

ORGANOCHLORINE PESTICIDE RESIDUES IN HUMAN BLOOD IN THE POPULATION OF MULTAN (PAKISTAN)

MUHAMMAD TAYYAB ANSARI, ZAFAR IQBAL, BASHIR AHMAD

Department of Pharmaceutical Chemistry,
Faculty of Pharmacy, University of Karachi.
Bahauddin Zakariya University,
Multan, Pakistan.

ABSTRACT:

Organochlorine pesticide residues were monitored in samples of human blood (N=32), obtained from concerned volunteers suspecting oral, dermal, or inhalation exposure to Endosulfan and Heptachlor, either environmentally, or occupationally, either recently or in the recent past of Multan Division, (Pakistan) during 1995-96. The pesticide residues were extracted with n-hexane; detection and quantification was performed by gas chromatography (Shimadzu GC 14-A) with a ⁶³Ni-Electron Capture Detector (ECD). Residues of Endosulfan *were* found higher as compared to Heptachlor in all samples. The highest concentration of Endosulfan residues in blood in the population of Multan and Mailsi regions was 90.29 g/kg and 82.14 g/kg, lowest as 58.13 g/kg and 60.13 g/kg while Heptachlor had highest level as 12.978 g/kg and 9.997 g/kg, minimum as 0.37 g/kg and 1.23 g/kg respectively.

INTRODUCTION

Pesticides are phytopharmaceutical agents, those are designed to kill or retard the growth of harming pests (both plants and animals) that interfere with man's comfort, health or economic well-being. Synthetic organic pesticides are used in large quantities to increase food by injuring some form of life (S.D.Murphy, 1986; C.F.Wilkinson, 1990).

The environment pollution by persistent organochlorine residues has received much attention in the past years because of its possible effects on wildlife and human health (Bertram *et al*, 1985; Splinder, 1983). During 1986-1987, blood samples were analysed in Spain for DDE and PCBs and the mean residue levels were as 3.84.0

g/kg and 9.1316.0 g/kg (R. Frank *et al.*, 1993) respectively. Williams *et al.*, (1988) published mean PCB and DDE residues in autopsy adipose tissue of Ontarians collected in 1984 showing levels of 2.11.5 gig and 3.22.6 gig, respectively. In another study residues of PCB and DDE in blood of the Ontario general public were below 10 and 0.2 g/kg respectively (Frank *et al.*, 1988). Sahl *et al.*, (1985) reported that PCB levels in blood plasma was 54 ppb. This level was considered to be the level expected from exposure to PCBs in the general environment. The mean residue of PCBs was reported in the Niagara region as 13.0 g/kg and in the Durham region at 6.2 g/kg (R. Frank *et al.*, 1993).

The widespread use of organochlorine compounds as insecticide during last decade has led to their ubiquitous presence in the environment. In Pakistan Endosulfan and Heptachlor are used extensively to meet the targets of crop yields. The organochlorine compounds are persistent lipophilic pollutants. Due to their widespread use and environmental stability, the Endosulfan and Heptachlor are the more ubiquitous pollutants in the eco-system.

The volunteers were selected using very narrow limits for the parameters of age, weight, diet, and smoking habits etc. so that the variations in the mean OCPs levels of the different groups could only be attributed to the environmental conditions of the areas selected for this study, as designed by C. Conde *et al.*, (1993).

The present study was conducted to investigate the levels of organochlorine residues with special concern to Endosulfan and Heptachlor in human blood from Multan region as well as agrarian area i.e. Mailsi, located at 85 Km in east from Multan.

MATERIAL AND METHODS

Blood samples were obtained from concerned volunteers (N=35) suspecting oral, dermal or inhalation exposure to Endosulfan and Heptachlor either occupationally or environmentally either recently or in the recent past. Blood samples were categorized into various categories, i.e. sex, origin and living in two different geographical areas i.e. Multan and Mailsi (as described in Tables 1 and 2), during 1995-1996.

