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Assessment of Contamination by The Organophosphorus of Human Matrix (Blood and Urine) at The Hygiene Agents in The City of Fez.

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ABSTRACT

The objective of this study was to evaluate the pesticide contamination in blood and urine within hygiene agents working in the city of Fez. An investigation of exposure of the hygiene agents to pesticides has been realized before the determination of organophosphorus in both matrices by gas chromatography coupled to a mass spectrometer (GC / MS). The noncash blood and urine were conducted among 30 persons for tubes and bottles appropriate respectively. Samples were transported under strict conditions for laboratory analysis. The survey results showed an exposure period of hygiene agents into pesticides that spread for one year to 40 years and a predominance of the family of pyrethroids followed by organophosphates. One third (1/3) of the subjects concerned did not use protective means when handling pesticides. The detection standards limit in the blood was found in the range of 1 ppm and the exploration of pesticides in the studied 60 samples (30 of blood and 30 of urine) that are taken from the hygiene agents, has been negative. The found results can comfort the exposed people and the authorities concerned. However, it would be necessary to recommend the rigorous use of protective means to prevent serious health risk.

Keywords: Hygiene Agents, Blood, Urine, Organophosphorus, Assessment, Health Risk, Fez-Morocco.

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INTRODUCTION

The use of insecticides, herbicides, fungicides ..., grouped under the name of pesticides or plant protection products, has proven benefits in terms of economic performance. However, behind these benefits, hide insidious effects that harm the environment, the quality of agricultural products and health including human and animal populations. Commonly used in agriculture as insecticides, organophosphates (OPs) are a common cause of accidental or suicidal poisoning, with high morbidity and mortality of which the estimate is very variable from one country to another [1-3].

Based on this last point, the World Health Organization estimates that more than a million of victims are poisoned annually and twenty thousand of which die [4-6].

OPs are toxic lethal, with a predominant systematic action; the main mechanism of which is to block the breakdown of acetylcholine at the level of the cholinergic synapses by inhibiting cholinesterase, while other poorly understood mechanisms also exacerbate toxicity [7,8].

In Morocco pesticides are generally used by three departments: health for the fight against vectors of disease, the interior for the fight against nuisance mosquitoes and agriculture for the fight against plant pests. Unfortunately these pesticides, be them toxic by oral, dermal or respiratory could affect human health [9].

The main objective of our study is to evaluate the pesticide contamination of human matrix (blood and urine), within hygiene agents in six districts of the city of Fez. A survey measure the exposure of these hygiene agents to pesticides. This was performed by using a questionnaire on the exposure period, the families of pesticides and chemical classes as well as the means of protection.

MATERIALS AND METHODS

Zone and the population under question

The survey is done in Fez, the central-north Morocco. This latter consists of six districts: Sais, Agdal, Zouagha, Merinides, Fez Medina and Jnane El ward. The survey is conducted via a questionnaire sent to 30 qualified members of technical staff, officials in the Office of Service of the Municipality of Hygiene (BCH) at the urban commune of Fez. The questionnaire focused on the period of exposure, the family and the chemical class of pesticides and finally the protection means. We have obtained the constantly concerned hygiene agents before starting the study.

Methods of sampling and routing

We have drown five milliliters of blood at the brachial vein in test tubes of Ethylene Diamine Tetra-Acetic Acid (EDTA) and fifteen milliliters of urine in suitable vials stored and transported in a cooler maintained at a temperature of 5 / 3 ° C to toxicology laboratory of the poison control center and pharmacovigillance in Morocco (CAPM), for analysis.

Qualitative determination of OPs

This assay is to identify pesticides including OPs in human matrices (blood and urine), via an extraction method adopted by Keiko kudo et al., in 2012 [10].

Conditions of chromatographic analysis of OPs

We used the machinery of gas chromatograph perkun Elmer Clarus 680 which is combined with a mass spectrometer in an electronic energy ionization with a mode of 70 eV and with an ion source temperatures of 250 °C. The type of injection is split/ splitless, column RESTEK RXI 5 ms, 30 mx0, 25mm of internal diameter, a film with a thickness of 250 µm; the carrier gas was helium with a flow rate of 0.1.

