INT. J. BIOL. BIOTECH., 14 (3): 467-474, 2017.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF PESTICIDE RESIDUES IN THE BLOOD AND RESULTANT HEALTH ISSUES IN THE PEOPLE OF KARACHI - PAKISTAN

Farkhunda Iftekhar[1] and M. Arshad Azmi[1*]

[1]*Department of Zoology, University of Karachi, Karachi-Pakistan*
[*]**Corresponding Author**

ABSTRACT

The present study aimed to quantify the status of pesticide residues in the blood sample of persons representing from different regions of Karachi, Pakistan. Total 45 persons exposed to pesticides were selected in the study for high performance liquid chromatographic quantification of residues. After analysis of all the blood samples, the results indicated that only one sample contains malathion, two samples showed the presence of polyrin-C while Cypermethrin and Deltamethrin were found in almost all the samples. Rest of the Pesticides such as DDE, DDT, diazinon and monocrotophos were not found in any sample.

Keywords: Pesticide, Residues, Blood, Health issues.

INTRODUCTION

Pesticides comprise of various chemicals that are deliberately inducted in the environment for the purpose of eliminating different pests. The main consumption of pesticides is in the agricultural sector. As pesticides are basically pollutants or mixture of poisonous substances that are found in our environment can be beneficial in eliminating or killing undesirable organisms but as they are toxic in nature so their application or use in the field or home should be controlled because their misuse can cause serious disturbances to human health. (Azmi and Naqvi, 2011).

In the agriculture sector most of the persons from Pakistan consider commercial-based pesticides for the protection of their agricultural products from the attack of harmful toxic organisms but these commercial or traditional pesticides causing harm to the people and to the environment as well. Based on environmental contamination the developing countries replaced these commercial pesticides with plant-based pesticides as they are not only effective for the field products but also safe to the environment and human health.

It is important to mention that there are number of important factors that can cause contamination or poisoning among farm workers in the developing countries like frequent use of pesticides by the persons or through dermal, oral, eye and inhalation routes of contact (Wesseling *et al.,* 2001; Konradson *et al.,* 2003, Coronado *et al.,* 2004). A number of studies in this connection have proved that misuse or mishandling of pesticides creates serious disturbances on human health and environment. (Soares *et al.,* 2003; Mancini *et al.,* 2005; Remor *et al.,* 2009).

Extensive research has been done in this connection and reported the detection of pesticides such as polychlorinated biphenyls and organchlorine pesticides in water and human blood through several techniques. They are potentially dangerous for human health (Kocan *et al.,* 1994; Ntow, 2001; Charlier and Plomteux 2002, Butler *et al.,* 2003, Van Oostadam., 2004; Lopez *et al.,* 2004).

Polychlorinated biphenyls and some common organchlorines have been reported from the sediments and coastal regions of Egypt and in the serum samples of people from Slovak. (Barkat *et al.,* 2013; Jana *et al.,* 2014). Similarly, Sharma *et al.,* (2015) monitored the status of pesticide residues of persons from India. Related to the pesticide exposure and health issues, Khan and Samalas (2015) also observed the same findings in the farmers of Punjab from Pakistan. In such concern Sharama *et al.,* (2016) reported the use of spectroscopic methods and Fourier transform infrared coupled with solid phase extraction method for the detection of malathion level in the blood plasma of human.

Keeping the hazardous effects in priority, present study has been under taken to determine the quantity of pesticides in the blood as well as to assess their harmful effects in persons belonging from different socio-economic regions of Karachi-Pakistan.

MATERIALS AND METHODS

Collection of blood Samples

Blood samples were collected from patients of Fatimid Foundation belonging from different regions of Karachi were selected. The blood samples of all persons were collected in well-cleaned heparinized glass tubes and saved in

ice box during collection. The samples were immediately brought to the laboratory for the centrifugation. Serum were separated out and transferred into glass vials. All the vials containing serum samples were placed in freezer for analysis.

