Occupational predictors of urinary dialkyl phosphate concentrations in Mexican flower growers

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ABSTRACT

Background: Flower growers have high potential for exposures to pesticides. Occupational factors, such as tasks performed, the production method (organic or conventional), the use of personal protective equipment (PPE), and workplace characteristics influence the intensity of pesticide exposure.

Objective: To evaluate occupational characteristics affecting urinary concentration of dialkylphosphate (DAP) metabolites of organophosphate pesticides among a group of Mexican floricultural workers.

Methods: A questionnaire was administered to 117 workers who also provided a first morning urine sample. According to tasks performed and the production methods, pesticide contact was defined as low, medium, or high. PPE use was categorized as acceptable, fairly acceptable, and unacceptable. Urinary concentration of DAP metabolites were determined using gas-liquid chromatography. Association between occupational characteristics and DAP urinary concentrations was assessed by means of linear regression models.

Results: After adjusting for potential confounders, the workers in the medium and high contact categories had significantly higher DAP concentrations than those in the low contact category (β : 0.3, CI 95%: 0.1–0.5). Greenhouse workers had greater DAP concentrations than outdoors workers (β : 0.3, CI 95%: 0.1–0.5). Compared with non-acceptable use of PPE, acceptable use of PPE was associated with lower DAP concentrations (β : –0.4, CI 95% –0.6 to –0.1).

Conclusion: Improved safety training is needed for correct PPE usage, especially among flower growers who use conventional pest control methods and who work in a greenhouse environment.

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Introduction

Exposure to pesticides is an occupational risk that agricultural workers face, as they use pesticides with more frequency and intensity compared to most other occupational groups [1]. Workers may be occupationally exposed either by direct contact during the mixing, loading or application of pesticides, or indirectly by contact with residues that remain in the crops, or when entering the fields without observing the safety period after the pesticide application [1,2].

Among agricultural workers, flower growers are a highly exposed group, since the flowers are easily attacked by various pests and require the application of large amounts of pesticides, fertilizers, and chemical preservatives (chemicals used in order to improve

post-harvest life of cut flowers) to make their cultivation profitable. Furthermore, since they are not used as food, restrictions regarding the levels of pesticide residues are less restrictive compared to other crops [3].

In Mexico, flowers are cultivated in areas of 20,000 hectares. The Mexican states with the largest production, involving approximately 10,000 producers, are Mexico, Puebla, and Morelos [4]. A wide variety of pesticides are used in floriculture, with one of the most utilized being the organophosphate pesticides (OP) group. Previous findings from our research group showed that Mexican flower growers regularly applied omethoate, oxydemeton, metamidophos, chlorpyrifos, and malathion, and 90% of workers showed detectable urine levels in at least one dialkyl phosphate metabolite (DAP).

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However, DAP concentrations ranged between 34.3 and 2270.8 µg/g creatinine [5], suggesting that occupational or environmental factors may influence the observed variation in metabolite levels. Among the occupational factors, the tasks performed, which may involve more or less contact with pesticides, the use of personal protective equipment (PPE) and the characteristics of the work site (outdoors or greenhouses), influence the intensity of pesticide exposures [1] both generally and to OPs in particular.

Appropriate use of PPE can protect workers from pesticide exposure [6]. Previous studies have assessed the role played by the use of PPE in exposure to these substances [7–13], but most have been carried out on workers with direct pesticide contact. Little is known about the pesticide exposure associated with performing specific agricultural tasks [2].

In Mexico, we are unaware of any studies having assessed the extent to which variables related to work activity are associated with pesticide biomarker concentrations in agricultural workers. The main objective of this study was to evaluate the occupational characteristics associated with the urine concentrations of DAP metabolites in Mexican flower growers from the states of Mexico and Morelos, where flower growing is one of the principal economic activities.

Materials and methods

Study population

This analysis is framed within a cross-sectional study carried out among flower growers of the states of Mexico and Morelos during the period of July–October 2004. The main objective was to evaluate the association between urine DAP concentrations and male hormone profile. The researched variables included the occupational characteristics of these workers. The details of the study design were previously published [14]. In short, information was collected from 136 healthy workers aged 18 to 52 years, all employed for at least 6 months as flower growers. Workers with a background of infertility were excluded. The workers were selected using the records of 57 flower-growing companies in Morelos and the State of Mexico. The workers performed tasks that involved different opportunities for contact with pesticides, from administrative tasks to pesticide mixing and application. Among the study participants, 15 were employees of a company that used organic means of production. Eligible workers were informed about the objectives of the study, as well as of the detailed required procedures, and were invited to participate. Those who agreed signed an informed consent letter. The study was approved by the Ethics Committee of the National Institute of Public Health (INSP) of Mexico.