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Method of analysis for the identification of the detection limit in the blood

Before starting the qualitative assay of OPs, we conducted a verification of the detection limit of a mixture of five standards of OPs by gas chromatography coupled with mass spectrometry (GC / MS) using five concentrations: 50ppm, 5ppm, 1 ppm, 0.5 ppm and 0.1 ppm. The mixture of standards was prepared from an initial concentration of 1000 ppm diluted with methanol.

We used blank plasma that has been doped by a mixture of OPs and extracted according to a liquid-liquid extraction technique. The extract obtained is so focused and then analyzed by GC / MS [10]. The identification of doped compounds was made by reading the retention time and the relative abundance of the mass by the Turbo Mass 6.0 software.

Method of OPs determination in human samples (blood and urine)

The molecules pesticides standards we have worked on have been proposed by the toxicology laboratory at (CAPM), they are: Malathion, Fenitrothion, Diazinon, Chlorpyrifos and Dimethoate.

The Thirty samples of blood and the thirty studied samples of urine were extracted with hexane, evaporated under nitrogen stream to obtain a dry residue eluted with methanol and then analyzed by GC / MS.

Processing and analysis of spectra

The analysis and treatment of spectra were performed by the turbo 6.0 mass software. Each peak appearing in the chromatogram is selected and compared with the spectral library. All likelihood, with the peak correspondence selected in a given molecule, exceeding 70% is considered appropriate.

RESULTS

Survey Results

The survey results carried out on hygiene agents showed that pesticide exposure period varies between one and forty years and that the pesticides used, belonged to two families of pesticides which are insecticides and rodenticides. They are mainly represented by two chemical classes: organophosphates (Temephos, Fenitrothion, and Chlopyriphos Phosphorothioate) and pyrethroids (Permethrin, Bioallethrin, Bifenthrin, Cypermethrin, Tetramethrin, Pyrethrin, Deltamethrin and Alphacypermethrin).

According to the collected data from survey (Figure l) we found that one-third (1/3) of the daily hygiene agents manipulators of pesticides, do not use appropriate means of protection against the exposure to pesticides.

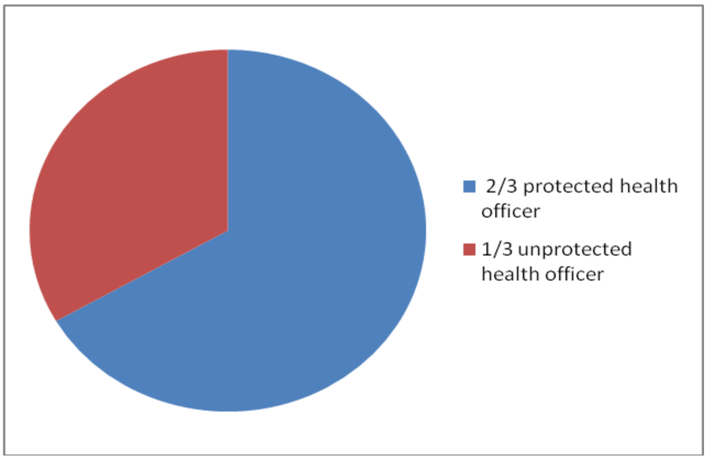

Figure 1: The share of protected agents.

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Results of the verification of the detection limit

Before carrying out the extraction of OPs within thirty noncash of blood and the thirty specimens of urine, we analyzed a mixture of OPs standards by GC / MS with different concentrations: 50 ppm, 5 ppm, 1 ppm, 0.5 ppm and 0.1 ppm and we have obtained the chromatograms 2a, 2b, 2c, 2d and 2e as shown in Figure 2.

