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Occurrence of DDT and HCH Insecticide Residues in Human Biopsy Adipose Tissues in Punjab, India

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The use of organochlorine pesticides (OCPs) like dichlorodiphenyl trichloroethane (DDT) and hexachlorocyclohexanae (HCH) has either been banned or restricted in agriculture in India but are still being used in public health programmes for the control of vector borne diseases such as malaria, filaria and kala azar etc. In view of the classical stability and persistency of organochlorine pesticides in environment and biological media, their residues through food chain enter the human body. The principal route of exposure is the consumption of food particularly leafy and root vegetables, fatty meat, fish and poultry. Monitoring studies done in India have revealed wide spread contamination of fruits, vegetables, milk, meat and their products with pesticide residues (Kang et al. 2000; Pandit et al. 2002; Bedi et al. 2005).

Indiscriminate use of OCPs in India till the 1990's resulted in widespread contamination of the ecosystem and therefore the potential of possible health hazards. Epidemiological studies on the association between exposure to OCPs and health hazards have been reviewed extensively and have been investigated as a potential risk factor for liver cancer, breast cancer, prostrate and testicular cancer (Ekbom et al. 1996), dysfunction of immune and endocrine systems, birth defects and lungs damage (Mansour 2004). The concentrations determined in adipose tissue of human populations are the best indices for estimating the extent of exposure to these pesticides and risk evaluation. The purpose of the present study was to determine the levels of DDT and HCH in adipose tissues of the general population in Punjab, India.

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Materials and Methods

Fifty-five human adipose tissue samples from surgical patients were collected from Dayanand Medical College and Hospital Ludhiana. The samples were transported to the laboratory in glass vials under chilled conditions, then frozen at –20°C till processing. A questionnaire was completed by each patient aiming to relate the possible residue levels of OCPs with epidemiological data. Age, sex, food habits, background (rural/urban), body mass index and cause for surgical procedure were recorded. Subjects with a history of occupational or accidental exposure to pesticides were excluded.

All the chemicals were of analytical grade and were obtained from M/s E. Merck (India) Ltd. The solvents were glass distilled before use. The suitability of the reagents/solvents was evaluated by utilizing blanks. The standards for DDT and its metabolites, HCH and its isomers (all >95% purity) were obtained from M/s Dr. Ehrenstorfer GmbH, Augsberg, Germany and were used as reference standards.

The organochlorine pesticide residues from the human fat samples were extracted and purified by as per the method of Nair and Pillai (1992) with modifications. Briefly, five grams of each fat sample were macerated with anhydrous sodium sulfate and Soxhlet extracted with hexane: acetone (1:1) mixture. The purification of residues was done by liquid–liquid partitioning. The human fat extract in hexane acetone mixture was dried and first separated in acetonitrile followed by hexane.

The residues of pesticides in the purified extract were quantified on a Nucon Gas Chromatograph (Model 5700) equipped with an electron capture detector (ECD, Ni^{63}) and a glass column of 2 m length and 3 mm internal diameter filled with 1.5% OV-17 + 1.95% OV-210 with solid support of chromosorb WHP 80-100 mesh. Nitrogen was used as carrier gas at flow rate of 1.5 Kg/cm^2 Psig. The injector, column and detector temperatures were maintained at 220, 210 and 240°C, respectively. Identification from retention times and quantification using external standards allow the determination of the α-HCH, β-HCH, γ-HCH, δ-HCH, op DDT, pp DDT, op DDE, pp DDE, op DDD and pp DDD. Confirmations of results were accomplished on an alternate glass column (2 m × 3 mm i.d.) packed with 3% DEGS coated on 80-100-mesh chromosorb WHP.

Recovery tests were performed to check the efficiency of the residue analysis method for the estimation of pesticide residues in the substrate analyzed during the study. Spiking was done at two levels, i.e. 0.5 and 1.0 mg kg^{-1}. The detection limit for DDT (metabolites) and HCH (isomers) was recorded as 0.01 mg kg^{-1} and average recoveries obtained were between 84% and 92%. Along with the spiked samples, matrix blanks and reagent blanks were also processed so as to estimate the background level and to find interference if any, from the substrate or from reagents. The results did not include recovery corrections. Mean values of organochlorine pesticides were calculated using basic statistics. To compare variability among residue levels and age, Pearson correlation coefficient was calculated. To analyze the presence of residues in relationship with the urban/rural population and food habbits, the chi-squared coefficient was applied using the SPSS statistical package.