Information collection

Collection of biological samples and questionnaire administration took place the day after a pesticide application. Trained nurses provided each worker with a plastic container for urine collection as well as a brochure with instructions to the appropriate self-collection of the first morning urine (before 8 AM). Urine samples were placed on frozen ice packs in a cooler after collection. Samples were then transported to laboratory of National Institute of Public Health of Mexico (INSP) where they were frozen and stored in freezers at –20 °C until they were sent on dry ice to the laboratory of toxicology at the Center for Advanced Studies and Research (CINVESTAV) for analysis.

Questionnaires gathered on the social-demographic characteristics (age, schooling, and marital status), alcohol and smoking habits, and personal pathological background. The workers were questioned in detail about the characteristics of their work: number of years worked in the flower growing job, tasks performed, use of PPE, workplace (outdoor or greenhouse), number of hours worked per day, and number of workdays per week. Questionnaires administration and anthropometric measurements (weight and height) were taken by trained nurses.

According to the production method and the occupational tasks of flower growers, workers were placed in one of the following categories:

- (1) High contact: Included flower growers working for companies that used pesticides in the work process and who had direct contact with these products – pesticide applicators, pesticide mixers, pesticide application equipment managers – regardless of whether or not they additionally performed other types of activities.
- (2) Medium contact: included flower growers working for companies that used pesticides in the work process, but who performed none of the tasks included in the “high contact” category.
- (3) Low contact: included those workers with occasional or no contact with pesticides (e.g. administrators) and workers who used organic production methods.

Among agricultural workers the main pesticide absorption pathways are through inhalation and dermal contact [15] therefore, we classified PPE into three categories:

- (1) Acceptable use: worker wore a filter mask plus at least one of the next waterproof garments: overalls, pants, jacket, or gloves.
- (2) Fairly acceptable use: worker wore either a filter mask or at least one of the next waterproof garments: overalls, pants, jacket, or gloves.

- (3) Unacceptable use: worker wore no filter mask or waterproof overalls, pants, jacket, or gloves, regardless of whether or not any other type of protective clothing was worn.

The workplace type was classified into outdoors, greenhouse, and both.

Laboratory procedures

Six common DAP metabolites of organophosphate pesticides were determined: dimethylphosphate (DMP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethylphosphate (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP) by gas chromatography-mass spectrometry following the protocol described by Ueyama et al. [16] For levels of DAP metabolites below the limit of quantification (50 µg/l) or below the limit of detection (22.5 µg/l), imputed values equals to one-half the LOQ (22.5 µg/l) or LOD (11.25 µg/l), respectively, were assigned.

The dimethyl (DMP, DMTP, and DMDTP) and diethyl (DEP, DETP, and DEDTP) metabolite concentrations were converted to their molar concentrations (µmol/l) and summed to produce a single methyl (ΣDMPs) or ethyl (ΣDEPs) phosphate concentration and total DAP (ΣDAP) concentration for each sample as proposed by Lu et al. [17] Metabolite concentrations were adjusted using creatinine concentration to correct variable urine dilutions. Urinary creatinine concentration was determined by spectrophotometry using a commercial kit (Randox Creatinine Kit).

Statistical analysis

The analysis was based on the data of 117 flower growers about whom complete information was available. General characteristics of the study population were described using frequencies and percentages or central tendency (arithmetic mean) and dispersion measures (standard deviation and range), according to the type of variable. DAP metabolites were described using geometric means (GM) and percentiles.

DAP concentrations were compared according to the established contact categories, as well as to the PPE use and workplace categories, by means of the non-parametric Kruskal–Wallis test. To evaluate the association between the characteristics of the flower growing work and the DAP concentrations (ΣDMPs, ΣDEPs, ΣDAP), three linear regression models with robust variance were used. The independent variables included in the models were the occupational characteristics of interest (contact category, workplace, and PPE use), as well as other potentially confounding variables and covariables (age, number of hours worked per day, smoking habits, years of experiences as flower growers). In all three models, the dependent variable was transformed to its natural

logarithm to meet the assumption of normality in residuals. The models were evaluated through a diagnosis of residuals and goodness-of-fit tests.