Quantification of pesticide residues

Quantification of residues of pesticides in the blood sample was carried out through high performance liquid chromatography (HPLC) which is attached with electron capture detector. The different steps involved for quantification are: Fat extraction method (Kadoum, 1967), Soxhlation method (Holden and Marsden, 1969) and Sorption process consisting of alumina chromatographic column method (Holden and Marsden, 1969) and Silica chromatographic column (Kadoum, 1967; 1968) method. All the parameters required for quantification process were set on HPLC Schimadzu SPD-10A apparatus attached with chart recorder. The standard pesticides and blood samples were prepared and processed according to Dale *et al.,* (1940) method. After processing ten (10) µL of each sample was injected into the HPLC apparatus through special chromatographic syringe for obtaining respective chromatograms. Quantification was then done by comparing the peaks of sample chromatogram with the standard peaks on the basis of retention time. The area of each sample peak was calculated to quantify the residues in the blood sample.

RESULTS AND DISCUSSION

All the blood samples after centrifugation and clean up procedures were brought to HPLC for residual analysis and were found to have sufficient quantity of pesticide residues **(Table 1).** In the present study 45 persons in total from different locations of Karachi were selected to find out the different pesticides in their blood. Out of these only 20 persons showed severe exposure to pesticides and were considered for HPLC analysis. Ten different standard pesticides were taken into consideration in this study and only five pesticides were detected in exposed persons. The pesticides that were detected included Cypermethrin, Deltamethrin, polytrin-C, malathion and permethrin **(Table 2; chromatogram I-V)**. The retention time (RT) of all the standards is given in **Table 2**. In addition 10 normal control persons were also included in the study for the quantification of pesticide residues.

It has been found that out of 20 samples, Cypermethrin was detected in 10 samples. Low quantity was found in 4 samples and the lowest 0.32 µg/mL was noted in **sample no. 32** in male person named **Sarfraz** (32 yrs). It was also found in higher quantity in 6 samples and the highest was found in female person in **sample no. 42** named **Nusrat** (32 yrs) i.e., 26.8 µg/mL **(chromatogram-Ia).** The high quantity in this subject may be due to longer exposure. In rest of the samples Cypemethrin was not detected.

Delthamethrin was noted in the blood samples of 13 persons and the highest quantity was in **samples no. 33** i.e., 34.86 µg/ mL **(chromatogram-IIa)** named **M. Yousuf** (M) aged 29 years possibly because of direct exposure or use of Deltamethrin frequently by this person. Similar finding is also reported by Jahan (1995) i.e., 12.22 µg/mL and Bissacot and Vassilieff (1997). Low quantity of Deltamethrin was noted in the blood of 7 persons where as in other persons Deltamethrin was not detected in a detectable quantity.

A high amount of polytrin-C i.e., 16.15 µg/mL was found in male person named **Shehzad** in **sample no.19** and a very high quantity 30.76µg/mL **(chromatogram-IIIa)** was noticed in a very young male subject in **sample no. 27.** This high quantity may be due to the greater exposure or improper use. No other worker showed detectable quantity in any of the samples.

Malathion was detected in high quantity i.e., 12.6µg/mL **(chromatogram-IVa)** in the person named **Ali Mohammad** aged 33 years in **sample no. 41**. No detectable quantity of malathion was seen in rest of the samples. Detection of malathion in the samples of human blood was also reported by many researchers (Abu-Qare and Abu-Donia, 2001; Musshoff *et al.*, 2002; Jaiswal *et al.*, 2008; Venugopal *et al.*, 2013).

Permethrin was detected in high quanity in 6 samples and the highest quantity 8.82µg/ml **(chromatogram –Va)** was found in the blood sample of very young male person named **Jan Sher** from **sample no.4** out of total 6 samples. No low quantity was detected in any sample. Permethrin level in the blood sample was also reported by many researchers (Abu-Qare and Abu-Donia 2001; Lestremau *et al.*, 2014) in the blood samples of human and rat model.

