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HCH and DDT in Surface Extractable Skin Lipid as a Measure of Human Exposure in India

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Organochlorine insecticides are usually determined in blood, urine, maternal milk or adipose tissue to determine exposure in human subjects. However, technical and ethical problem often prevent the collection of these samples under field conditions. The skin is rich in lipids and is a major storage site of halogenated hydrocarbons. Human skin lipids consist of triacyl glycerol, squalene, fatty acids and wax esters (Nicolaides 1974). HCH and DDT, like most organochlorines are lipid soluble and tend to accumulate in adipose tissue, and lipid rich organs, such as brain, liver and lipid fractions of biological membranes (de Velieger et al. 1968; Slater 1984). Wolff (1984) has reported utilization of surface lipids as a non-invasive technique to estimate DDE in the human body and related contamination with DDE levels in serum. Recently, Sasaki et al (1991a) reported good correlation of β-HCH and p,p'-DDE concentrations in skin lipids and adipose tissue and further used skin lipids as an index for the accumulation of organochlorine chemicals in the human body. We report the accumulation of HCH-isomers and DDT/DDT metabolites in skin lipid and its correlation with whole blood concentrations to investigate the use of skin lipids as a non-invasive technique to monitor HCH and DDT body burdens under field conditions.

MATERIALS AND METHODS

n-Hexane, petroleum ether (40-60°C), diethyl ether, acetone, sulphuric acid, anhydrous sodium sulphate were all of analytical grade reagents. Neutral alumina was of column chromatographic grade. Authentic standards of HCH-isomers and of DDT and its metabolites were obtained from the Industrial Toxicological Research Centre, Lucknow, India.

Paired samples of skin lipids and whole blood were collected from Farah PHC of the Mathura district. 24 were taken from the general population and 15 samples from occupationally exposed individuals involved in the spraying of HCH and DDT for malaria control. Samples were collected in August 1994. Skin lipid samples were collected according to the method of Sasaki et al. (1991a). In brief, the face was washed with soap and 2h later wiped with de-fatted cotton wool soaked in 70% ethanol to collect skin lipid. 100 µl whole blood was taken by finger-prick

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method on the Whatman No.1 paper with the help of heparinized capillary glass tubes (Drumand Scientitic Co. USA) (Dua et al., 1996). All samples were stored in a refrigerator until analysis.

Whole blood samples, collected on filter papers were extracted according to Dua et al. (1996). In brief, the filter papers were cut into small pieces and extracted three times with 10 ml of hexane : acetone mixture (1: 1 v/v).

Cotton wool containing the skin lipid samples were dried in desiccator and then extracted three times with petroleum ether (25 ml) after gentle heating in water bath (40-50°C) with continuous shaking. Extracts were then filtered and evaporated to dryness in water bath at 60°C. The residual lipid was weighed.

For the extraction of HCH and DDT residues, the weighed skin lipid samples were dissolved in petroleum ether and were treated with concentrated sulphuric acid (97-99%) (10 ml) until a clear layer of acid was separated. The petroleum ether layer was collected and washed with distilled water then dried with anhydrous sodium sulphate.

Whole blood and lipid extracts were cleaned-up by chromatography on a 5% deactivated Florisil column using 2% diethyl ether in petroleum ether as eluting solvent **(25** ml).

A Hewlett-Packard Model 5890A gas chromatograph equipped with Ni^{63} electron capture detector and coupled to an integrator 3390A was used for the analysis of HCH and DDT residues in whole blood and skin lipid samples. A fused silica capillary column PTE^{TMS} (30m length, 0.25 mmm i.d. and 0.25 nm thick) supplied by Supelco ParkUSA was used. The column, injector and detector temperature were 190,210 and 220°C. Ultrapure nitrogen was used as carrier gas at a flow rate of 2ml min^{-1}. The extraction recoveries of HCH-isomers and DDT and its metabolites varied from 84 to 92.1% from whole blood and 82.8 to 88.4% from skin lipid samples. The minimum detection limit of HCH-isomers and DDT metabolites were 0.005 mg/L and 0.01 mg/kg for whole blood and lipid samples respectively. The residue values reported have not been corrected for recovery.