In a first analysis, DAP concentrations corresponding to non-quantifiable or undetectable values were assigned by the laboratory. Additionally, an alternative analysis was carried out, in which random values with a normal distribution were generated for those values below the quantification and detection limit [18].

Results

General and occupational characteristics of the study population

The workers were primarily from Morelos (80.3%) and married (74.4%) (data not shown in table). Their average age and education was 33 and 8.75 years, respectively; 52.1% of them were overweight or obese. Around 80% reported current alcohol consumption and 79.5% reported currently or formerly smoking. Most workers (85%) performed more than one task during their work day, with the most frequent activities being: pesticide application (50.4%) and mixing pesticides (42.7%). Workers performed an average of two tasks (range: 1–9), and over 50% of the participants worked in a greenhouse (only 20.5% worked exclusively outdoors). As for the use of PPE, 38.5% reported wearing none. Among those who used PPE, the most frequently worn item was the filter or cartridge mask. Likewise, the use of waterproof clothing (overalls, jacket, pants, gloves, and boots) was below 10%. Most flower growers had worked for more than five years as flower growers (Table 1). They worked an average of 7.5 h per day and an average of 6 days per week. More than half (61.5%) of the workers lived close to (less than 500 m) the greenhouses or flower crops and 29.1%, near other cultivation fields (data not shown in Table 1).

Urine concentrations of OP pesticide metabolites

In general, dimethyl metabolites (DMP, DMTP, and DMDTP) were more frequent and appeared in higher concentrations than diethyl metabolites (DEP, DETP, and DEDTP). Specifically, 78% of the flower growers had DMP levels above the quantification limit, with the highest concentration of all the assessed metabolites (GM of 0.6 µmol/g creatinine). For diethyl metabolites, the most frequently detected was DEP, whereas 90% of the samples having DEDTP concentrations below the limit of detection. The GM for the sum of dimethyl metabolites (ΣDMPs) was 1.1 µmol/g creatinine, and for the total DAP metabolites (ΣDAPs), of 1.5 µmol/g creatinine (Table 2).

ΣDMPs concentrations showed significant differences according to contact with pesticide and workplace categories. Flower growers in the medium and high contact categories had significantly higher ($p = 0.005$)

Table 1. General and occupational characteristics of the study population.

Variable	N: 117	Frequency (%) ^a
<i>Age</i>		
Mean $\pm$ SD (range)		33.1 $\pm$ 8.9 (18–52)
<i>Education (years completed)</i>		
Mean $\pm$ SD (range)		8.75 $\pm$ 4.22 (0–19)
<i>BMI</i>		
Normal (17.5–24.9)	56	47.9
Overweight (25–29.9)	46	39.3
Obesity (≥ 30)	15	12.8
<i>Alcohol consumption (g/day)</i>		
No drinker	24	20.5
<30 g/day	78	66.7
≥ 30 g/day	15	12.8
<i>Tobacco consumption</i>		
Never smoked	24	20.5
Past smoker	34	29.1
Current smoker	59	50.4
<i>Task</i>		
Administrative task	9	7.7
Facility maintenance	2	1.7
Earth mixer	6	5.1
Pesticides mixer	50	42.7
Pesticide applicator	59	50.4
Fumigation equipment manager	5	4.3
Planting	12	10.3
Laying and removing plastic mulch	3	2.6
Flower cutting	12	10.3
Flower bud removal	3	2.6
Sapling cutting	5	4.3
Weeding	10	8.6
Watering	23	19.7
Packaging of flowers	4	3.4
<i>Workplace</i>		
Outdoor	24	20.5
Greenhouse	73	62.4
Both	20	17.1
<i>Protection equipment</i>		
No one	45	38.5
Hat or cap	21	18.0
Filter or cartridge mask	34	29.1
Paper mask	14	12.0
Waterproof overalls	10	8.6
Waterproof jacket	1	0.9
Waterproof pants	1	0.9
Waterproof gloves	8	6.8
Waterproof boots	15	12.8
Goggles	5	4.3
<i>Years spent as a floriculture worker</i>		
<5	21	18.0
5–10	42	36.0
>10	54	46.2

^aFor age and education arithmetic mean, standard deviation(SD) and range were calculated.

concentrations than those included in the low contact category. However, there were no significant differences between the medium and high contact groups. Also, those who performed their tasks exclusively in a greenhouse had significantly higher urine concentrations of these metabolites than those who worked outdoors ($p = 0.002$). On the other hand, although the DMPs concentrations were lower in workers with acceptable PPE use, the differences between categories were not significant. A similar result was observed for the sum of all the DAP metabolites. In the case of Σ DEPs, no significant differences were observed between the categories of variables (Table 3).