Results and Discussion

DDT was found in all the 55 samples with a frequency occurrence of 100% and a mean concentration of 6.86 mg kg^{-1} while HCH was detected in 46 samples with a frequency occurrence of 83.6% and a mean concentration of 5.72 mg kg^{-1} (Table 1). Distribution of DDT and HCH residues based on sex, food habits, occupation, obesity and urban/rural population did not clearly indicate any pattern.

However, age wise distribution revealed that the mean levels of HCH and DDT increase with age, with maximum residue levels seen in persons above 50 years of age. The positive correlation of age with DDT ($r = 0.362$) and HCH ($r = 0.342$) indicate that with aging the burden of pesticides on body increases The results were in accordance with those reported by other authors (Waliszewski et al. 1995; Gomez-Catalan et al. 1995). Non- significant relation was detected in DDT and HCH residue levels with food habbits and background of subjects. Distribution of organochlorine pesticide residues in human fat according to body mass index indicates that there was no relationship of organochlorine residues with obesity. As earlier reported by Gomez-Catalan et al. (1995) that obesity increment the accumulated body burden, but not the concentration.

An interesting finding of the present study was the presence of residues in a 7-month-old female child with levels of DDT and HCH as 1.92 and 0.59 mg kg^{-1}, respectively. However, substantially higher levels of DDT (15.2 mg kg^{-1}) have been reported from adipose tissue of a 1-day-old child from Mexico (Waliszewski et al. 1995). High levels of pesticide residues at this age might be due to the exposure to organochlorine compounds both in utero and through breast-feeding. Infants who were breast fed had higher concentration of OCPs than those who were formula fed (Lackman et al. 2004).

DDT residues mainly occur as op DDE and pp DDE with mean levels of 3.57 and 1.54 mg kg^{-1}, respectively. β-HCH was the dominant isomer found among various HCH isomers with frequency of 80% and mean level of 4.74 mg kg^{-1} followed by γ-, α-, and δ-isomers (Table 2). The results of present study regarding the presence of DDE residues was not unexpected since DDE is the most abundant and most persistent metabolite of the group and is in accordance with other authors both in India and other countries (Jani et al. 1988; Vladimir et al. 2002). DDE residue presence may reflect the past use of DDT, which has been metabolized in the environment and years later might be incorporated into the body through the diet (Kutz et al. 1991). The levels of op DDT and pp DDT were also similar to those reported by other investigators (Ramachandran et al. 1984; Nair and Pillai 1992). In human body DDT is first dechlorinated to DDD and is either metabolized to DDA or excreted directly as DDD. The short life of DDD in human beings after exposure to DDT suggests an isolated and recent exposure to DDT in these cases (Kutz et al. 1991). The source of this exposure may either be direct by a sudden misusage of this insecticide (as this is banned for agriculture use in India) or indirect, through the consumption of foods that are contaminated due to the DDT impurities of other authorized products.

β-HCH was the dominant isomer found among various HCH isomers with frequency of 80% and mean level of 4.74 mg kg^{-1} which could be related to its persistence as it takes five times longer period to be excreted from the human body than other isomers (Kutz et al. 1991). This results in a 10–30 times stronger ability of this isomer, compared to γ-HCH (lindane) to accumulate in the human fat (Sugaya 1971). Moreover α- and γ-isomers of HCH are converted into the β-isomer in living organisms. As a result of this conversion as much as 90% of HCH detected in human tissue and breast milk is β-HCH (Jensen and Slorach 1991). In an earlier study conducted in Ludhiana in 1976–1977, β-HCH

Table 1 Residues of organochlorine insecticides (mg kg^{-1}) in biopsy human fat samples