RESULTS AND DISCUSSION

Analyses of residual levels of HCH and DDT in whole blood from occupationally exposed persons and general population are shown in Table 1. The mean value of α-, γ-, β- and δ-HCH in occupationally exposed persons were 0.34±0.21, 0.32±0.25, 0.36±0. and 0.17±0.24 mg/L while the mean value of p,p'-DDE, p,p'-DDD and p,p'-DDT were 0.36±0.42, 0.02±0.07 and 0.64±0.80 mg/L, respectively. Similarly, the mean value of α-, γ-, β- and δ-HCH in general population were 0.28±0.15, 0.20±0.11, 0.28±0.19 and 0.13±0.19 mg/L while the mean concentrations of p,p'-DDE and p,p'-DDT were 0.30±0.24 and 0.49k±0.64 mg/L, respectively.

Table 1. HCH and DDT concentrations (mg/L) in whole blood of occupationally exposed and general population

Insecticide	Concentration (Mean±S.D.)	
	Occupationally exposed group	General population
α-HCH	0.34±0.21 (0.11-0.72)	0.28±0.15 (0.07-0.54)
γ-HCH	0.32±0.25 (0.10-0.85)	0.20±0.11 (0.04-0.59)
β-HCH	0.36±0.27 (0.07-0.90)	0.28±0.19 (<0.005-0.72)
δ-HCH	0.17±0.24 (<0.005-0.80)	0.13±0.19 (<0.005-0.84)
Total HCH	1.19±0.61 (0.39-2.58)	0.89±0.31 (0.21-1.45)
p,p'-DDE	0.36±0.42 (<0.005-1.13)	0.30±0.24 (<0.005-0.98)
p,p'-DDD	0.02±0.07 (<0.005-0.31)	ND -
p,p'-DDT	0.64±0.80 (<0.005-2.40)	0.49±0.64 (<0.005-2.00)
Total DDT	1.02±0.80 (0.00-2.58)	0.79±0.74 (0.00-2.44)

ND: <0.005; Figures in parentheses are range

The concentrations of HCH and DDT residues in skin lipid samples of occupationally exposed persons and general population are presented in Table 2. The mean values of α-, γ-, β- and δ-HCH in lipids from exposed persons were 9.69±7.39, 12.00±9.90, 22.04±10.89 and 10.84±12.45 mg/kg, respectively, whereas the mean value of p,p'-DDE, p,p'-DDD and p,p'-DDT were 10.52±8.31, 4.89±9.96 and 11.76±12.97 mg/kg, respectively. p,p'-DDE was detected from all samples whereas p,p'-DDD and p,p'-DDT were found in only 12 and 8 samples, respectively. β-HCH was dominant (39.1%) followed by γ-HCH (22.7%), α-HCH (19.4%) and δ-HCH (18.8%). For the DDT metabolites, p,p'-DDT was dominant (42.3%) followed by p,p'-DDE (38.7%) and p,p'-DDD (18.0%) in skin lipid samples. Similarly, the mean values of α-, γ-, β- and δ-HCH in general population were 3.63±3.39, 3.54±3.22, 5.98±4.52 and 4.77±4.42 mg/kg, respectively while the mean values of p,p'-DDE, p,p'-DDD and p,p'-DDT were 5.79±6.12, 1.30±1.97 and 6.77±7.23 mg/kg, respectively.