Association results

In the bivariate analysis, age was positively associated with both Σ DMPs ($\beta = 0.01$, CI 95%: 0.01, 0.02) and Σ DEPs ($\beta = 0.01$, CI 95%: 0.01, 0.02), and with the total DAP ($\beta = 0.01$, CI 95%: 0.01, 0.02). Current smoking was positively associated with Σ DMPs ($\beta = 0.2$, CI 95%: 0.01, 0.4) and total DAP concentrations ($\beta = 0.2$, CI 95%: 0.02, 0.4). Furthermore, flower growers who reported consuming more than 30 g/day of alcohol had significantly higher total DAP concentrations than those that never consumed alcohol ($\beta = 0.2$, CI 95%: 0.01, 0.5). Occupational variables, such as days worked/week, hours worked/day or seniority at work were not associated with DAP concentrations. Likewise, these variables were not associated with the type of activities carried out, the correct use of PPE, or the place of work (data not shown).

After adjusting for age, number of hours worked per day, years working as a flower grower, and smoking, flower growers with high and medium contact with pesticides had on average Σ DMPs concentrations of 0.3 $\mu\text{mol/g}$ creatinine and 0.4 $\mu\text{mol/g}$ creatinine higher, respectively, than flower growers categorized as having low contact with pesticides. For total DAP, those with medium and high contact had on average 0.3 $\mu\text{mol/g}$ creatinine more of these metabolites than flower growers with low contact with pesticides. There were no significant associations for DEPs metabolites

Table 2. Urinary DAPs levels distribution: selected percentiles 25th, 50th, 75th, 90th; and geometric mean (GM).

Dialkylphosphates ($\mu\text{mol/g}$ creatinine)	P25	P50	P75	P90	GM	$\geq \text{LOQ}^a(\%)$	$\geq \text{LOD}^b(\%)$	< LOD (%)
DMP	0.3	0.6	1.2	3.3	0.6	78.0	14.4	7.6
DMTP	0.1	0.2	0.4	1.1	0.2	47.5	22.0	30.5
DMDTP	0.04	0.05	0.1	0.4	0.1	17.8	22.0	60.2
DEP	0.1	0.1	0.2	0.6	0.1	35.6	44.9	19.5
DETP	0.04	0.1	0.10	0.3	0.1	17.0	33.1	50.0
DEDTp	0.03	0.04	0.05	0.1	0.04	2.5	7.6	89.8
Σ DMPs ($\mu\text{mol/g}$ creat.)	0.6	1.1	2.2	4.4	1.1			
Σ DEPs ($\mu\text{mol/g}$ creat.)	0.2	0.2	0.4	0.9	0.3			
Σ DAPs ($\mu\text{mol/g}$ creat.)	0.8	1.4	2.7	5.2	1.5			

^aLOQ = quantification limit (50 $\mu\text{g/l}$).

^bLOD = detection limit (22.5 $\mu\text{g/l}$).

Table 3. Creatinine adjusted urinary DAPs by category of occupational exposure to pesticides.