Exposure to pesticides and resultant health problems in the highly exposed persons were also assessed indicated that their health being affected by the pesticide residues e.g., persons that were highly exposed with Cypermethrin in sufficient quantity were **Nusrat** 26.8µg/mL **(sample no. 42)**, **Asif** 17.31µg/mL **(sample no.43)** and **M. Yousuf** 13.93 µg/mL **(sample no.33)**. These persons complained about liver disorder and therefore suffered with hepatitis repeatedly. They might be highly affected due to the frequent exposure of this pesticide. In addition to hepatitis these people also complained about the backache and headache frequently while **M. Khan** complained about the burning sensation during micturition and hematuria. Some researchers also reported the health issues that are

produced due to the frequent exposure of Deltamethrin e.g., **Abdul Rahman (sample no. 29)** and **M. Yousuf (sample no. 33)** had high quantity of Deltamethrin i.e., 22.42µg/mL and 34.86µg/mL respectively in their blood. These people complained about the history of respiratory illness, altered lung functions and disturbed some of the hematological profiles. These findings are almost in line with the findings of some researchers (Goswamy *et al.*, 1994; Fareed *et al.*, 2013). **Shehzad** from **(sample no.19)** and **Aamir (sample no. 27)** with high values of profenofos 16.15µg/mL and 30.76µg/mL suffered with dermatitis, irritation and redness of eye, headache and dizziness during examination. The same findings were also noted by Mehboob *et al.*, 2012; Khan and Damalas, 2015).

Table 1. Pesticide residues in the blood samples of 20 persons from Karachi.

Blood Samples	Name Age (Sex)	Pesticide Residues (µg/mL)					
		Cypermethrin	Deltamethrin	Polytrin-C		Malathion	Permethrin
				Cypermethrin	Profenophos		
Sample # 4	Marium 31 yrs (F)	-	1.71	-	-	-	-
Sample # 7	Akhter Hussain 26 yrs (M)	0.42	0.97	-	-	-	-
Sample # 8	Asim 30 yrs (M)	↑1.34	↑3.84	-	-	-	-
Sample # 10	Zeeshan 35 yrs (M)	-	-	-	-	-	↑3.58
Sample # 15	Ghulam Mustafa 28 yrs (M)	↑4.31	-	-	-	-	-
Sample # 19	Shehzad 31 yrs (M)	0.51	0.36	-	↑16.15	-	↑8.10
Sample # 20	Jan Sher 23 yrs (M)	-	-	-	-	-	↑8.82
Sample # 22	Fazal Ghani 45 yrs (M)	-	0.34	-	-	-	↑6.66
Sample # 24	Younus 30 yrs (M)	0.76	↑3.52	-	-	-	-
Sample # 25	M. Khan 35 yrs (M)	↑9.36	-	-	-	-	-
Sample # 27	Aamir 22 yrs (M)	-	0.34	-	↑30.76	-	↑6.15
Blood Samples	Name Age (Sex)	Pesticide Residues (µg/mL)					
		Cypermethrin	Deltamethrin	Polytrin-C		Malathion	Permethrin
				Cypermethrin	Profenophos		
Sample # 29	Abdul Rahman 25 yrs (M)	-	↑22.42	-	-	-	↑4.0

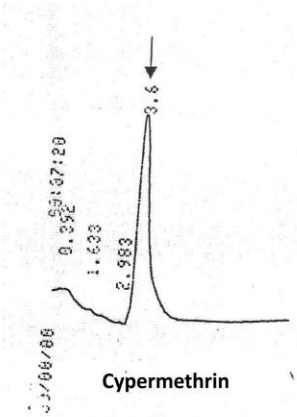
Sample # 31	Imran Ahmed 27 yrs (M)	-	1.54	-	-	-	-
Sample # 32	Sarfraz 32 yrs (M)	0.38	↑4.64	-	-	-	-
Sample # 33	M. Yousuf 29 yrs (M)	↑13.93	↑34.86	-	-	-	-
Sample # 35	Irfan 30 yrs (M)	-	1.46	-	-	-	-
Sample # 37	Samad 26 yrs (M)	-	↑2.71	-	-	-	-
Sample # 41	Ali Muhammad 33 yrs (M)	-	-	-	-	↑12.6	-
Sample # 42	Nusrat 32 yrs (M)	↑26.8	-	-	-	-	-
Sample # 43	Asif 29 yrs (M)	↑17.31	-	-	-	-	-

Under line = Low value , ↑= High value, - = Not detected,

Table 2. Different parameters of standard pesticides.