Blood samples were collected into fresh test tubes (stoppered). All samples were refrigerated immediately at 5°C and the refrigeration was maintained until the start of clean up. After careful collection of blood samples from volunteers, clean up

process was performed within 12 hours of collection and fractioned on Florisil as described Holdrinet (1974).

CHEMICALS :

n-Hexane, Diethyl ether, Benzene, Methanol, Florisil.

All the solvents used were of pesticide grade throughout the analysis procedure.

ANALYTICAL PROCEDURE :

Blood samples were analyzed for Endosulfan and Heptachlor by using packed column gas chromatography (Shimadzu GC 14-A) with a ^{63}Ni Electron Capture Detector (ECD) operated at 290 C . The analytical column was maintained at 250 C. The carrier gas was oxygen (O₂) free nitrogen (N₂) at a flow rate of 50 ml/min. Determination of Endosulfan and Heptachlor was based in comparison with a standard reference solution of Endosulfan and Heptachlor. The levels of residue are reported as g/kg extracted blood.

RESULTS AND DISCUSS

Thirty five blood samples from volunteers were analyzed. Volunteers were inquired to get information regarding dietary habits, socio-economic conditions as well as geographical characteristics, as summarized in Table 1. Where Table 2, summarizes the mean age, sampling period (1995-96), and male/female ratio of the volunteers under study.

Table 3, illustrates the results about concentrations of Endosulfan and Heptachlor residues which were found in all the samples studied (N=35). The two populations showed a very similar pattern; Endosulfan was present in higher concentrations as compared to Heptachlor residues in all samples, the maximum Endosulfan residues in blood plasma taken from different kind of population of Multan Division and Mailsi (85 km from Multan) were 90.29 g/kg and 82.14 g/kg and minimum level as 58.38 g/kg and 60.13 g/kg respectively (Table 3). After studying various other factors in studied areas it is assumed that the higher concentrations of Endosulfan residues in human blood are due to following factors:

1:- massive use of Endosulfan and other OCPs since last couple of decades.

2:- the several potential sources i.e. a) impurity of other pesticides b) the direct use of Endosulfan and Heptachlor as pesticides (J.Gomez-Catalan *et al.*, 1993) c) lack of technical personnel and equipment for monitoring network of

TABLE 1
GEOGRAPHICAL AND SOCIO-ECONOMIC CHARACTERISTICS
OF THE POPULATIONS STUDIED.

ORIGIN	RESIDENTS	LOCATION	MAIN ECONOMIC ACTIVITIES
MULTAN	10	Urban	Agro Services.
	10	B.Z.U*.	Services, Students.
SUB-URB.	5	Rural	Agriculture.
MAILSI**	5	Urban	Agrarians, Pesticide-business.
	5	Rural	Agriculture.

(*) Bahauddin ZAKARIYA University

(**) Distt. Vehari, Division Multan.

TABLE 2
SAMPLES: ORIGIN, NUMBER (N), SAMPLING PERIOD (SP), MEAN AGE OF
VOLUNTEERS S.D. (AGE) AND MALE /FEMALE RATIO (M / F).

ORIGIN	N	S.P.	AGE	M/F
MULTAN	25	1995-1996	24±5	20 / 5
MAILSI	10	1995-1996	38±6	5 / 5

(*) DISTT. VEHARI, DIVISION MULTAN.

TABLE 3
MEAN RESIDUES OF ENDOSULFAN AND HEPTACHLOR IN WHOLE
BLOOD FROM RESIDENTS OF MULTAN DIVISION 1995-96

ORIGIN	NUMBER	ENDOSULFAN		HEPTACHLOR (ug/kg)		
		Mean $\pm$ SD	Highest-	Lowest- Mean $\pm$ SD	Highest	Lowest
MULTAN	25	62.5 $\pm$	90.29	58.38	2.35 $\pm$	12.9780.37
			11.36			2.95
MAILSI	10	61.4 $\pm$	82.14	60.13	1.68 $\pm$	9.997 1.23
		11.29			3.23	

TABLE : 4
MEAN RESIDUES OF ENDOSULFAN AND HEPTACHLOR IN
WHOLE BLOOD FROM URBAN AND RURAL AREAS OF MULTAN
DIVISION (1995-1996) [ug/kg $\pm$ SD].