Variable	N: 117	Mean	Median (range)	<i>p</i> ^a
ΣDMPs (μmol/g creat)				
<i>Categories of contact</i>				
Low contact	16	0.74	0.38 (0.12–1.98)	0.005
Medium contact	37	2.15	1.25 (0.23–12.24)	
High contact	64	1.99	1.13 (0.15–15.75)	
<i>Work place</i>				
Outdoor	24	0.77	0.70 (0.12–1.88)	0.002
Outdoor/Greenhouse	20	2.01	0.89 (0.17–15.75)	
Greenhouse	73	2.20	1.40 (0.13–12.90)	
<i>Protective equipment</i>				
Unacceptable use	80	2.03	1.18 (0.12–15.75)	0.10
Fairly acceptable	28	1.76	1.11 (0.15–5.85)	
Acceptable	9	0.78	0.60 (0.17–2.08)	
ΣDEPs (μmol/g creat)				
<i>Categories of contact</i>				
Low contact	16	0.21	0.23 (0.08–0.29)	0.48
Medium contact	37	0.36	0.24 (0.08–2.90)	
High contact	64	0.59	0.24 (0.06–6.82)	
<i>Work place</i>				
Outdoor	24	0.49	0.21 (0.06–4.74)	0.41
Outdoor/Greenhouse	20	0.39	0.21 (0.08–1.74)	
Greenhouse	73	0.48	0.24 (0.08–6.82)	
<i>Protective equipment</i>				
Unacceptable use	80	0.48	0.24 (0.06–6.82)	0.99
Fairly acceptable	28	0.45	0.21 (0.09–4.74)	
Acceptable	9	0.43	0.22 (0.08–1.50)	
ΣDAPs (μmol/g creat)				
<i>Categories of contact</i>				
Low contact	16	0.95	0.63 (0.24–2.21)	0.004
Medium contact	37	2.51	1.44 (0.41–12.71)	
High contact	64	2.59	1.55 (0.30–17.50)	
<i>Work place</i>				
Outdoor	24	1.25	0.90 (0.24–6.15)	0.004
Outdoor/Greenhouse	20	2.40	1.27(0.30–17.50)	
Greenhouse	73	2.68	1.80 (0.24–13.35)	
<i>Protective equipment</i>				
Unacceptable use	80	2.51	1.47 (0.24–17.50)	0.31
Fairly acceptable	28	2.22	1.34 (0.30–6.15)	
Acceptable	9	1.20	0.90 (0.30–2.81)	

^aBy the Kruskal–Wallis test.

(Table 4). Likewise, flower growers who worked in a greenhouse had on average significantly higher levels of ΣDMPs and total DAP compared to outdoor workers ($p < 0.01$). Flower growers with acceptable PPE use had significantly lower levels of ΣDMPs and total DAP than those with unacceptable PPE use ($p < 0.01$) (Table 4).

When the analysis was repeated using the results of the assignment of random values with a normal distribution for concentrations below the quantification and detection limit, the regression coefficients (β) obtained were 100% higher than those resulting from the analysis with laboratory-assigned values; however, they were significant for the same variables. For this reason, only the results of the analysis with laboratory-assigned values are presented.

Discussion

According to the findings of this study, workers with medium and high contact with pesticides, and those who worked indoors had higher urine DAP concentrations

compared to low contact and outdoor workers. Acceptable use of personal protective equipment (PPE) reduces DAP concentrations. Flower growers had higher dimethyl than diethyl metabolites concentrations, consistent; with the fact that the pesticides most widely used are omethoate, metamidophos, and methyl parathion, all of which form methyl derivatives [2]. Studies from other North American locations also found that methyl metabolites are more frequent than ethyl and in higher concentrations [19–21]. The highest concentrations of measured metabolites found in our own and in some other populations were those of DMP [9,22]. However, other studies show DMTP concentrations to be the highest [20,23,24]. The differences are accounted for by the type of pesticides most frequently used in the studied populations.

With regard to work tasks, most flower growers performed more than one, the most frequent being those that involve direct contact with pesticides. This is in agreement with findings from other researchers [1,2,25]. In this study, this may be explained by the fact that workers are often employed in small, family operated flower growing companies where specialized work is less common than in larger companies.

The correct use of PPE was rare: 39% of the workers wore no PPE, 61% reported using at least one form of PPE (most frequently being the filter or cartridge mask and least frequently waterproof clothing), and only nine workers were included into the “acceptable use” category. Furthermore, 12% mentioned wearing handkerchiefs as masks, potentially counterproductive, as sprayed pesticides may impregnate the handkerchiefs, saturate them with chemicals, and produce an increased exposure, causing more harm than benefit [26]. The low frequency of use of PPE or incorrect use of PPE has been reported by other studies with agricultural workers in different countries [1,9,11,25,27–32]. In the Mexican context, factors influencing the use of PPE could be related to their lack of availability in the workplace, lack of economic accessibility, uncomfortable use, lack of specific safety training and cultural reasons, such as the use of PPE may be considered in some communities as a sign of weakness [1].