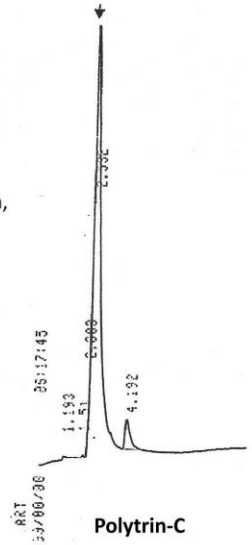
No.	Pesticides	Retention Time (RT)	Concentration (%)	Quantity (µg/10µL)
1.	Match	3.243	0.019	1.9
2.	Cypermethrin	3.662	0.009	0.9
3.	Deltamethrin	2.638	0.016	1.6
4.	Polytrin- C • Cypermethrin • Profenophos	2.532	0.014	1.4
		4.192	0.014	1.4
5.	Diazinon	5.572	0.012	1.2
6.	DDE	5.297	0.014	1.4
7.	Monocrotophos	4.15	0.012	1.2
8.	Malathion	3.223	0.009	0.9
9.	DDT	6.153	0.006	0.6
10.	Permethrin	2.658	0.008	0.8

Flow rate = 1.0 mL/min, Absorbance = 0.02nm, Chart speed = 0.5 mm/min, Pressure = 200kg/mm, Wave length = 250nm, Sample injected = 10µL



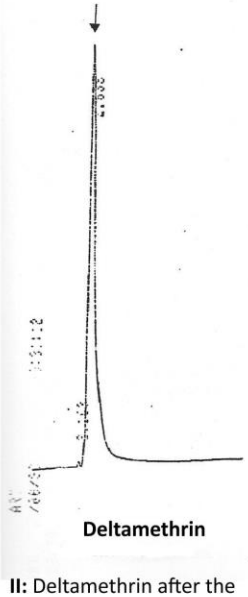
Cypermethrin

I: Cypermethrin after the injection of 0.9µg fr = 1.0 ml/min, abs - 0.02nm cs =0.5mm/min, pressure = 200kg/mm, wavelength = 250nm.



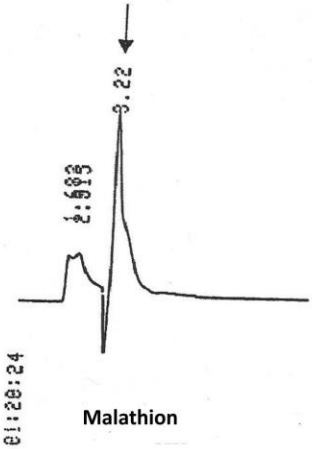
Polytrin-C

III: Polytrin-C after injection of 1.4µg fr=1.0ml/min, abs=0.02nm, cs=0.5mm/min, pressure=200kg/mm, wavelength=250nm.



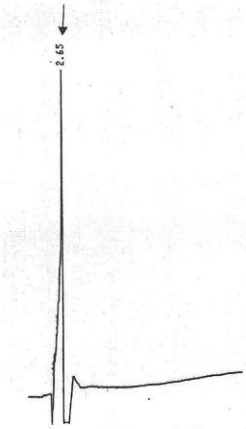
Deltamethrin

II: Deltamethrin after the injection of 1.6µg fr=1.0ml/min, abs=0.02nm, cs=0.5mm/min, pressure=200kg/mm, wavelength=250nm.



Malathion

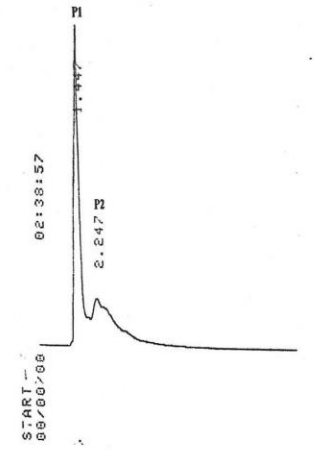
IV: Malathion after the injection of 0.9µg fr=1.0ml/min, abs=0.02 nm, cs=0.5mm/min, pressure= 200kg/mm, wavelength=250nm.