ORIGIN	NO	ENDOSULFAN (ug/kg)	EPTACHLOR (ug/kg)
MULTAN URBAN	10	60.50 $\pm$ 9.08	0.34 $\pm$ 2.33
RURAL	15	72.05 $\pm$ 8.08	0.44 $\pm$ 0.33
MAILSI URBAN	5	62.4 1 $\pm$ 2.39	0.73 $\pm$ 2.37
RURAL	5	72.4 1 $\pm$ 1.34	0.37 $\pm$ 1.22

pesticide residues and effects d) illiteracy, poverty, and lower sanitation and medicare standards result in higher health risks during occupational, and long-term exposure to pesticide and their residues e) lack of real choice when they depend on foreign aid for their pest control agents.

TABLE 5
RESIDUES OF ORGANOCHLORINE PESTICIDES IN MALE AND FEMALE
POPULATIONS (ug/kg ext. blood).

OCPs	GM		N	
	MALE	FEMALE	MALE	FEMALE
ENDOSULFAN	63.54	64.31	25	10
HEPTACHLOR	1.74	1.64	25	10

3: The population of Multan Division is domestic cattle flesh oriented, impetuous use of dairy products, because those cattle are fed from the same cultivated lands, where Endosulfan and Heptachlor has been used. Due to the accumulative properties of organochlorine chemicals in the soil, the residues of OCPs are transformed to the cattle feeds and ultimately to the human.

The turnover in generations of animal making up the food basket is rapid i.e. 16 Weeks for poultry, one to two years for beef, hence giving a rapid cleansing of food system, the turnover in generations of human is slow (R. Frank *et al.*, 1993).

Measurements have shown that organochlorine pesticide (OCP) levels in blood and milk fat are proportional with blood levels of approximately 1/10 (Kodama and Ota, 1980) to 1/20 (Mes *et al.*, 1984) of those found in milk. Our values cannot be compared to those reported by other authors (Baluja *et al.*, 1982; Gomez *et al.*, 1991) because of high difference.

The highest level of Endosulfan (90.29 g/kg) was found in the population of Multan region and higher mean level in the rural area was 72.40 11.34 g/kg that is

remarkable (Table 4). Mean residual level of Endosulfan and Heptachlor of Multan and Mailsi was 62.511.36 and 61.411.29 g/kg while Heptachlor was 2.35 2.95 and 1.68 3.32 g/kg respectively. The residual level of organochlorines in human blood in Multan and Mailsi are much higher than those from USA (Sahl *et al.*, 1985) and Canada (R. Frank *et al.*, 1993; Williams *et al.*, 1988; Frank *et al.*, 1988).

Concentration of Endosulfan and Heptachlor was found more in rural areas than urban areas (Table 4). This is an agreement with other countries reports i.e. higher levels of organochlorine residues on agrarian populations than in the general ones and have been attributed to a heavier labour and environmental exposure (Greer *et al.*, 1980). Special incidence in agrarian areas could be indicative of a pesticide - use - linked origin.

Residue levels in male and female population (Table 5) did not show any statistically significant difference. This result is in accordance with those of M.Camps *et al.*, 1989; J. Gomez-Catalan *et al.*, 1993; however, some other suggest the existence of some sex-linked factor in the accumulation of organochlorine residues (Ferrer *et al.*, 1992).

The residue level of Heptachlor in human blood in the population of Multan is much lower than Endosulfan due to its limited use. Heptachlor residue level is higher (12.978 g/kg) in the population of Multan than Mailsi (9.997 g/kg) in whole blood. So the residue level of Heptachlor should also be monitored time to time.

