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Organochlorine Pesticide Residues in Human Blood Samples Collected from Haryana, India and the Changing Pattern

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Abstract Blood samples were collected during August 1992 and August 2002 from various hospitals of Haryana state and analyzed for the presence of HCH and DDT residues and the change in concentration of pesticide residues was calculated in terms of % reduction. The study revealed that the mean residue levels of total HCH in human blood samples have declined by 87.6 % while those of total DDT have decreased by 98.9 % during a gap of 10 years. The obtained results reveal that during 1992 p,p′-DDT was the major component with the mean value of 6.125 mg/L followed by p,p′-DDE, γ-HCH, α-HCH and β-HCH while in 2002, β-HCH and p,p′-DDE were comparable with mean value of 0.053 and 0.052 mg/L, respectively followed by p,p′-DDT, α-HCH and p,p′-DDD.

Keywords Organochlorines · Pesticide residues · Human blood · DDT metabolites · HCH isomers · % reduction

With the advent of green revolution in mid sixties resulting from introduction of high yielding varieties, new pest problems have arisen. In the recent past, different types of pesticides came into evolution to kill various pests. These pesticides remain persistent in soil and water bodies and get incorporated in food chain, make their entry into the human body and get deposited in body fat. DDT was identified as one of the 12 POPs during the Stockholm Convention on persistent organic pollutants (POPs) in 2001. The use of pesticides to prevent losses by pest in agriculture or in sanitation was started in India in 1948–1949. India banned the agricultural use of DDT in 1989 (Anonymous 1991) with a mandate to use a maximum of 10,000 tons of DDT per year for the control of malaria and kala-azar (Dash et al. 2007).

Pesticides being persistent in nature get accumulated in various components of the environment like soil (Yadav et al. 1981), water (Agarwal et al. 1986), air (Kaushik et al. 1987). The principal source of human exposure in tropical countries is inhaled vapors (Jury et al. 1982) followed by the consumption of contaminated food of animal origin such as milk and meat (Waliszewski et al. 1997). The remarkable biological persistence combined with great lipophilicity leads to accumulation of the pesticide residues in food chain and in human tissues (Gallelli et al. 1995). Pesticides affect various systems of the body such as nervous, renal, gastro intestinal and haemopoietic system (Ray 1992) and also result in chromosomal aberration (Sharma and Sobti 1988). The environmental impact of chemicals has become a matter of great concern and the broad spectrum synthetic insecticides which were commonly used are now being phased out. Restriction on the use and banning of some of the OCP compounds have resulted in reduction of their residues in the environment (Weisenberg et al. 1985). Serum is considered as a suitable matrix since it is quite homogenous and does not readily coagulate during freezing (Atuma and Aune 1999). The previous

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work showed significant presence of pesticides in the human serum of rural population as compared to urban area due to intensive use of pesticides (Rugama et al. 1993). The present study was undertaken in order to determine the status of pesticide residues in human blood from Haryana and the changing pattern of various organochlorine pesticide residues calculated in terms of % reduction in a decade.

Materials and Methods

Blood samples were collected randomly from the pathology lab of Post Graduate Institute of Medical Sciences, Rohtak and from Blood Bank of Hisar in Haryana. Blood samples were collected by venipuncture in 25 mL vials without any anticoagulant. The age of volunteers ranged from 10 to 80 years. For separation of plasma the sample vials were placed in Styrofoam container with wet ice. In the laboratory blood samples were routinely spun down at 2,000 rpm and the serum was collected in separate tubes kept in freezer. Two mL of blood sample was taken and vortexed for about 20 min till RBCs were separated. To the above clear serum, 6 mL of hexane was added and vortexed. Emulsion when formed was separated by centrifugation and few drops of saturated sodium sulfate solution. Top hexane layer was taken out and evaporated to dryness on rotary evaporator. The final volume was made to 1 mL of hexane before analyzing on GLC.

The used stock standards of HCH and its isomers were obtained from Environmental Protection Agency (EPA), while the standards of DDT and its metabolites, aldrin and dieldrin were obtained from the department of entomology, CCS Haryana Agriculture University, Hisar and Central Pollution Control Board, Delhi. Dried pure standards of α and β-endosulfan were gifted by C.C. Shroff Research Institute, Mumbai. Stock standards (100 ppm) for α- and β-endosulfan were prepared by dissolving pure pesticides standards in pesticide grade n-Hexane separately in volumetric flask. The standards were stored in dark in the refrigerator. Working standards of the pesticides of 1–2 ppm were prepared from the stock solutions by diluting with hexane using micropipette. Analysis of pesticide residues was carried out on a Chemito series 2,865; microprocessor controlled gas chromatograph equipped with ECD having Nickel (63Ni) foil as electron source. The IOLAR Grade-O nitrogen gas of ultra purity (SMS Multitech India Pvt. Ltd., Delhi, India) was used as the carrier gas. The glass column (2 m length, 6.25 mm (1/4″) diameter) packing is 1.5 % OV-17/1.95 % QF-1 on chromosorb WHP, 80/100 mesh size (Chemito 2,865 column). The glass column was conditioned for 24 h by keeping the oven temperature 15–20°C higher than the operating column temperature. An aliquot (2–5 μL) of the sample extract in the known quantity of hexane was loaded on the column with micro syringe and peaks appearing in the chromatogram so obtained were identified by comparing their retention times with that of the known standards.