As expected, flower growers in the medium and high contact categories had higher DAP metabolite concentrations than those in the low contact category. Paradoxically, concentrations were slightly higher in flower growers who performed medium contact tasks than in those who performed high contact activities, but the difference was not significant. These results are consistent with those reported by Coronado et al. [2], who found that the percentage of agricultural workers with detectable DAP levels was lower among those workers who mixed, loaded, or applied pesticides than among those who did not perform this kind of tasks; other authors have found that exposure levels during re-entry tasks were similar or may exceed those observed during

Table 4. Multivariate models analyzing the association between work characteristics and urine DAPs, β coefficients and 95% confidence intervals.

Variable	Model 1: Σ DMPs ($\mu\text{mol/g creatinine}$)	Model 2: Σ DEPs ($\mu\text{mol/g creatinine}$)	Model 3: Σ DAPs ($\mu\text{mol/g creatinine}$)
<i>Categories of contact</i>			
Low contact			
Medium contact	0.4 (0.1, 0.6)*	0.1 (−0.2, 0.3)	0.3 (0.1, 0.5)**
High contact	0.3 (0.1, 0.6)**	0.2 (−0.1, 0.4)	0.3 (0.1, 0.5)**
<i>Workplace</i>			
Outdoor			
Outdoor/Greenhouse	0.2 (−0.03, 0.4)	0.01 (−0.3, 0.3)	0.1 (−0.1, 0.3)
Greenhouse	0.3 (0.2, 0.5)**	0.1 (−0.2, 0.3)	0.3 (0.1, 0.5)**
<i>PPE Use</i>			
Unacceptable use			
Fairly acceptable	−0.2 (−0.4, 0.02)	−0.10 (−0.3, 0.1)	−0.1 (−0.3, 0.02)
Acceptable	−0.5 (−0.8, −0.2)**	−0.1 (−0.4, 0.2)	−0.4 (−0.6, −0.1)**

All models are adjusted by age, hours worked/day, years spent as a floricultural worker and tobacco consumption.

* $p < 0.05$; ** $p < 0.01$.

treatment operations [33,34]. This may be partly due to the fact that workers who mix, load, and apply pesticides receive training in the proper handling of these substances more often than other agricultural workers, whereby following good hygiene, protection, and safety practices may be encouraged. In this sense, Thompson et al. [35], found that workers who mixed, loaded, or applied pesticides followed hygiene practices more frequently than those who did not perform these activities. This may also be the case among the flower growers in this sample, although no information was collected on work safety and hygiene training or on the frequency of hygiene practices. Alternatively, the exposure duration may be greater in workers who perform tasks that do not involve direct contact with pesticides [36].

The positive association between work in a greenhouse and urine metabolite concentrations is in agreement with other studies. In Ecuador, Colosio et al. [37], found higher concentrations of ethylene thiourea, a pesticide metabolite of the dithiocarbamate family, in greenhouse flower growers compared to outdoor flower growers. The conditions of greenhouses (high humidity levels, high temperatures, and scarce ventilation) favor pesticide absorption [38].

On the other hand, the results of studies evaluating the effectiveness of PPE to prevent pesticide absorption have been heterogeneous, although more indicate a protective effect. In Kenya [7], farmers who wore both boots and overalls had less acetylcholinesterase (AChE) inhibition than those who only wore boots (40% vs 44% AChE inhibition, respectively). Also, Arbuckle et al. [8] found that the use of PPE (specifically, waterproof gloves and rubber boots) among Ontario workers who applied herbicides reduced urinary herbicide concentrations during the first 24 h after starting an application. Among Washington (U.S.A.) workers who handled agricultural pesticides, the use of a full-face respirator mask and chemical-resistant boots reduced butyrylcholinesterase (BuChE) inhibition; handlers who did not

wear boots resistant to these products had an average of 11.4% greater BuChE inhibition, and an estimated 7.6-fold increased risk of BuChE depression. However, handlers who wore chemical-resistant aprons had higher levels of BuChE inhibition than those who did not [12]. Gomes et al. [39], found that AChE inhibition in workers of the United Arab Emirates was negatively associated with the use of work gloves and overalls and the implementation of safety and hygiene procedures. However, among Tanzanian farmers, the use of gloves, long boots, and overalls was not associated with changes in AChE activity [40]. It is important to mention that most of these studies evaluated PPE individually, i.e. garment by garment, whereas our study built a variable that encompasses all elements of PPE given the low frequency of their use, and therefore cannot be easily compared with other studies.