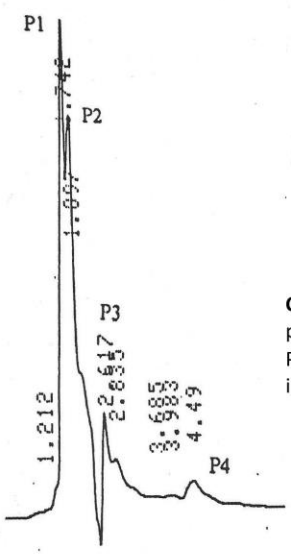
Permethrin

V: Permethrin after the injection of 0.9µg fr=1.0ml/min, abs=0.02nm, cs=0.5mm/min, pressure=200kg/mm, wavelength=250nm.

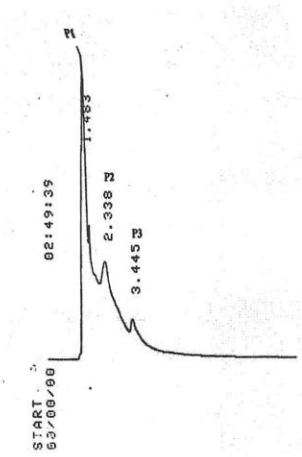
Fig.1. HPLC Chromatograms Showing the arrowed peaks (↓) of standard pesticides Cypermethrin, Deltamethrin, polytrin-C malathion and permethrin.



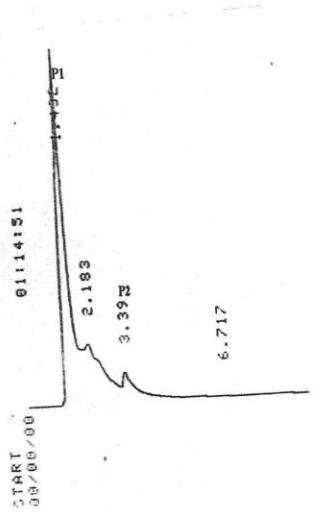
Chromatogram Ia: Showing P1= Solvent, P2=Cypermethrin 26.8µg/10µl in the blood sample of Nusrat.



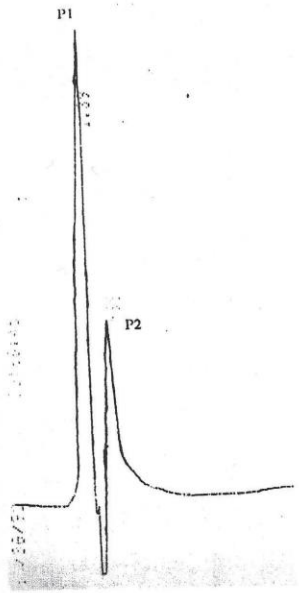
Chromatogram IIIa: Showing pesticide residues P4=Profenophos 30.76µg/10µl in the blood sample of Aamir.



Chromatogram IIa: Showing pesticide residues P3=Deltamethrin 34.86µg/10µl in the blood sample of Yousuf.



Chromatogram IVa: Showing pesticide residues P2=Malathion 12.6µg/10µl in the blood sample of Ali Mohammad.



Chromatogram-Va: Showing the pesticide residues P2=Permethrin 8.82 µg/10µl in the blood sample of Jansher.

Fig. 2. HPLC chromatograms showing high quantity of different pesticide residues in the blood samples of highly exposed persons from different regions of Karachi.

Regarding malathion high quantity 12.6µg/mL was seen only in one person named **Ali Mohammad** of **sample no. 41**. In addition it is also noted that based on the level of exposure this person complained the regular sign and symptoms on examination of numbness, in hands backache, irritation of eye, lethargy, shortness of breath, in breathing weakness and on examination. (Choudhary *et al.,* 2008; Venugopal *et al.*, 2013; Moreira *et al.*, 2015). Similarly, **Jan Sher** of **sample no. 20** with permethrin 8.82 µg/mL remained sick throughout the year and complaining the frequent gastric pain and difficulty in breathing. The hazardous effects and health deterioration has also been reported by (Lestremau *et al.*, 2016).

It is thus concluded that on the basis of experimental data obtained it has been observed that Cypermethrin and Deltamethrin were present in sufficient quantity in some persons. Likewise polytrin-C, and permethrin also showed enough quantity but malathion was seen only in one person and other such as match, diazinon, DDT and monocrotophos were not found in any sample. So, it is clear that most of the detected pesticides were found in higher level than the maximum residual limit. Therefore, on the basis of experimental data and health issues it is suggested that as pesticides are basically poisonous in nature and the persons handling any pesticides must adopt safety measures by using gloves, spectacles and masks to protect them from the hazardous effects of pesticides.