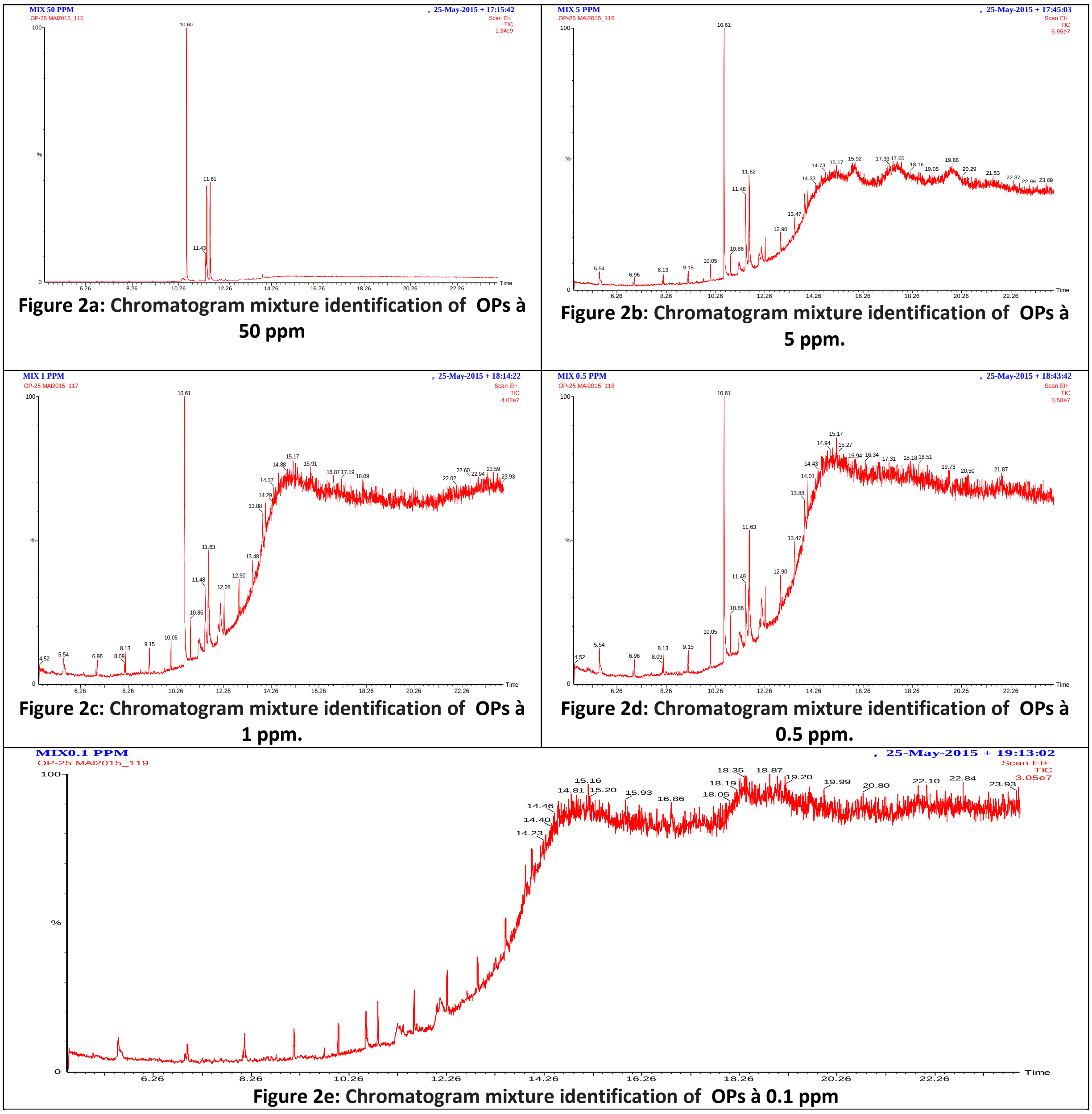

Figure 2a: Chromatogram mixture identification of OPs à 50 ppm

Figure 2b: Chromatogram mixture identification of OPs à 5 ppm.

Figure 2c: Chromatogram mixture identification of OPs à 1 ppm.

Figure 2d: Chromatogram mixture identification of OPs à 0.5 ppm.

Figure 2e: Chromatogram mixture identification of OPs à 0.1 ppm

Figure 2: Chromatograms mixture identification of OPs to 50 ppm, 5 ppm, 1 ppm, 0.5 ppm and 0.1 ppm.

OPs Mixture

50 ppm Mixture

Figure 2a shows the mixture identification chromatogram of OPs to 50 ppm, the mass spectra of each peak are shown in Figure 3.

