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Determination of HCH and DDT in Finger-Prick Whole Blood Dried on Filter Paper and Its Field Application for Monitoring Concentrations in Blood

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The organochlorine insecticides HCH and DDT have been extensively used in India for the control of vector borne diseases. However due to their chemical nature, they became major environmental pollutants. On account of their extreme stability, persistance and probable indiscriminate use, they have found their entry into the food chain (Agnihotri et al. 1974 ; Bhupendra and Seth 1981 ; Battu et al. 1989) and ultimately into the human system where they are stored in body fat after partial metabolism in various tissues (Ramachandran et al. 1974; Kaphalia and Seth 1983). Whole blood concentration are preferred for the assessment of persistance of HCH and DDT in general population (Brown and Chow 1975 ; Sasaki et al. 1991). A large volume of blood through venous sampling and freezing facilities are required for the determination of HCH and DDT residues in whole blood which under tropical field conditions are difficult to get. We present a method for the assay of HCH isomers and DDT metabolites in whole blood samples taken by finger prick and dried on filter paper. This method has been applied for finding HCH and DDT level in whole blood during Ardh Kumbh congregation held at Hardwar in 1992 where these insecticides were extensively used for the control of mosquitoes and house flies. Results are presented in this paper.

MATERIALS AND METHODS

n-Hexane, acetone and benzene (analytical-reagent grade) were purchased from S.D. Fine-Chem-Ltd. Bombay. Anhydrous sodium sulphate and silica gel (60-120 mesh) obtained from E. Merk (India) Ltd. Bombay, were used for packing the column. All others chemicals of AR grade were used without further purification. Primary standards of HCH-isomers, DDT metabolites and aldrin (internal standard) were obtained from Industrial

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Toxicological Research Centre, Lucknow. Ultrapure nitrogen was obtained from Indian Oxygen Ltd. Delhi for gas chromatographic analysis.

Whole blood samples (100 µl) were collected through finger prick with the help of heparinized capillary tubes filled up to the red mark (Drumond Scientific Co. U.S.A.) from 47 occupationally exposed group i.e. persons involved in the spraying of HCH and DDT and 37 from general population during Ardh Kumbh congregation held during January to April 1992 at Hardwar, U.P., India. Ardh Kumbh is an important congregation of Hindus in which about one million devotees assamble in every six year at Hardwar to take holi dip in Ganga river. About 6 metric tons of HCH and 2 metric tons of DDT was used covering an area of 130 sq km for the control of mosquitoes and house flies. All the samples were collected on Whatman No. 1 filter paper (Whatman International Ltd Maidstone, England). The filter papers were dried in hanging position and protected from the insects. After drying, each filter paper was placed between the plain paper and kept at ambient temperature. Proper care was taken to avoid cross contamination during the Packing.

The recovery (extraction yield) was determined at conc-entration of 0.2 ng/0.l ml for HCH isomers, aldrin and DDT metabolites from spiked whole blood samples applied on filter paper. Within day reproducibility was obtained by analysis of samples (n=5) containing 0.2 ng/0.l ml of each HCH isomers, aldrin and DDT metabolites. Similarly day to day reproducibility was also determined by assaying different concentrations on different days. Some samples of same concentrations were also analysed with different time intervals to see the effect of storing the samples for different time at ambient temperature.

The dried blood spots on the papers were cut into small pieces and immersed into 5 ml of n-Hexane : Acetone (1:1 v/v) for 20 minutes and vortexed for 10 minutes. Solvent phase was separated. The filter paper was re-extracted twice with 5 ml of n-hexane : acetone mixture. The extracted solvent was pooled and evaporated to 2 - 5 ml on Vortex evaporator (Haake Buchler, Saddle Brook N.J. U.S.A.). The concentrated samples were cleaned up by passing through sodium sulphate-silica column using n-Hexane:benzene (50:50 v/v) as eluting solvent. The eluent was collected and dried. The dried samples were dissolved in 0.5 ml n-hexane and 2-8 µl were injected for gas chromatographic analysis.

All samples were analysed for HCH isomers and DDT

Table 1. Extraction recovery of HCH, aldrin and DDT from whole blood

Insecticide	% Recovery (mean)	Standard deviation	Standard error	% Coefficient of variation
α-HCH	91.0	5.4	2.4	5.9
ß-HCH	85.9	2.9	1.3	3.3
ϒ-HCH	89.3	5.4	2.5	6.0
δ-HCH	92.1	8.4	3.7	9.1
Aldrin	90.6	4.1	1.8	4.5
o,p'-DDE	89.7	6.8	3.0	7.5
p,p'-DDE	86.9	3.3	1.5	3.8
o,p'-DDT	84.0	2.7	1.2	3.2
p,p'-DDT	89.2	8.4	3.7	9.4

a: Mean of five sets

metabolites on Hewlett-Packard 5890 A gas chromatograph equipped with Ni^{63} electron capture detector on 5% OV-17 coated on Gas Chrom Q (80-100 mesh) packed glass column. Nitrogen (flow @ 120 ml/min) was used as carrier gas with injector 210° C, oven 190° C and detector 220°C temperature. The identity of HCH isomers and DDT metabolites were confirmed on 5% DEGS column coated on Gas Chrom Q (100 - 120 mesh) (Kaushik et al. 1987). The identities of the residues were also comfirmed by studying the disappearances or shifts in the peak patterns in alkali-derivatized samples as compared to underivatized samples (EPA 1980).