Results and Discussion

Forty-nine samples were analyzed for the presence of DDT (p,p′-DDT, p,p′-DDD, p,p′-DDE) and HCH residues (α-HCH, β-HCH, γ-HCH) in Haryana in August 1992 and thirty serum samples collected from Haryana during August 2002 were analyzed for DDT and its metabolites (o,p′-DDT, p,p′-DDT, o,p′-DDE, p,p′-DDE, o,p′-DDD, p,p′-DDD), isomers of HCH (α-HCH, β-HCH, γ-HCH), α-endo, β-endo, aldrin and dieldrin. o,p′-DDE, o,p′-DDD, aldrin and α-endosulfan could not be detected in any of the 30 serum samples. The concentration of pesticide residues obtained are expressed in ppm i.e. μg/mL. None of the DDT isomers were detected in 8 samples out of 30 samples in 2002. Results obtained in 1992 indicated that except p,p′-DDD all the DDT metabolites and HCH isomers were detected in the samples. ΣHCH and ΣDDT were calculated on the basis of sum of their respective metabolites and isomers.

The range (min–max), arithmetic mean ± SD, mean of positive samples ± SD values of various pesticide residues observed on wet weight basis (μg/mL) collected from various areas of Haryana in August 2002 are shown in Table 1. Table 2 shows the changing pattern of OCP residues in human blood samples collected from Haryana in a decade. The minimum quantity which could be detected by the used method were 0.5 ng for α-HCH and p,p′-DDD, 0.4 ng for γ-HCH, 0.6 ng for β-HCH, 0.8 ng for δ-HCH, 0.04 ng for aldrin, 0.14 ng for dieldrin, 0.3 ng for α-endosulfan, 0.4 ng for β-endosulfan, 0.7 ng for o,p′-DDE, o,p′-DDT and p,p′-DDT, 0.4 ng for p,p′-DDE. About 10–15 % samples on each occasion were analyzed for percent recoveries. For all the pesticides following the above mentioned method the percent recoveries were more than 80 %.

The results obtained showed that during 1992, among the DDT isomers p,p′-DDT was the major component followed by p,p′-DDE with the mean value of 6.125 and 0.32 μg/mL, respectively. The mean residue level of DDT metabolites, reported in 2002 were in the order of p,p′-DDE > p,p′-DDT > p,p′-DDD > o,p′-DDT having the mean values of 0.0524, 0.0122, 0.0078, 0.0031 μg/mL, respectively as shown in Table 1. The mean values of p,p′-DDE was quite higher compare to other metabolites. It may be due to persistence or non reactive nature of this isomer. The total mean value of p,p′-DDE, observed in 2002

Table 1 Concentration (μg/mL) of OCP residues in human blood samples collected from Haryana in 2002

Pesticide residues	o,p′-DDT	p,p′-DDT	p,p′-DDE	p,p′-DDD	ΣDDT	α-HCH	β-HCH	γ-HCH	ΣHCH
Range	ND-0.0787	ND-0.0866	ND-0.0329	ND-0.1186	ND-0.329	ND-0.119	ND-0.242	ND-0.055	ND-0.242
Arithmetic mean ± SD	0.0031 ± 0.014	0.0122 ± 0.0280	0.0524 ± 0.082	0.0078 ± 0.022	0.069 ± 0.084	0.008 ± 0.02	0.053 ± 0.065	0.0041 ± 0.011	0.066 ± 0.074
Mean of positive samples ± SD	0.0239 ± 0.036	0.0366 ± 0.0392	0.074 ± 0.090	0.0196 ± 0.033	0.090 ± 0.085	0.030 ± 0.042	0.072 ± 0.067	0.017 ± 0.018	0.080 ± 0.074