The chromatogram (Figure 2a) shows the result of analysis by GC / MS of the mixture of OPs to 50 ppm. We note the appearance of five studied OPs with good separation. Referring to the spectral library, the different peaks were identified through these five molecules: Dimethoate, Diazinon, Malathion, Fenitrothion and Chlorpyrifos.

Each peak in the chromatogram is automatically associated with a mass spectrum.

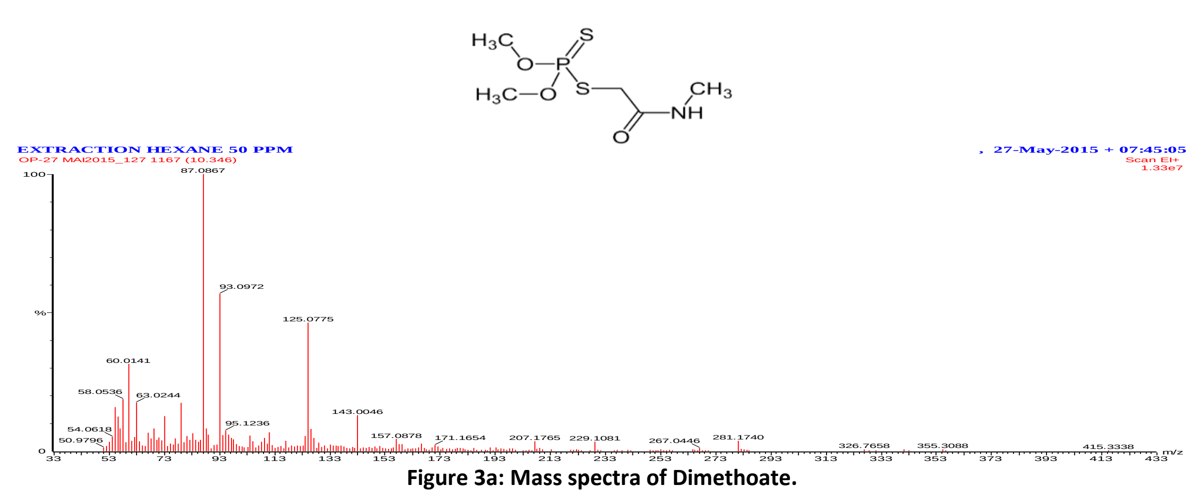

Figure 3a: Mass spectra of Dimethoate.

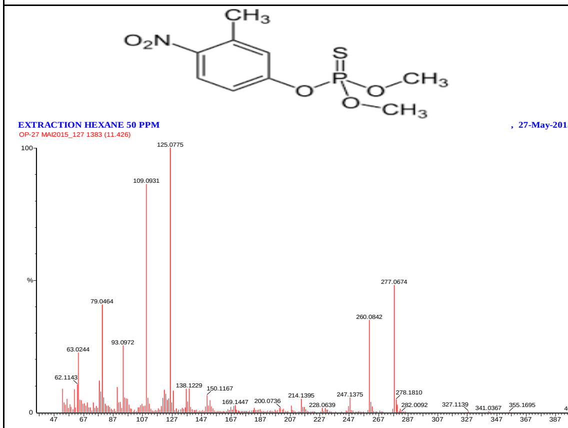

Figure 3b: Mass spectra of Fenitrothion.

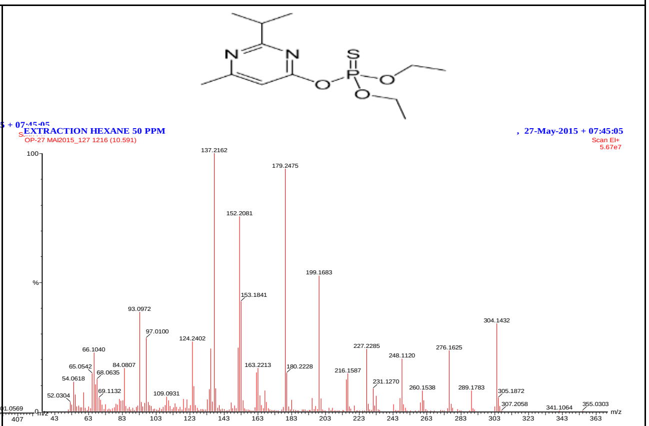

Figure 3c: Mass spectra of Diazinon.