RESULTS AND DISCUSSION

The extraction recoveries of the HCH-isomers, aldrin and DDT metabolites from whole blood spiked on filter paper are given in Table-l. The extraction recoveries of HCH isomers varied from 85 to 92% with the standard deviations of 2.9 to 8.4 while that of DDT and its metabolites, the recoveries varied between 84 to 92% with the standard deviations of 2.7 to 8.4. Standard curves from HCH isomers, DDT and metabolites were linear over a concentration range of 2 to 1.2 /µg/L. The correlation of concentration verses peak height (area) ratio was 0.97 (n=5) and the intercept did not differ significantly from zero value. Within day coefficient of variation for 0.2 ng/0.l ml and 1.2 ng/0.lml for HCH and DDT were between 2 to 9%. Concentrations of the samples stored upto one month did not differ from each other. Significant correlations were recorded between finger-prick and venipucture whole blood concentration for HCH ($r = 0.93$, $p < 0.001$)

Table 2. Residual levels of HCH and DDT in whole blood of the general population

Insecticide	Concentration(μg/L)			
	Mean (n=37)	Standard deviation	Standard error	Range
α-HCH	6.32	3.80	0.62	1.76 - 16.75
ß-HCH	13.44	5.04	0.82	4.87 - 23.80
Y-HCH	1.75	3.00	0.49	ND - 12.15
Total-HCH	21.50	7.78	1.27	8.36 - 33.17
o,p'-DDE	4.67	7.57	1.24	ND - 25.15
p,p'-DDE	16.13	14.33	2.30	ND - 69.41
Total DDT	20.79	14.81	2.43	ND - 69.41

ND : Not detectable

and DDT ($r = 0.95$, $p < 0.001$). The minimum detection limit of HCH isomers and DDT metabolites were 0.003 and 0.01 μg/L, respectively. These values were based on the amount of the pesticide residues that gave 10 % full-scale deflection, without interferences from the co-extractives. The value below the minimum detection limit was recorded as not detectable.

An analysis of the technical sample of HCH used for spraying showed 27.2% (α-HCH, 49.4% γ-HCH and 23.4% δ-HCH isomers while the technical sample of DDT consisted of p,p'-DDT 60.2%, o,p'-DDT 31.92%, p,p'-DDE 1.68% and o-p DDE 6.22%. Average HCH and DDT levels in whole blood determined by filter paper method from 37 male which were not envolved in spraying operation (age group 20 - 50 years, average weight 47 kg) from District Hardwar, U.P. are given in Table-a. Mean HCH and DDT contents in general population were 21.50 μg/L (range 8.36-33.17 μg/L) and 20.79 μg/L (range ND-69.41 μg/L) respectively. β-HCH contributed 62.5% followed by (α-HCH 29.4% and γ-HCH 8.1% of the total HCH present. Similarly p,p'-DDE was 77.6% followed by o,p'-DDE 22.4% of DDT. No p,p'-DDT was detected in any sample.

47 samples from the occupationally exposed persons who were involved in the spraying operation of HCH and DDT during Ardh Kumbh Congregation at Hardwar in April, 1992 for the control of mosquitoes and flies, were also analysed for HCH and DDT contamination in their blood and the results are given in Table 3. Mean HCH concentration in whole blood was 68.0 μg/L (range 33.43-231.8 μg/L) while mean DDT was 58.43 μg/L (range 11.18-238.3 μg/L). β-HCH contributed 48.6%

Table 3. Residual levels of HCH and DDT in whole blood of the occupationally exposed population

Insecticide	Concentration (µg/L)			
	Mean (n=47)	Standard deviation	Standard error	Range
α-HCH	24.05	13.13	1.92	7.81 - 75.58
ß-HCH	33.08	23.40	3.42	9.89 - 156.3
ϒ-HCH	8.50	15.41	2.25	ND - 61.67
δ-HCH	2.37	6.77	0.99	ND - 34.09
Total HCH	68.01	34.12	4.98	33.43 - 231.8
o,p'-DDE	3.28	10.09	1.54	ND - 44.45
p,p'-DDE	44.42	48.02	7.33	11.18 - 211.6
o,p'-DDT	1.48	4.48	0.68	ND - 20.09
p,p'-DDT	9.24	21.71	3.31	ND - 95.24
Total DDT	58.43	53.72	8.20	11.18 - 238.3

ND: Not detectable

followed by α, γ, and δ of 35.4%, 12.5% and 3.5% respectively of the total HCH. Similarly p,p '-DDE contributed 76% followed by p,p'-DDT 15.8%, o,p'-DDE 5.6% and o,p '-DDT 2.5% of total DDT found.