Sample no.	Sex	Age (years)	Back-ground	Food habits	BMI	∑ DDT	∑ HCH
1	F	40	Urban	Veg	26.4 (O)	1.57	2.41
2	F	42	Rural	Non-Veg	23.2 (N)	2.92	3.64
3	F	65	Urban	Non-Veg	27.0 (O)	6.31	2.37
4	M	34	Urban	Non-Veg	24.0 (N)	3.73	1.65
5	M	18	Urban	Veg	20.2 (N)	8.90	4.57
6	F	20	Urban	Veg	23.3 (N)	5.50	8.22
7	F	28	Urban	Veg	20.6 (N)	7.23	6.78
8	F	57	Rural	Veg	19.5 (N)	5.18	3.27
9	F	40	Urban	Non-veg	20.7 (N)	4.82	2.79
10	F	40	Rural	Veg	21.6 (N)	5.47	18.74
11	M	32	Rural	veg	25.1 (O)	3.29	3.67
12	F	37	Urban	Non-veg	20.3 (N)	10.62	33.91
13	M	65	Urban	Veg	25.1 (O)	7.58	3.25
14	F	35	Rural	Veg	23.1 (N)	3.64	2.60
15	M	55	Urban	Non-veg	16.7 (U)	7.60	10.89
16	F	23	Urban	Veg	22.7 (N)	12.42	BDL
17	F	49	Urban	Non-veg	23.8 (N)	4.56	BDL
18	F	23	Urban	Veg	19.9 (N)	1.82	3.22
19	M	24	Rural	Veg	17.9 (U)	6.02	4.41
20	F	25	Urban	Non-veg	18.7 (N)	1.75	2.26
21	F	24	Urban	Veg	18.8 (N)	5.10	1.57
22	F	24	Rural	Non-veg	24.6 (N)	3.67	2.11
23	M	28	Urban	Non-veg	43.0 (Ob)	1.43	2.17
24	F	29	Rural	Non-veg	24.4 (N)	3.16	5.42
25	F	43	Rural	Non-Veg	23.7 (N)	5.63	5.76
26	F	45	Rural	Non-veg	27.8 (O)	28.40	5.38
27	F	28	Rural	Non-veg	16.4 (U)	6.43	4.96
28	F	22	Rural	Veg	18.3 (U)	3.86	3.98
29	F	50	Rural	Veg	24.7 (N)	9.38	6.22
30	F	38	Urban	Veg	25.3 (O)	11.27	8.05
31	F	29	Urban	Non-veg	22.8 (N)	1.57	0.93
32	F	22	Rural	Veg	20.9 (N)	2.84	BDL
33	F	40	Urban	Veg	30.4 (Ob)	27.60	10.8
34	F	34	Urban	Veg	41.6 (Ob)	4.47	BDL
35	M	45	Rural	Non-veg	23.2 (N)	8.72	BDL
36	F	33	Urban	Veg	36.5 (Ob)	3.05	BDL
37	F	30	Urban	Veg	21.5 (N)	9.17	5.91
38	F	32	Rural	Non-veg	23.8 (N)	4.83	BDL
39	M	65	Urban	Non-veg	24.2 (N)	5.60	1.52
40	F	7 month	Urban	Veg	(U) [a]	1.92	0.59
41	F	33	Urban	Veg	43.1 (Ob)	5.34	6.41
42	F	42	Urban	Non-veg	24.2 (N)	2.72	3.37
43	F	44	Rural	Veg	22.9 (N)	9.49	14.30
44	F	50	Urban	Non-veg	24.8 (N)	11.90	15.27
45	F	32	Rural	Non-veg	23.6 (N)	5.22	5.56
46	M	44	Urban	Non-veg	25.1 (O)	4.29	2.18
47	F	40	Urban	Veg	27.3 (O)	2.31	BDL
48	F	77	Rural	Veg	27.0 (O)	31.60	24.12

Table 1 continued

Sample no.	Sex	Age (years)	Back-ground	Food habits	BMI	∑ DDT	∑ HCH
49	M	71	Urban	Veg	22.7 (N)	2.55	30.6
50	F	40	Urban	Veg	17.1 (U)	3.72	3.45
51	F	35	Rural	Non-veg	22.2 (N)	1.51	4.03
52	F	39	Rural	Veg	22.7 (N)	0.81	2.49
53	F	27	Urban	Veg	19.0 (N)	10.53	15.65
54	F	60	Rural	Veg	24.8 (N)	17.20	3.56
55	F	42	Rural	Non-veg	18.3 (U)	9.36	BDL
Mean ± SD		37.99				6.86 ± 6.38	5.72 ± 7.2
Range						0.81–31.60	BDL-33.91

BDL below detectable limits
BMI-18.5 under weight (*U*), 18.5–25.0 normal (*N*), 25.0–30.0 over weight (*O*), >30.0 obese (*Ob*)

Table 2 Frequency of occurrence and levels of various metabolites of DDT and HCH isomers in human fat samples

Metabolite/isomer	Frequency	Per cent	Level (mg kg^{-1})
o,p′ DDE	43/55	78.1	3.57 (BDL-14.4)
p,p′ DDE	8/55	14.5	1.54 (BDL-31.6)
o,p′ DDD	2/55	3.6	0.03 (BDL-1.37)
p,p′ DDD	13/55	23.6	0.69 (BDL-8.72)
o,p′ DDT	4/55	7.2	0.61 (BDL-27.6)
p,p′ DDT	11/55	20.0	0.42 (BDL-3.8)
α-HCH	3/55	5.4	0.12 (BDL-5.4)
β-HCH	44/55	80.0	4.74 (BDL-33.9)
γ-HCH	16/55	29.0	0.83 (BDL-9.84)
δ-HCH	1/55	1.8	0.03 (BDL-1.73)