A comparison of the levels of total HCH and DDT in occupationally exposed work

Table 2. HCH and DDT concentrations (mg/kg) in skin lipids of occupationally exposed group and general population

Insecticide	Concentration (Mean±SD.)	
	Occupationally exposed group	General population
α-HCH	9.69±7.39 (1.42-23.49)	3.63±3.39 (0.36±13.18)
γ-HCH	12.00±9.90 (0.65-37.85)	3.54±3.22 (<0.01-12.58)
β-HCH	22.04±10.89 (3.16-38.52)	5.98±4.52 (<0.01-18.50)
δ-HCH	10.84±12.45 (<0.01-36.36)	4.77±4.42 (<0.01-19.64)
Total HCH	54.59±20.50 (3.67-94.22)	17.92±12.40 (0.67-58.12)
p,p'-DDE	10.52±8.31 (1.08-29.62)	5.79±6.12 (<0.01-23.39)
p,p'-DDD	4.89±9.96 (<0.01-29.64)	1.30±1.97 (<0.01-5.91)
p,p'-DDT	11.76±12.97 (<0.01-44.54)	6.77±7.23 (<0.01-17.35)
Total DDT	27.17±19.66 (10.49-67.14)	13.86-12.70 (0.00-45.06

Figures in parentheses are range

ers and general population is given in Figure 1. The whole blood samples from occupationally exposed individuals contained 1.4 and 1.3 times higher values of total HCH and DDT than their corresponding value from general population. Similarly, lipid samples from occupationally exposed individuals contained 3.6 and 1.9 times higher concentrations of total HCB and DDT residues than the general population. Significant difference in the mean values of total HCH and DDT were observed between occupationally exposed individuals and general population whole blood samples ($\gamma_{(HCH)}$=2.533; $p<0.05$ and $\gamma_{(DDT)}$= 2.500; $p<0.05$). Similar observations were also recorded for lipid samples between two groups ($\gamma_{(HCH)}$ = 7.202; $p<0.001$ and $\gamma_{(DDT)}$== 2.492; $p<0.05$).

Skin lipid and whole blood samples collected from occupationally exposed individuals and the general population were analysed to determine a possible correlations between lipid and blood concentrations of HCH and DDT residues (Table 3). Significant correlations were observed for β-HCH ($r = 0.5594$, $p < 0.01$), total HCH ($r = 0.4862$, $p < 0.05$) and p,p'- DDE ($r = 0.8092$, $p < 0.001$). However, for the general population, significant correlations were observed for γ-HCH ($r = 0.4250$, p

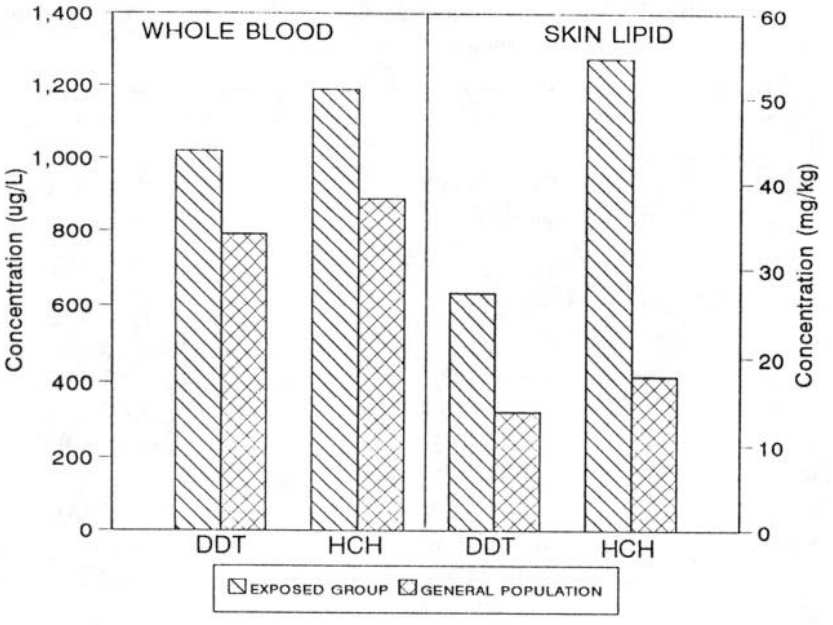

Figure 1. DDT and HCH residues in exposed group and general population

< 0.05) β-HCH (r = 0.5019, p < 0.01), total HCH (r = 0.5388, p < 0.001) p,p'-DDE (r = 0.6275, p < 0.001), p,p'-DDT (r = 0.7260, p < 0.001) and total DDT (r = 0.7856, p < 0.001).