A comparison of the levels of HCH and DDT in occupationally exposed and general population is given in figure 1. It is clear that the level of HCH in blood from occupationally exposed population was 3.1 times more as compared to general population. Similarly DDT level was 2.8 times more in exposed group as compared to general population. Comparatively higher concentrations of p,p'-DDT and γ-HCH in exposed cases resulted due to recent exposure by spraying operations. A wide range of concentration difference in exposed group as compared to general group was due to their nature of duties and extent of involvement in spraying operation.

Statistical comparison of the residual level of HCH in whole blood from two different group of population i.e. occupationally exposed and ii) general population showed the significant difference in the mean value ($t = 5.214$, $p < 0.001$). Significant difference was also observed for DDT residues in blood from two group populations ($t = 4.137$, $p < 0.001$).

In the present study, the mean HCH and DDT concentrations are quite lower than earlier reports from Delhi population (Agarwal et al. 1976; Ramachandran et al. 1984) but similar to Lucknow and Ahmedabad population

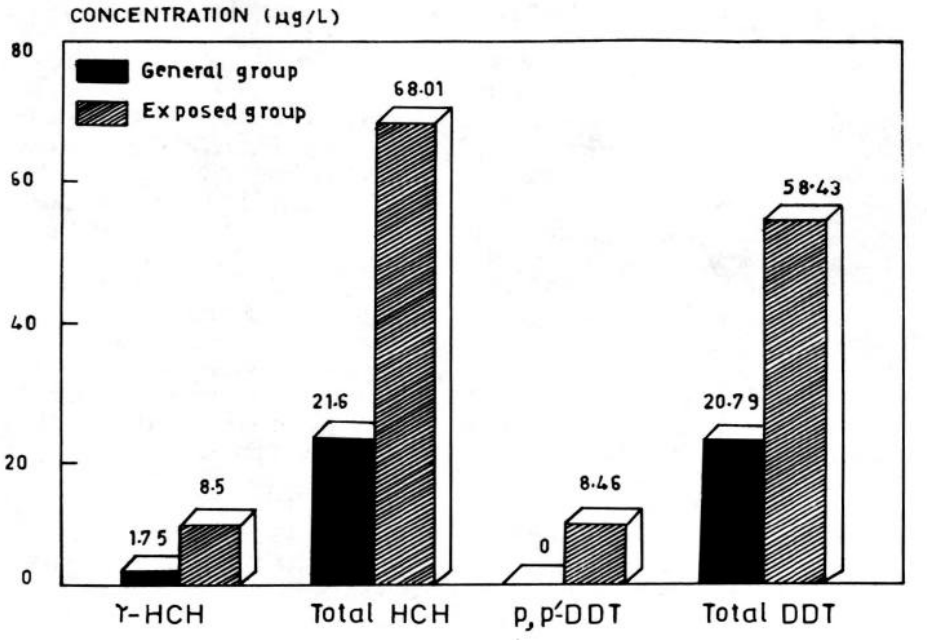

Figure 1. Comparison of HCH and DDT residues in whole blood from general and occupationally exposed population.

(Kaphalia and Seth 1983 ; Bhatanagar et al. 1992). This could be due to lesser use of these insecticides in public health and in agriculture or descending trend in the bioaccumulation of HCH and DDT in body fat. The average HCH in whole blood concentration in exposed group was 3.1 times more as compared to general population which is similar to that reported by Bauman et al. (1980) and Kaphalia and Seth (1983) while the total DDT in occupationally exposed population was only 2.8 times more than the general population which is low as compare to the earlier report (Kaphalia and Seth 1983). This might be due to the lesser use of DDT by exposed population for spraying operation.

The stability during storage upto one month, recovery and precison of HCH and DDT determination in whole blood sample spiked on filter paper was satisfactory and their was no concentration difference between venous blood samples and capillary blood samples applied on filter paper. In tropical field work, venous punctures are often not accepted by the people besides strictly standarized handling of blood samples is required which is difficult in field work (Dua et al. 1986). The use of dried finger tip blood samples have three advantages i) it is accepted by the people ii) the handling of these samples is not complicated

and iii) samples can be stored and transported easily.

It is clear from the present study that considerable amount of chlorinated pesticides are present in the general population in Hardwar area, however the level of contamination is not as high as reported from Delhi (Agarwal et al. 1976 ; Ramachandran et al 1984). B-HCH contributed more than 50 % to total HCH isomers which is known to possess the highest chronic mamalian toxicity of all isomers of HCH (Battu et al. 1989). The results therefore emphasised the need for appropriate care and restriction in the application of these insecticides in order to regulate their residues in different food items and eco-system. Significantly higher concentrations of HCH and DDT in the exposed group suggest that the necessary precaution should be taken for the application of these insecticide during congregations in India.

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