Concentration of Endosulfan residues in human blood in the population of Multan Division is *very* alarming and big health risk as various toxic and deleterious effects may be occurred as are reported by different authors such as liver/body ratio imbalance, organochlorine pesticides stimulate xenobiotic metabolizing enzymes, the scattering of ribosomes can be an indication of cytotoxicity of a reversible nature (Arvind, K. Chutervedi, 1994), and decrease in sperm count, so, the presence of organochlorines can affect reproductive success (Michael D. 1996; Addison 1989; Casillas *et al.*, 1991; Elliot *et al.*, 1988; Westernhagen *et al.*, 1987), lower birth weights and smaller head circumference (Fein *et al.*, 1984), Parkinson's disease (Fleming, L. *et al.*, 1994), breast/liver carcinoma (Falck *et al.*, 1992).

Results obtained will form part of an up-to-date report on Endosulfan and Heptachlor pollution in Multan and Mailsi regions including all kinds of populations of different geographical and socio-economic characteristics, that will make it possible to identify the sources and trends of this contamination.

From these reports, it is concluded that the higher mean residue level of Endosulfan is being dragged the whole population towards many health risks, so for the time being, the international restrictions for the use of Endosulfan and other organochlorine pesticides (OCPs) should be observed in Pakistan from time to time rather agrarian areas in special and this study should be extended to substantiate the results.

ACKNOWLEDGEMENT

We are grateful to our volunteers and concerning people who co-operated us during this study, especially Dr. Waqar Ahmed (Late) Scientific Officer PCSIR Lab. Lahore.

REFERENCES

- Addison, R.F. (1989). Organochlorines and Marine mammals reproduction. *Can. J. Fish Sci.*, **46**: 360-368.
- Arvind, K.C. (1993). Toxicological evaluation of mixture of Ten widely used pesticides. *J. App. Toxicol.*, **13**(3): 183-188.
- Baluja, G., Hernandez, L.M., Gonzalez, M.S., Rico, M.C., (1982). Presence of organochlorine pesticides, polychlorinated biphenyls and mercury in Spanish human milk samples. *Bull. Environ. Contain. Toxicol.*, **28**: 573-577.
- Bertram, H.P., Kemper, F.H., Zenzen. C. (1985). Man -- a target of ecotoxicological influences. In: Nurnberg HW (ed) pollutants and their Ecotoxicological Significance. John Wiley, Chichester., pp: 415.
- Camps, M., Planas, N., Gomez-Catalan, J., Sabroso, M., TO-Figueras, J., and Corbella, J. (1989). Organochlorine residues in human adipose tissue in Spain: Study of an agrarian area. *Bull. Environ. Contam. Toxicol.*, **4**: 195-201.
- Casillas, E., Misitano, D., Johnson, L.L., Rhodes, L.D., Coolier, T.K., Stein, J.E., McCain, B.B. and Varanasi, U. (1991). Inducibility of spawning and reproductive success of female English sole (*parophrys vetulus*) from urban and non-urban areas of Puget Sound, Washington. *Mar. Environ. Res.*, **31**: 99-122.
- Conde, C., Maluenda, C. and Arrabal, C. (1993). Organochlorine Residues in Human Milk in Spain. Polychlorinated Biphenyls (PCBs) from 1988-1991, *Bul./Environ. Contam. Toxicol.* **51**: 832-837.
- Elliott, J.E., Norstrom, R.J., Keith, J.A. (1988). Organochlorines and egg shell thinning in Northern Gannets (*Sula hassanus*) from Eastern Canada 1968-1984. *Environ. Pollut.*, **52**: 81-102.