In the present study, the mean HCH and DDT concentrations are higher than the earlier reports from India (Ramchandran et al. 1984; Kaphalia and Seth 1983) which is due to extensive use of these insecticides in the Mathura district for malaria control because of high endemicity. Presence of high contents of γ-HCH and p,p'-DDT in both the groups (exposed individuals and general population) further confirmed the recent exposure. β-HCH followed by α-HCH contributed maximum among HCH isomers while p,p'-DDT and p,p'-DDE was present maximum amount of the total DDT in both groups. Similar results were reported in blood samples from Delhi (Ramchandran et al. 1984). Statistical comparison shows the significant difference of residual levels of total HCH and DDT between exposed and general population. Kaphalia and Seth (1983) have also reported similar difference in blood plasma and adipose tissue samples from normal and exposed human population.

The results of skin lipid for pesticide body burden in the present study are reported for the first time for an Indian population in order to establish the technique to provide a bioindicator. Significant correlation has been observed between skin lipid and whole blood for γ-HCH, β-HCH, total HCH, p,p'-DDE, p,p'-DDT and total DDT residues from general population, while significant correlation between lipid and blood from occupationally exposed individuals were observed only for β-HCH,

Table 3. Coefficients of correlation between skin lipid and whole blood concentratio of HCH and DDT

Insecticide	r-Value	P.E.R.	N	Significance level
Occupationally Exposed group				
α-HCH	0.0350	0.041	15	$p > 0.05$
γ-HCH	0.1127	0.032	15	$p > 0.05$
β-HCH	0.5594	0.009	15	$p < 0.01$
δ-HCH	0.3076	0.022	15	$p > 0.05$
Total HCH	0.4862	0.012	15	$p < 0.05$
p, p'-DDE	0.8092	0.002	15	$p < 0.001$
p, p'-DDT	0.1122	0.035	15	$p > 0.05$
Total DDT	0.3745	0.022	15	$p > 0.05$
General population				
α-HCH	0.3547	0.011	24	$p < 0.05$
γ-HCH	0.4250	0.009	23	$p < 0.05$
β-HCH	0.5019	0.007	23	$p < 0.001$
δ-HCH	0.1286	0.034	15	$p > 0.05$
Total HCH	0.5388	0.005	24	$p < 0.001$
p, p'-DDE	0.6275	0.004	23	$p < 0.001$
p, p'-DDT	0.7260	0.002	17	$p < 0.001$
Total DDT	0.7856	0.001	23	$p < 0.001$

r: Coefficient of correlation; N: No. of paired samples P.E.R.: Probable error ratio

total HCH and p,p'-DDE residues. The correlations for γ-HCH and p,p'-DDT were insignificant which might be due to the recent dermal exposure in exposed persons who were engaged in spraying of these insecticides in vector borne disease control programme. The experiment was repeated by an independent group and a significant correlation of HCH and DDT was obtained between skin lipid and whole blood from the general population. Sasaki et al. (1991 a,b) have reported correlation coefficient between adipose tissue and skin lipid for β-HCH (0.92) and p,p'-DDE (0.93). Wolff (1984) has reported 0.79 coefficient for DDE between adipose tissue and serum and 0.73 between adipose tissue and skin lipid.

Correlation of HCH and DDT residue between skin lipid and whole blood from paired samples implies that the collection of skin lipid as a non-interventive sampling method for halogenated hydrocarbons has a potential application as an indicator of body burden particularly in general population. However, skin lipid may not be suitable bioindicator for occupationally exposed population because dermal ex-

posure occurs in work place and skin lipid may contain chemicals representative of occupational exposures but not related to body burden which is evident by the low correlation of exposed workers. Similar observations have been made for Aroclor 1016 in capacitor manufacture and with polycyclic aromatic hydrocarbons in roofing workers (Wolff 1984).

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