Values in parenthesis indicates range of pesticide residues

was the most frequently occurring isomer with mean levels of 5.11 mg kg^{-1} (Chawla et al. 1978). γ-HCH was found in 16 samples with mean level of 0.83 mg kg^{-1}, which are comparable to the levels of 0.77 mg kg^{-1} reported by Kaphalia and Seth (1983) from Lucknow, India. It could be attributed to although γ-HCH has been banned for indoor use in India, it is still permitted to be used on field crops for pest control which may be the likely cause of levels reported. α-HCH was found in 5.4% of the samples with mean residue levels of 0.12 mg kg^{-1} while Ramachandran et al. (1984) reported levels upto 3.77 mg kg^{-1}. In the present study, the lowest frequency occurrence was that of δ-HCH (1.8%) with average concentration of 0.03 mg kg^{-1}, which is also lower than as reported earlier (Nair and Pillai 1992). The decline in levels of α- and δ-HCH might be due the imposition of ban on the usage of technical HCH in India since 1997.

Bans and restrictions do not immediately yield decreased detection of DDT and HCH in human adipose tissues because of the chemical's persistence. However, it is clear that banning or restricting the usage of DDT and HCH can eventually lead to declines in exposure and drops in detectable residue levels. Since exposure can occur through food and since global security relies on the import and export of food crops, a worldwide ban is the only solution to exposure.

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References

Bedi JS, Gill JPS, Aulakh RS, Joia BS, Sharma JK (2005) Contamination levels of DDT and HCH residues in different goat tissues. Indian J Anim Sci 75(1):11–13

Chawla RP, Sharda VM, Joia BS, Kalra RL (1978) A survey of residues of organochlorine insecticides in biopsy human fat samples. In: Proc. Symp. Pesticide Residues in Environment in India, UAS, Bangalore, pp 62–69

Ekbom A, Wicklund-Glynn A, Adami HO (1996) DDT and testicular cancer. Lancet 347:553–554

Gomez-Catalan J, Lezaun M, To-Figueras J, Corbella J (1995) Organochlorine residues in the adipose tissue of the population of Navarra (Spain). Bull Environ Contam Toxicol 54:534–540

Jani JP, Patel JS, Shah MP, Variya MR, Shah YH, Gupta S, Kashyap S (1988) Levels of DDT and HCH in human adipose tissue of Indian population. Scand J Work Environ Health 14 (3):201–204

Jensen AA, Slorach SA (1991) Chemical contaminants in human milk. Boca Raton. CRC press, Ann Arbor Boston

Kang BK, Singh B, Chahal KK, Battu R (2000) Insecticide residues in market samples of cucumber and reddish. Pestology 24(4):57–59

Kaphalia BS, Seth TD (1983) Chlorinated pesticide residues in blood plasma, and adipose tissue of normal and exposed human population. Indian J Med Res 77:245–247

Kutz FW, Wood PH, Bottimore DP (1991) Organochlorine pesticides and polychlorinated biphenyls in human adipose tissue. Rev Environ Contam Toxicol 120:1–82

Lackman GM, Schaller KH, Angerer J (2004) Organochlorine compounds in breast fed Vs. bottle fed infants: preliminary result at six weeks of age. Sci Total Environ 329(1-3):289–293

Mansour SA (2004) Pesticide exposure-Egyptian scene. Toxicology. 198(1-3):91–115

Nair A, Pillai MKK (1992) Trends in ambient levels of DDT and HCH residues in human and the environment of Delhi, India. Sci Total Environ 121:145–157

Pandit GG, Sharma S, Srivastva PK, Sahu SK (2002) Persistent organochlorine pesticide residues in milk and dairy products in India. Food Addit Contam 9(2):153–157

Ramachandran M, Banerjee BD, Gulati M, Grover A, Zaidi SSA, Hussain QC (1984) DDT and HCH residues in the body fat and blood samples from some Delhi hospitals. Indian J Med Res 80:590–593

Sugaya A (1971) Organochlorine pesticide residues in humanmilk. J Jpn Assoc Rural Med 19:379–380

Vladimir T, Valery R, Lorenzo T (2002) Dichlorodiphenyltrichloroethane (DDT): ubiquity, persistence and risks. Environ Hlth Persp 181(2):125–131

Waliszewski SM, Sedas V T, Infanzon RM, Rivera J (1995) Determination of organochlorine pesticide residues in human adipose tissue: 1992 study in Mexico. Bull Environ Contam Toxicol 55:43–49