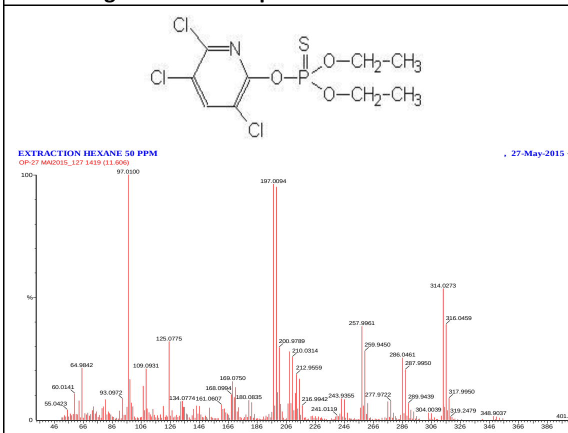

Figure 3d: Mass spectra of Chlorpyriphos.

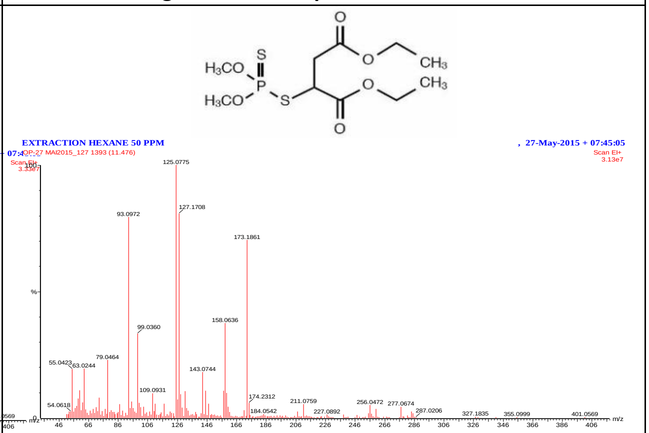

Figure 3e: Mass spectra of Malathion.

Figure 3: Mass spectra of OPs to 50 ppm.

5 ppm Mixture

The chromatogram 2b in Figure 2 shows the result of analysis by GC / MS of the mixture of OPs to 5ppm. This chromatogram shows the appearance of 3 OPs. According to the spectral library, the three peaks were identified on these three molecules: Diazinon, Malathion and Chlorpyrifos.

1 ppm Mixture

The chromatogram 2c Figure 2 shows the result of analysis by GC / MS of the mixture of OPs to 1 ppm. In this chromatogram 3 OPs appear. According to the spectral library, the three peaks were identified on these three molecules: Diazinon, Malathion and Chlorpyrifos.

0.5 ppm Mixture

The 2d chromatogram of Figure 2 shows the result of analysis by GC / MS of the mixture of OPs to 0.5 ppm. This chromatogram also shows three OPs, but the peaks are not well separated, they are overlapped with background nuance due to the GC / MS. According to the spectral library, the three peaks were identified through these three molecules: Diazinon, Malathion and Chlorpyrifos.

0.1 ppm Mixture

The second chromatogram in Figure 2 shows the result of analysis by GC / MS of the mixture of OPs to 0.1 ppm. In this chromatogram no molecule was detected. Table 1 shows the retention time and abundance of each peak in the series of OPs detected. They are mainly: 50 ppm, 5 ppm, 1 ppm, 0.5 ppm and 0.1 ppm.

Table 1: List of OPs detected at 50 ppm, 5 ppm , 1 ppm , 0.5 ppm and 0.1 ppm .