- Falck, F., Ricci, A., Wolff, M.S., Godbold, J. and Deckers, P. (1992). Pesticides and polychlorinated biphenyl residues in human breast lipids and their relation to breast cancer. *Arch. Environ. Nish.*, **47**(2): 143-146.
- Fein, G-G., Jacobson, J.L., Jacobson, S.W., Schwartz, P.W. and Dowler, J.K. (1984). Prenatal exposure to polychlorinated biphenyls: Effects on birth size and gestational age. *J. Pediatr.*, **105**: 315-320.
- Ferrer, A., Bona, M.A., Castellano, M., TO-Figueras, J. and Brunet, M. (1992). Organochlorine residues in human adipose Tissue of the population of Zaragoza (Spain). *Bull. Environ. Contam. Toxicol.*, **4**: 561-566.
- Fleming, L., Mann, J.B., Briggles, T. and Sanchez-Ramos, J.R. (1994). Parkinson's disease and brain levels of organochlorine pesticides. *Ann. Neural.*, **36**(1): 100-103.
- Frank, R., Braun, H.E. and Thrope, B. (1993). Comparison of DDE and PCB Residues in the General Diet and in Human Blood Ontario 1986-1987. *Bull. Environ. Contam. Toxicol.*, **51**: 146-152.
- Frank, R., Rasper, J., Smouth, M.S., Braun, H.E. (1988). Organochlorine residues adipose tissues, blood and milk from Ontario residents. *Can. J. Pub. Hlth.*, **79**: 150-158.
- Gomez-catalan, J., Planas, J., To-figueras, J., Camps, M. and Corbella, J. (1993). Organochlorine pesticide residues in the population of Catalonia (Spain). *Bull. Environ. Contam. Toxicol.*, **51**: 160-164.
- Gomez-catalan, J., Sabroso, M., To-figueras, J., Planas, J. and Corbella, J. (1991). PCBs residues in the adipose tissue of the population of Barcelona (Spain). *Bull. Environ. Contam. Toxicol.*, **47**: 504-507.
- Greer, E.S., Miller, D.J., Bruscato, F.N. and Holt, R.L. (1980). Investigation of pesticide residues in human adipose tissue in the Northeast Louisiana area. *J. Agric. Food Chem.*, **28**: 76-78.
- Holdrinet, M.V.H. (1974). Determination and confirmation of Hexachlorobenzene in fatty samples in the presence of other residual halogenated hydrocarbon pesticides and polychlorinated biphenyls. *J. Asso. Off Anal. Chem.*, **57**: 580-584.
- Kodama, H. and Ota, H. (1980). Transfer of polychlorinated biphenyls in infants from their mothers. *Arch. Environ. Hlth.*, **35**: 95-100.
- Mes, J., Doyle, J.A., Adams, B.R., Davies, D.J. and Turton, D. (1984). Polychlorinated biphenyls and organochlorine pesticides in milk and blood of Canadian women during lactation. *Arch. Environ. Contam. toxicol.*, **13**: 217-223.
- Michael, D. (1996). What is wrong with our sperms. *Time*. March **18**: 42-43.
- Murphy, S.D. (1986). Toxic effects of pesticides. In Casarett and Doull's

- Toxicology: The Basic Science of Poisons, 3rd Edn, ed. by C.D. Klaassen, M.O. Amdur and J. Doull, pp.519-581. Macmillan Publishing, New York.
- Sahl, J.D., Crocker, T.T., Gordon, R.J. and Rieder, E.J. (1985). Polychlorinated biphenyl concentrations in blood plasma of a selected sample of non-occupationally exposed southern California working adults. *Sci. Toxicol. Environ.*, **4**: 9-18.
- Splinder, M. (1983). DDT: Health aspects in relation to man and risk/benefit assessment based there upon. *Residue Rev.*, **90**: 1-34.
- Westernhagen, H.V., Sperling, K.R., Janssen, D., Dethlefsen, V., Cameron, P., Kocan, It, Landold, M., Furstenverg, G. and Kremling, K. (1987). Anthropogenic contaminants and reproduction in marine fish. *Der. Biol. Anstalt. Helgoland.*, **3**: 70pp.
- Wilkinson, C.F. (1990). Introduction and overview. In *The Effects of Pesticides on Human Health*, ed. by S.R. Baker and C.F. Wilkinson, pp. 5-33. Princeton Scientific Publishing, Princeton, NJ.
- Williams, D.T; LeBel, G.L. and Junkins, E. (1988). Organochlorine residues in human adipose autopsy samples from six Ontario municipalities. *Assoc. Off Anal. Chem.*, **71**: 410-414.