survey, was 0.0524 μg/mL which was lower than the average value of 0.32 mg/L, observed in 1992 survey but higher than 0.0270 μg/mL as reported by Chand et al. (1992) from Jaipur (Rajasthan) and 44.42 ppb, reported by Dua et al. (1996) from Haridwar but lower than 0.083 μg/mL reported in maternal blood by Sharma and Bhatnagar (Sharma and Bhanagar 1996) from Jaipur (Rajasthan). The mean value of p,p′-DDT was 0.0122 μg/mL which was quite lower than 6.125 mg/L obtained in 1992 survey but higher than 9.24 ppb as reported by Dua et al. (1996) from Haridwar. The mean value of p,p′-DDD was 0.0078 μg/mL which was found to be absent in 1992 survey. The mean value of o,p′-DDT was 0.0031 μg/mL which is higher than 1.48 ppb as reported by Dua et al. (1996) from Haridwar, but very less than 0.07 mg/L as reported by Ramchandran et al. (1984). The o,p′-DDT was estimated to be below detection limit.

Among the DDT metabolites, the comparison of two studies revealed that p,p′-DDT showed maximum per cent reduction of 99.9 % with 97.9 % frequency of occurrence in 1992 while the frequency of p,p′-DDT in 2002 survey was 30 %. p,p′-DDE showed per cent reduction of 83.63 % with 97.90 % frequency of occurrence in 1992 while the frequency in 2002 was reduced to 70 % (Table 2). p,p′-DDD was found to be absent in 1992 while it's frequency of estimated occurrence in 2002 was 40 %.

The mean value of ΣDDT registered in 1992 was 6.445 μg/mL which was quite higher than 0.069 μg/mL observed in 2002. The mean value of ΣDDT of 0.069 μg/mL in 2002 was lower than 120 ppb as reported by Sharma and Bhatnagar (Sharma and Bhanagar 1996) from Jaipur, Rajasthan but higher than the level of 58.43 ppb as reported by Dua et al. (1996) from Haridwar. The comparison of two studies shown in Table 2 revealed that the mean residues of total DDT have declined by 98.93 % during a gap of 10 year. In 1992 survey, γ-HCH contributed the maximum with the mean value of 0.293 mg/L followed by δ-HCH, α-HCH and β-HCH with the mean value of 0.251, 0.201 and 0.039 mg/L, respectively. The levels of HCH in 2002, were found in order of β-HCH > α-HCH > γ-HCH with a mean value of 0.053, 0.008, 0.004 μg/mL, respectively. The major contaminant was β-HCH having the mean value 0.053 μg/mL which is similar to the level reported by Pauwels et al. (2000) but higher than 33.08 ppb reported by Dua et al. (1996) from Haridwar. The mean value of 0.008 μg/mL of α-HCH was also very less as compared to 24.05 ppb, reported by Dua et al. (1996) from Haridwar. The mean value of 0.004 μg/mL of γ-HCH was also lower than the mean value of 8.5 ppb as reported by Dua et al. (1996) from Haridwar. The mean total of ΣHCH was 0.066 μg/mL which is quite lower than the mean value of 245.6 ppb reported by Sharma and Bhatnagar (Sharma and Bhanagar 1996) from

Table 2 Changing pattern of OCP residues (μg/mL) in human blood samples collected during August 1992 and August 2002 from Haryana

Sr. no.	Pesticide	Range, mean ± SD		% reduction	% samples exceeding MRL values		Frequency of detection (%)	
		1992 (n = 49)	2002 (n = 30)		1992	2002	1992	2002
1	α-HCH	ND-0.671	ND-0.119					
		0.204 ± 0.205	0.008 ± 0.02	96.08	63.26	03	63.26	26.6
2	β-HCH	ND-0.103	ND-0.242					
		0.039 ± 0.0366	0.053 ± 0.065	ND	65.3	50	59.18	73.3
3	γ-HCH	ND-0.699	ND-0.0240					
		0.293 ± 0.220	0.0041 ± 0.011	98.61	75.5	10	75.5	23.3
4	ΣHCH	ND-2.247	ND-0.242					
		0.536 ± 0.431	0.066 ± 0.074	87.69	75.5	20	75.5	80.0
5	p,p′-DDT	ND-12.052	ND-0.0921					
		6.125 ± 3.424	0.0122 ± 0.0280	99.90			97.9	30
6	p,p′-DDE	ND-0.711	ND-0.328					
		0.32 ± 0.169	0.0524 ± 0.082	83.63			97.9	70
7	p,p′-DDD	BDL	ND-0.1186					
		BDL	0.0078 ± 0.022	ND			BDL	40
8	ΣDDT	ND-120.052	ND-0.328					
		6.445 ± 0.338	0.069 ± 0.084	98.93	97.9	40	97.9	3.3

n number of blood samples

Jaipur (Rajasthan). However, the value is higher than 0.0125 μg/mL reported by Bikram Chand (Chand et al. 1992) from Jaipur (Rajasthan). Also the mean total of ΣHCH registered in 1992 was 0.536 μg/mL which was quite high compared to that obtained in 2002. The comparison of two studies shown in Table 2 reveals that mean residues of ΣHCH have declined by 87.69 % during a gap of 10 year.