REFERENCES

Abu-Qare, A.W. and M.B. About – Donia (2001). Simultaneous determination of malathion, permethrin, DEET (N,N-diethyl-m-toluamide), and their metabolites in rat plasma and urine using high performance liquid chromatography. *JPharma, Biomed.*, 26 (2): 291-299.

Azmi MA and S.N.H. Naqvi (2011). Pesticide pollution, resistance and health hazards. Pesticides: The impacts of pesticide exposure. INTECH., 1-24.

Barkat, A.O., A. Mostafa, T.L. Wade, S.T. Sweet and N.B. EL-Sayed (2013). Distribution and ecological risk of organochlorine pesticides and polychlorinated biphenyls in sediments from the Mediterranean Coastal environment of Egypt *Chemosphere*, 93 (3): 545-554.

Bissacot, D.Z. and I. Vassilieff (1997). HPLC determination of flumethrin, Deltamethrin, Cypermethrin and cyhalothrin residues in the milk and blood of lactating dairy cows. *J.Anal. Toxicol.*, 21: 397-402.

Butler, W.J., L. Saddon, E. McMullen, J. Houseman, K. Tofflemire, A. Corriveau , *et al.* (2003). Organochlorine levels in maternal and umbilical cord blood plasma in Arctic Canda. *Sci. Total. Environ.*, 2: 27-52.

Charlier, C.J. and G.J. Plomteux (2002). Determination of organochlorine pesticide residues in the blood of health individuals. *Clin. Chem. Lab. Med.*, 40: 361-364.

Choudhary, N., R. Goyal and S.C. Joshi (2008). Effect of malathion on reproductive system of male rats. *J. Environ Biol.*, 29 (2): 259-262.

Coronado, G.D., B. Thompson, L. Strong, W.C. Griffith and I. Islas (2004). Agricultural task and exposure to organophosphate pesticides among farm workers. *Environ. Health 18 Pessp.*, 112: 142-147.

Dale, We, J.W. Miles and T.B. Gainers (1940). Quantitative method of determination of DDT and DDT metabolites in the blood serum. *J. Assoc. Off Anal Chem.*, 53: 1287-1292.

Fareed, M., M.K. Pathak, V. Bihari, R. Kamal, A.K. Srivastava and C.N. Kesavachandran (2013). Adverse respiratory health and hematological alterations among agricultural workers occupationally exposed to organophosphate pesticides: a cross-sectional study in North India. *Plos One.*, 8 (7): 69755

Goswamy, R., A. Chaudhri and A.A. Mahashur (1994). Study of respiratory failure in organophosphate and carbamate poisoning. *Heart. Lung.*, 23: 466-472.

Holden, A.V. and M. Marsdon (1969). Single stage cleanup of animal tissue extracts for organochlorine's residue analysis. *Journal of Chromatography*, 44: 481.

Jahan, M. (1995). Pesticide residues in random blood samples of human population in Karachi. *JCPSF.*, 6: 151-153.

Jaiswal, A.K., K. Ashmeet, T. Millo and M. Gupta (2008). Determination of malathion in blood and urine using thin-layer chromatography. *J. Indian Acad. Forens Med.*, 30 (1): 15-21.

Jana, C., D. Beata, F. Anna, C. Kamil, W. Sona and P. Marian (2014). Polychlorinated biphenyls and selected organochlorine pesticides in serum of Slovak population from industrial and non-indusrial areas. *Environ Monitor Assess.*, 186 (11): 7643-7648.

Kadoum AM (1967). The process of sorption was carried out in a chromatographic column of silica. *Bull Environ. Contamin Toxicol.*, **2** (5): 65-70.

Kadoum, A.M. (1968). Clean up animal tissue extract. *Bull. Environ Contam Toxicol.*, 3 (2) : 65-70.

Khan, M. and C.A. Damalas (2015). Occupational exposure to pesticides and resultant health problems among cotton farmers of Punjab, Pakistan. *Int. J. Environ Health.*, 25 (5): 508-521.

