OCCUPATIONAL EXPOSURE TO ORGANOPHOSPHORUS INSECTICIDES IN FLORICULTURE IN ECUADOR

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Introduction

Floriculture represents one of the major sources of income in the Andean Region, and Ecuador is one of the greatest worldwide producers of ornamental flowers. Floriculture can be carried out both in open fields and in greenhouses with an extensive use of chemicals, as fertilizers, growth hormones, and pesticides. Among pesticides, organophosphorous insecticides (OPs) represent one of the most widely used group, due to their wide insecticide action coupled with low persistence. Organophosphorus insecticides elicit their toxicity through the inhibition of the nervous tissue anticholinesterase (AchE), the enzyme responsible for the destruction of the neurotransmitter acetilcholine (ACh), which, in turn, brings to the accumulation of free Ach at all cholinergic endings and to continuous stimulation of electrical activity. Organophosphorus insecticides undergo extensive biotransformation – mainly through cleavage by A- esterase - and metabolites are mostly excreted in the urine within 24 h. Alkylphosphates can be found in urine samples of both workers exposed to OPs and general population at exposure levels much lower than those inhibiting AChE activity; though alkylphosphates can be considered specific metabolites of OP compounds, different chemical classes can reflect exposure to specific families: dimethyl metabolites can be found in urine samples of workers exposed to dymethil OPs, while diethyl metabolites are the measurable outcome for exposure to diethyl OPs. For instance, omethoate breaks down into dimethylthiophosphate (DMTP) and dimethylphosphate (DMP), while exposure to ethyl parathion is followed by increase of urinary excretion of diethyl phosphate (DEP) and diethylthiophosphate (DETP). Moreover, others more specific urinary metabolites can be used in biological monitoring of workers exposed to OPs: paranitrophenol for ethylparathion and 3,5,6-trichloro-2-pyridinol for chlorpyrifos-methyl. Despite the wide use of pesticides, data on occupational exposure of Ecuadorian floriculture workers are lacking.

Aim of the study

The aim of the study was the assessment of exposure to OPs in Ecuadorian floricultural workers by the determination of the urinary excretion of selected metabolites of organophorus insecticides: dimethylphosphate (DMP), diethyltiophosphate (DETP) and diethylditiophosphate (DEDTP). In particular, in this phase, differences between pre – and post-exposure and between greenhouse and open field work have been investigated.

Methods

Subjects

Thirty-six floriculture workers from an Andean region close to Quito, Ecuador, were recruited for the study. Workers were exposed to OPs while applying OPs-based plant protection products, and during re-entry activities or crop maintenance in one open field and two greenhouse farms.

Biological monitoring

Biological monitoring was performed by analysis of urinary alkylphosphates (DMP, DETP, DEDTP). Urine samples were collected in the morning (pre-exposure specimen, n= 36) and after workshift (post-exposure specimen, n = 34). The results were expressed as µg/L.

Analysis of samples

Alkylphosphates were determined by highly sensitive high-performance liquid chromatography/selective reaction monitoring mass spectrometry method (HPLC-SRM-MS/MS). The assay was linear in the range concentration of 1-50 µg/L for the three compounds in human urine. Results below 5 µg/L were considered as 2,5 µg/L.

Statistical analysis

The SPSS package has been used. Non parametric Wilcoxon test for paired data has been performed to compare urinary excretion of alkyiphosphates.