α-Endosulfan and aldrin were found to be absent in all the 30 samples in 2002. The total mean value of β-endosulfan in serum was 0.0143 g/mL and the mean value of dieldrin was 0.0011 μg/mL. It may be due to more persistence of dieldrin and β-endosulfan in environment. Figure 1 shows the total pesticide residues found in human blood samples from Haryana in 2002.

The figure reveals that the major contribution towards the pesticide contamination is from DDT isomers with mean DDT concentration of 0.069 ± 0.084 μg/mL. The second major contaminant with almost comparable

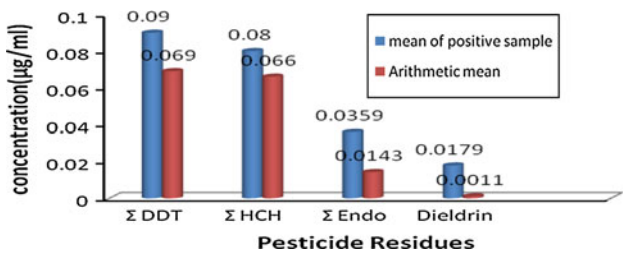

Fig. 1 Levels of organochlorine pesticide residues estimated in human blood samples collected from various area of Haryana in 2002

concentration of 0.066 ± 0.074 μg/mL is total HCH followed by endosulfan and dieldrin with the mean value of 0.0143 ± 0.046 μg/mL and 0.0011 ± 0.004 μg/mL, respectively. The higher contribution of HCH and DDT is due to β-HCH and p,p′-DDE in which p,p′-DDE contributed more than 75 % of total DDT and β-HCH contributed more than 60 % of total HCH. The latter is known to possess highest chronic mammalian toxicity of all the isomers of HCH (Battu et al. 1989).

Table 2 shows that there is reduction in percentage of OCP residue concentration during 10 years of gap except β-HCH and p,p′-DDD. The high frequency (73 %) among all the HCH isomer, no reduction and significant contribution (63 %) of β-HCH in 2002 blood indicates widespread contamination of human blood by this isomer. The β isomer of HCH is least reactive and maximum persistent out of the HCH isomers (Wang et al. 2003), eliminated more slowly from body than lindane (Pfeilsticker 1973) and has a longer half life in fatty components (Kutz et al. 1991). Among HCH isomers, maximum reduction of 98.61 % was recorded for γ-HCH followed by 96.08 % for α-HCH, and no reduction for β-HCH. Among the DDT isomers, maximum reduction of 99.9 % was observed in p,p′-DDT, followed by 83.63 % for p,p′-DDE and no reduction of p,p′ DDD (Fig. 2).

In 1992 study, 75.5 % of samples exceeded the maximum residue limit (MRL) of 0.10 mg/kg of ΣHCH as recommended by WHO (1973), 63.26 % samples of 0.05 mg/kg for α-HCH, 75.5 % samples of 0.01 mg/kg for γ-HCH, 65.3 % samples of 0.02 mg/kg for β-HCH as

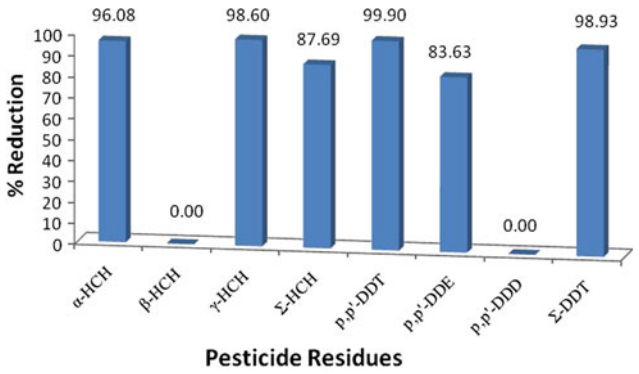

Fig. 2 Reduction of HCH and DDT residues in human blood samples collected during August 1992 and August 2002 from Haryana

recommended by PFAA (1954). The percentage of blood samples exceeding MRL in 2002 study reduced to 3, 50, 10 and 20 % in case of α-HCH, β-HCH, γ-HCH and ΣHCH, respectively (Table 2). 97.9 % of samples exceeded the MRL of 0.05 mg/kg of ΣDDT (FAO 1986) in 1992 while in 2002 it got reduced to 40 % as shown in Table 2.

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