Extraction	Numéro des pics	Molécules des OPs	Formule	Temps de rétention en mn	L'abondance des pics en %
50 ppm	1	Dimethoate	$C_5H_{12}NO_3PS_2$	10.43	96.1
	2	Diazinon	$C_{12}H_{21}N_2O_3PS$	10.61	89
	3	Fenitrothion	$C_9H_{12}NO_5PS$	11.42	94
	4	Malathion	$C_{10}H_{19}O_6PS_2$	11.46	98.2
	5	Chlorpyriphos	$C_9H_{12}NO_3PS_2$	1.61	9.2
5 ppm	1	Diazinon	$C_{12}H_{21}N_2O_3PS$	10.63	93.9
	2	Malathion	$C_{10}H_{19}O_6PS_2$	11.47	94.3
	3	Chlorpyriphos	$C_9H_{12}NO_3PS_2$	11.63	89.4
1 ppm	1	Diazinon	$C_{12}H_{21}N_2O_3PS$	10.62	93.9
	2	Malathion	$C_{10}H_{19}O_6PS_2$	11.48	94.3
	3	Chlorpyriphos	$C_9H_{12}NO_3PS_2$	11.62	89.4
0.5 ppm	1	Diazinon	$C_{12}H_{21}N_2O_3PS$	10.60	88.29
	2	Malathion	$C_{10}H_{19}O_6PS_2$	11.50	28.6
	3	Chlorpyriphos	$C_9H_{12}NO_3PS_2$	11.62	90.3
0.1 ppm	0	-	-	-	-

Extracting the mixture of OPs

We have realized the extraction of plasma boosted by five OPs according to the same procedure adopting the same concentrations of mixtures. They are mainly: 50 ppm, 5 ppm, 1 ppm, 0.5 ppm and 0.1 ppm.

50 ppm extraction

Five OPs molecules were detected respectively: Dimethoate, Diazinon, Fenitrothion, Malathion and Chlorpyrifos.

5 ppm extraction

Three OPs molecules were found: diazinon, malathion and chlorpyrifos.

1 ppm extraction

A single molecule of OPs was detected which is Diazinon.

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0.5 ppm extraction

No molecule has been detected.

0.1 ppm extraction

No molecule has been detected. Table 2 presents the retention time and abundance of each peak of the mixture with an extraction of different detected OPs concentrations: 50 ppm, 5 ppm, 1 ppm, 0.5 ppm and 0.1 ppm.

Table 2: List of OPs detected in the extraction mixture with 50 ppm, 5 ppm, 1 ppm.

Extraction	Numéro des pics	Molécules des OPs	Formule	Temps de rétention en mn	L'abondance des pics en %
50 ppm	1	Dimethoate	$C_5H_{12}NO_3PS_2$	10.36	36.2
	2	Diazinon	$C_{12}H_{21}N_2O_3PS$	10.60	92.4
	3	Fenitrothion	$C_9H_{12}NO_5PS$	11.41	92
	4	Malathion	$C_{10}H_{19}0_6PS_2$	11.46	98.2
	5	Chlorpyriphos	$C_9H_{12}NO_3PS_2$	11.60	89.4
5 ppm	1	Diazinon	$C_{12}H_{21}N_2O_3PS$	10.61	38.2
	2	Malathion	$C_{10}H_{19}0_6PS_2$	11.47	62.2
	3	Chlorpyriphos	$C_9H_{12}NO_3PS_2$	11.61	76.20
1 ppm	1	Diazinon	$C_{12}H_{21}N_2O_3PS$	10.61	38.2

Result of detection limit of the assay mixture of OPs in urine

We found the same result as that found in the mixture of OPs in blood.

Results of assay of OPs at the samples (blood and urine) of hygiene agents

The assay result of organophosphates in urine and blood for hygiene agents was negative.

DISCUSSION

This work had an objective to assess the pesticide contamination in human matrices (blood and urine), in hygiene agents in six districts of the city of Fez and achieve an investigation to determine the exposure period, the families of pesticides and chemical classes as well as the means of protection adopted.

The results of the survey showed that particularly the period of exposure of individuals studied ranged from 1 to 40 years and that more than a half (50%) of the studied individuals were exposed to pesticides over a period exceeding 10 years.

These results are interesting because they have demonstrated the existence of possible contamination of human matrices (blood and urine) by the pesticides mainly OPs used by the health services department within the Office of the Municipality of Hygiene (BMH).

The survey results also allowed us to identify the most common families which are insecticides and rodenticides. This information is not consistent enough with the results of a study conducted in Morocco by Fennane et al., in 2014 [11], which found that most pesticides used by manipulators are three families: herbicides, fungicides and insecticides.