FINDINGS

Table 1: Urinary concentrations of dimethylphosphate (µg/L) in 36 ecuadorian floriculture workers (greenhouses and open field)

	DMP pre-exposure	DMP post - exposure
N	36	34
MEAN (±DS)*	19.6 (±6.9)	32.6 (±16.9)
MEDIAN	21	27
MIN	10	12
MAX	45	77

*P value for mean differences = 0.0001

Post-exposure urinary concentrations of DMP are statistically different from pre-exposure ones (p=0.0001) when both greenhouses and open field workers are considered (mean differences= 15.7 µg/L);

Table 2: Urinary concentrations of dimethylphosphate (µg/L) in 23 ecuadorian floriculture workers (greenhouses)

	DMP pre-exposure	DMP post - exposure
N	32	22
MEAN (±DS)*	21(±18.44)	34.8 (±7.3)
MEDIAN	21	29
MIN	7.3	15
MAX	45	77

* P value for mean differences = 0.0043

Table 3: Urinary concentrations of dimethylphosphate (µg/L) in 10 ecuadorian floriculture workers (open field)

	DMP pre-exposure	DMP post - exposure
N	10	10
MEAN (±DS)*	16.6 (±5.3)	27.9 (±12.5)
MEDIAN	15.5	24.5
MIN	10	12
MAX	26	48

* P value for mean differences = 0.05

Means are still statistically different when greenhouses workers and open field workers are considered as separate populations; by the way, difference is bigger (14.8 µg/L) and more significant (p=0.0043) for greenhouse workers in comparison to open field ones (mean difference= 13 µg/L; p=0.05).

Table 4: Urinary concentrations of diethyltiophosphate (µg/L) in 36 ecuadorian floriculture workers (greenhouses and open field)*

	DETP pre-exposure	DETP post - exposure
N	36	34
MEAN (±DS)*	4.54 (±4.75)	6.3 (±4.6)
MEDIAN	<5	4.8
MIN	<5	<5
MAX	25.5	18.5

*P value for mean differences = 0.02

- Mean post-exposure DETP urinary excretion is higher than pre-exposure one (p=0.02);
- On the other hand, both pre- and post-exposure urinary DETP concentrations are statistically different from controls ones (pre-exposure/controls: p=0.01; post-exposure/controls: p=0.001)

Table 5: Urinary concentrations of diethylditiophosphate (µg/L) in 36 ecuadorian floriculture workers (greenhouses and open field)

	DEDTP pre-exposure	DEDTP post- exposure
N	36	34
MEAN (±DS)*	6.9 (±12.54)	12.46 (±10.11)
MEDIAN	<5	11.3
MIN	<5	<5
MAX	65.5	48.6

* P value for mean differences = 0.008

- DEDTP urinary excretion nearly doubles after workshift (6.9 versus 12.46 µg/L) (p=0.008);
- Moreover, DEDTP concentration does not significantly differ between pre-exposure samples and controls (p=0.15), while it reaches significativity when post-exposure and controls levels are compared (p=0.003)

Discussion and Conclusions

Analysis of urine samples from workers engaged in floriculture activities shows that post-exposure excretion of DMP, DETP and DEDTP in the study population is significantly higher than controls' ones. Moreover, pre-exposure and post-exposure concentrations are significantly different for DMP, DEDTP and DETP. DMP excretion increases more and with a more significant p value when only greenhouses workers are considered.

In order to compare these preliminary results with those available in scientific literature, the metabolite concentration (µg/L) has been divided by specific moleculare weight (unit of grams per mole); then it has been corrected by urine creatinine and the comparison has been performed considering nmol/g creatinine.

The post exposure urinary excretion of DMP is in general lower than the one found by other Authors in occupationally exposed population (Aprea 1997) and in general population (Aprea 2000 and Heudorf 2001), especially when children are considered.

Due to the fact that residues of DMP are directly attributable to pesticide exposure, while DETP and DEDTP are less directly associated with pesticide exposure and rapidly degrade to other alkyl phosphate metabolites and the interpretation can be counfounded by non - pesticides sources, it can be concluded that ecuadorian workers engaged in floriculture activities are exposed to organophosphorus compounds, both methylated and ethylated. The exposure level, derived from urinary excretion of DMP, is stronger for greenhouses workers, when compared to open field ones, as a result of exposure in an indoor environment. Due to the well known correlation between organophosphorus pesticide exposure and DMP urinary excretion (Aprea 2004), it can be concluded that ecuadorian workers are likely to be exposed to low doses of OPs

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