Other occupational variables, such as hours/day and days/week worked, were not associated with DAP concentrations, likely explained by the low variability in the distribution observed (95 + % of employees worked 8–10 h/day and 90% worked 6 days/week). Nor was length of time in the job associated with DAPs concentration. This is consistent with the fact that there were no significant differences in the correct use of PPE, the place of work or the type of activities carried out depending on length of time in the job, meaning that experience does not seem to change behavior, for example the correct use of PPE, as a priori one might expect. Previous studies have found that, compared with less experienced workers, use of protective clothing was lowest for farmworkers working in agriculture for more than 10 years [41] or for more than 9 years [42]. On the other hand, DAPs are not persistent compounds, meaning that their concentration in urine reflects recent exposure (approximately prior 48 h). As a result, the number of years worked would not necessarily mean a greater concentration of these metabolites in a single sample. The only covariate consistently associated with increased concentration

of DAPs was employee age. Given that no significant relationship was found between age and the correct use of PPE, the type of activities carried out or the place of work, we believe that the increase observed depending on age could be due to a reduction in the metabolism of OPs.

This study was cross-sectional in design, limiting our ability to establish a temporal relationship between the occupational characteristics and the DAPs concentrations, however organophosphates are non-persistent compounds and the urine concentration of DAPs is an indicator of recent exposure (24–48 h prior to sampling). Since all samples were taken the day after fumigation and the questionnaire referred to workers' practices during the aforementioned fumigation, we believe that it is unlikely there is a problem of temporality related to exposure-outcome. Additionally, cross-sectional design raising concerns of health worker selection bias.

On the other hand, the DAP metabolites measured in the urine are not specific for each organophosphate pesticide and may have also entered the body from other sources, such as foods [43], however they provide an acceptable measure of the joint exposure to OPs. Occupational characteristics were measured via questionnaire, so we cannot rule out a potential information error, although we believe this to be unlikely, as the information refers to recent conditions and, if there is an error, it would be non-differential, as the workers did not know their DAP levels, and the laboratory staff did not know the occupational characteristics of the workers. Furthermore, characteristics such as the hygiene practices of flower growers in the workplace were not assessed. This would have provided us with relevant information for estimating the exposure and allowed us to adjust the statistical analysis accordingly.

Despite these limitations, the study has certain strengths, e.g. the availability of biomarkers of exposure to pesticides and a relatively large sample size compared to other studies on this topic. To our knowledge, this study is the first to assess the main occupational predictors associated to exposure to pesticides among flower growers in Mexico. Although the data were collected in 2004, we consider them to be relevant because, unfortunately, working conditions in floriculture have not changed substantially in Mexico in subsequent years. In 2014, a study among floriculturists from the same region showed that the frequency of the correct use of PPE continues to be very low (20%) [44]. Also, recently, a study conducted in Mexican farmers showed that only 15% use some protection equipment when applying or handling pesticides [45]. Lack of strict regulation of the use of PPE that places the provision of this in the hands of the employer without taking into account the fact that, very often, the employer is a small producer, combined with the conditions of poverty in which many floriculturists live, limits the use of PPE and increases the risk of poisoning.

Results of this study can provide evidence to establish standards and design programs with the purpose of reducing health risks among these workers to improve the quality of the working environment and provide the necessary training to avoid pesticide exposure and intoxication, as well as to promote the frequent and appropriate use of the PPE, both in workers who have direct contact with pesticides and in workers who perform task involving an indirect contact with them. Additionally, studies are called for that combine qualitative and quantitative methods that would enable the reasons underlying the limited correct use of PPE among these employees to be identified.

Finally, development of technology to improve work processes, as well as safer products to substitute for the toxic ones is needed. Integrated pest management and organic production practices are required. The use of directional ionization systems for purifying contaminated irrigation water and the pasteurization of soil, consequently reducing the incidence of plant pests and diseases, are some of the technological alternatives that have enabled the physical characteristics of flowers and the quality of ornamental plants to be improved while limiting the use of pesticides [46]. Similarly, other pest control techniques, such as the manual removal and destruction of insects, the reproduction of beneficial predatory insects, the installation of pest traps, crop rotation and diversification and the use of pest-resistant plant varieties, would enable chemical pesticide use to be reduced, allowing small quantities of a restricted variety of these to be applied as a last resort.

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Disclosure statement

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