A predominance of pyrethroids following organophosphates was observed. These results differ from those of Saliou et al., in 2013 [12]. Who noted the employ by users of a wide variety of pesticides represented by all chemical classes (organochlorine, OPs, carbamates and pyrethroids).

The survey results also allowed us to see that 1/3 of exposed health officers did not use the means of protection during the handling of pesticides. Similarly to our results, the survey of Saliou et al. [12] showed ignorance of good pesticide application practices by users.

The determination of the detection limit by GC / MS with a mixture of five organophosphorus (Dimethoate, Diazinon, Malathion, Fenitrothion, and Chlorpyrifos) was performed at five levels namely: 50 ppm, 5 ppm, 0.5 ppm, 0.1 ppm. This OPs detection limit in the studied mixture was found in the order of about 1 ppm.

According to a study published by Lacassie et al., in 2001 [13], dealing with the determination of 29 multi pesticide residues (OPs) in blood and urine by GC / MS, the detection limit of 0.005ppm was adopted to 0.025ppm. The results obtained by this method were very satisfying. In 2009 in Korea, Lee et al., [14] studied the search of 66 pesticides using a 1 ppm and 0.1 ppm detection limit. They were able to detect pesticides, to 1 ppm, with more than 70% to 18 pesticides and 50 to 70% to 15 pesticides.

A study published in 2014 by Nishimiyiyimana FX et al., [15], has developed a pesticide residue analysis method by GC / MS in the biological matrix. The method was used to determine significant percentages of four pesticides: Chlorpyrfos, Dichlorvos, Dichlorodiphenyl Trichloroethane(DDT) and Heptchlor.

Recently in 2015, a research conducted by Valente Nuno IP et al., [16], in Portugal, revealed a law detection limit of 50 ng / ml (0.066 ppm) for the determination of 9 OPs GC / MS: Chlorfenvinphos, Chlorpyrifos, Diazinon, Dimethoate, Fenthion, Malathion, Parathion, Phosalone, Pirimiphos-methyl and Quinalphos.

According to this work, especially those of Lee et al., [14], our detection limit of around 1 ppm conforms to detect one of the five researched OPs.

The search for OPs within thirty urine samples and thirty blood samples for hygiene agents was negative. This has led us to conclude and deduct two hypotheses: either our assay OPs, a 1 ppm detection limit, is far from sensible and effective or that no studied individual is contaminated by one of the sought OPs.

These results differ from those of Karen et al., in USA in 2012 [17], who found from 0 to 0.5 ng / ml Diazinon and from 0 to 1726ng / ml of Chlorpyrifos in blood samples from a population. The same authors recommended significant associations between the levels of organophosphate pesticides in the blood and metabolites in the urine.

The results found for the determination of OPs in human matrix (blood and urine) does not allow if not formally eliminate contamination with organophosphate insecticides. In any event, we need to educate users about the dangers of pesticides and training them on good practice (wearing gloves, a face mask and boots) and finally they must comply with strict rules and spray pesticide use [18-22].

CONCLUSION, RECOMMENDATION AND PERSPECTIVES

The main objective of our study was the assessment of pesticide contamination in the human matrix (blood and urine), among hygiene agents in six districts in the city of Fez.

The results of the survey we conducted first are: the period of exposure, the families of pesticides and chemical classes and the means of protection adopted by health officers of the Municipal Hygiene Office. They showed a predominance of pyrethroids tracking OPs and the families of the most used pesticides are insecticides and rodenticides. The exposure of health officer's period ranges from 1 year to 40 years and the third of these items are not strictly protected while handling pesticides.

The assay results of OPs in samples of blood and urine were negative.

The information obtained in this study provides stakeholders and specifically those of the Department of Health and Interior Department with the elements and tools to help prevent the risk of exposure to insecticides. These results are relative with the search for traces of OPs in human matrices (blood and urine) as well as reassuring people exposed and the authorities concerned. The rigorous use of protective means to prevent serious health risk should be recommended.

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In view of this work, it would be desirable to extend the range of analysis to other pesticide families and using more sensitive analytical methods such as GC / MS / MS.

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