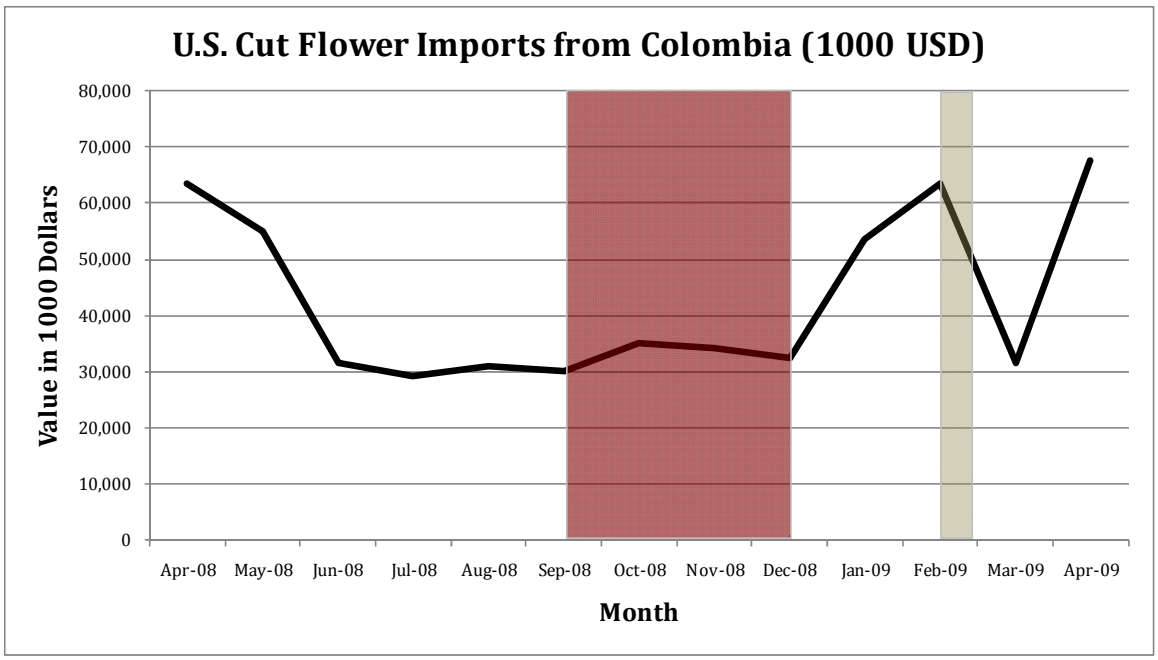


Figure S2. U.S. Cut flower imports from Colombia from April 2008 to April 2009 (Data source: U.S. Department of Agriculture, National Agricultural Statistic Service: Data and Statistics). Shaded red area is the sampling time period corresponding to Sabana de Bogota; shaded brown corresponds to sampling period in Antioquia.