Kocan, A., J. Petrik, B. Drobna and J. Chovancova (1994). Levels of PCB's and some organochlorine pesticides in human population of selected areas of the Slovak Republic J. Blood. *Chemosphere.*, 29: 2315-2325.

Konaradsen, F., W. Van der Hoek, D.C. Cole, G. Hutchinson, H. Daisley and S. Singh *et al*. (2003). Reducing acute poisoning in developing countries option for restricting the availability of pesticides. *Toxicology.*, 192: 249-261.

Lestremau, F., M.E. Willemin, C. Chatellier, S. Desmots and C. Brochot (2014). Determination of cis-permethrin, trans-permethrin and associated metabolites in rat blood and organs by gas chromatography-ion trap mass spectrometry. *Anal. Bioanal Chem.*, 406 (14): 3477-3487.

Lestremau, F., F.A. Zeman, E. Leclerc, C. Moesch and C. Brochot (2016). Modeling of Cis and transpermethrin isomers and their major urinary metabolities in rats. *Toxicol Appl Pharmacol.*, 249 : 65-77.

Lopez, R., F. Goni, A. Extandia and E. Millan (2007). Determination of organocholirne pesticides and polychlorinated biphenyls in human serum using head space solid – phase micro extraction and gas-chromatography-electron capture detection. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.*, 846 : 298 – 305.

Mancini, F., A.H.C. Van Bruggen, J.L.S. Jiggins, A.C. Ambatipudi and H. Murphy (2005). Acute Pesticide poisoning among female and male cotton grower in India. Int. *J. Occup. Environ Health.*, 11: 221-232.

Mehboob, U., M.A. Azmi, M.A. Azmi and S.N.H. Naqvi (2012). Effect of pesticide residues on health and different enzyme levels in the milk of women from Karachi, Pakistan *J. Basic Appl Sci.*, 8: 444-450.

Moreira, J.P., B.L. Oliveira, C.D. Muzi, C.L. Cunha, S. Brito Ados and R.R. Luiz (2015). Rural workers health in Brazil. *Cad. Saude Publica.*, 31 (8): 1698-1708.

Musshoff , F., H. Junker and B. Madea (2002). Simple determination of 22 organophosphorus pesticides in human blood using headspace solid – phase micro extraction and gas chromatography with mass spectrometric detection. *J. Chromatogr. Sci.*, 40: 29-34.

Ntow, J.W. (2001). Organochlorine pesticides in water, Sediments, Crops and human fluid in a farming community in China. *Arch Environ. Contam. Toxicol.*, 40: 557-563.

Remor, A.P., C.C. Totti, D.A. Mareira, G.P. Dutra, V.D. Heuser and J.M. Boeira (2009). Occupational exposure of farm workers to pesticides: Biochemical parameters and evaluation of genotoxicity. *Environ Int.*, 35 (2): 273-278.

Sharma, A., J.P. Gill and J.S. Bedi (2015). Monitoring of pesticide residues in human blood from Punjab, India. *Bull. Environ Contam Toxicology.*, 94 (5): 640-646.

Sharma, A.K., U. Tiwari, M.S. Gaur and R.K. Tiwari (2016). Assessment of malathion and its effects on leukocytes in human blood samples. J.Biomed Res. 30 (1): 52-59.

Soares, W., R.M.V.R. Almeida and S. Moro (2003). Rural work and risk factor associated with pesticide use in Minas Gerais, Brazil. *Cand. Sande. Publica.*, 19 : 1117-1127.

Van Oostadam, J.C., E. Dewailly, A. Gilman, J.C. Hansen, J.O. Old land and V. Chaschin *et al*. (2004). Circumpolar maternal blood contaminant Survey, 1994-1997 Organochlorine compounds. *Sci Total Environ.*, 330 : 55-70.

Venugopal, N.V.S., B. Sumalatha and S. Bonthula (2013). Spectrophotometric determination of malathion (an organophosphorous insecticide) with potassium bromate. *Eurasian J. Anal Chem.*, 8 (3): 131-135.

Wesseling, C., A. Aragon, L. Castillo, M. Corriols, F. Chaverri and E. de la Cruz (2001). Hazardous pesticides in Central America. *Int J. Occup. Environ Health.*, 7: 287-294.

(*Accepted for publication June 2017*)