Bull Environ Contam Toxicol (2012) 88:497–500
DOI 10.1007/s00128-012-0546-6

Organochlorine Pesticide Residues in Blood Samples of Agriculture and Sheep Wool Workers in Bangalore (Rural), India

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Received: 12 October 2011 / Accepted: 25 January 2012 / Published online: 10 February 2012
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Abstract To describe exposure level of organochlorine pesticides (OCP) among workers occupationally engaged in agriculture and sheep wool associated jobs, the present study was carried out in rural neighborhood of Bangalore city, India. Thirty participants were interviewed and obtained informed consent before blood sample collection. The maximum concentrations of OCP were detected in blood samples of agriculture workers than sheep wool workers. Among the metabolites of HCH and DDT, lindane (γ-HCH) and *p,p'*-DDE were the most contributed to the total OCP. There were no differences in pesticide residues found between sex and work groups. It was observed that about 30% of samples exceeded the tolerance limits of 10 μg/L prescribed for HCH under the prevention of food adulteration act. Therefore, the present study recommends continuous monitoring with larger sample size.

Keywords Organochlorine pesticides · Human blood · Sheep wool workers · Agriculture workers

The large-scale use of persistent organochlorine pesticides in modern agriculture has caused serious concern due to the presence of their residues in the environment. Besides combating insect pests, insecticides also get accumulated in many parts of the ecosystem and exert toxic effects on organism including human. Human are exposed to these hazardous chemicals through consumption of contaminated food or through occupational exposure. The occupational exposure could be during manufacture and formulation of pesticides and their distribution in field condition during application of pesticides. Presence of these compounds in human adipose tissues (Alawi et al. 1999), human milk (Behrooz et al. 2009), maternal serum (Bloom et al. 2009) are reported from various part of the world. Increasing incidence of cancer, chronic kidney diseases, suppression of the immune system, sterility in male and females, endocrine disruption, neurological disorders, have been attributed to chronic pesticide poisoning (Hosie et al. 2000).

Organochlorine pesticides, such as hexachlorocyclohexane (HCH) and DDT account for two-thirds of the total consumption in India (Kumari et al. 2001) for agriculture and public health purposes respectively. Currently, there are about 165 pesticides registered for use in India, of which 40% are organochlorines (FAO 2005). The consumption pattern of these chemicals in India differs from the rest of the world. In India there are studies on the presence of organochlorine residues in human breast milk from the general population of different cities (Kumar et al. 2006; Subramanian et al. 2007) and from metropolitan (Devanathan et al. 2009). However, there is little information available on human blood concentration (Bhatnagar et al. 2004; Subramaniam and Solomon 2006). The present study was aimed to determine OCP in human blood samples of people engaged in agriculture and sheep wool work in neighborhood of Bangalore city, South India.

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Materials and Methods

This study was initiated as part of a research plan on assessing the health status of agriculture, sheep breeding and wool shearing workers at villages of neighborhood of

Bangalore city. This rural population in the southern part of Karnataka is largely engaged in agriculture work. During 2009, human blood samples were collected from 30 males and females with age ranging between 22 and 45. Informed consents were obtained from all blood donors. Subjects were requested to provide information on their demographics, dietary habits and smoking status (Table 1). About 4–5 ml of blood was collected by venepuncture from all the 30 participants and placed in refrigerator until analysis. Special care was taken to avoid contamination of glassware by pesticides.

Blood samples were extracted for OCP analysis by the addition of 5 mL formic acid and 5 mL hexane in a 25 mL centrifuge tube and the contents were shaken for 2 h in a slow speed rotating machine. The contents were centrifuged for 10 min at 2,000 rpm and then the clear top layer of hexane was collected in a clean graduated test tube and

Table 1 Demographical profile of study subjects

Characteristics	Numbers
Total number of participants	30
Male	20
Female	10
Age	
Years	22–45
Education	
Illiterate	11
Intermediate (10 + 2) pass or less	13
Graduate	6
Marital status	
Married	28
Unmarried	2
Water source for drinking	
Ground water	30
Fuel source for cooking	
Firewood	30
Smoking habits	
Smokers	11
Non smokers	19
Tobacco chewer	13
Non chewer	17
Dietary habits	
Non vegetarian	30
Occupation	
Agriculture	9
Shepard, sheep breeding and its associated jobs	21
Common symptoms	
Back pain, cough, headache, watering of eyes	8
Cough, dizziness, throat irritation	4
Joint pain (knee, shoulder, finger etc.)	11
Chest Pain	2

concentrated under steam of N_2 in a waterbath at 40°C. The residue was dissolved in 1 mL of hexane. Samples (1 µL) were injected into a Hewlett Packard 5890 Series II gas chromatograph equipped with Ni^{63} electron capture detector (ECD). A fused silica capillary column (30 m × 0.32 mm × 0.5 µm) DB-608 (5% diphenyl and 95% dimethyl polysiloxane) stationary phase (J & W Scientific Co., USA) was used for quantification. Chromatographic conditions for organochlorine pesticide analysis were as follow; detector 300°C; injector 250°C; oven temperature was programmed as 180°C-3 min; 4°C/min-260°C-15 min. All the samples were analysed for alpha-hexachlorocyclohexane (α-HCH), *β*–HCH, *δ*-HCH, lindane, heptachlor, heptachlor epoxide (HE), aldrin, dieldrin, *p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, endrin, endrin aldehyde, α-endosulfan, *β*-endosulfan and endosulfan sulfate.

Spiked and duplicate samples were also analysed. Pesticide residues were analysed and quantified from individually resolved peak areas with the corresponding peak area of standard (Dr. Ehrenstorfer—Germany). The recovery of pesticides averaged from 94% to 103% and the residue levels were not corrected as per the recovery calculation. Procedural blanks were run with every set of six samples to check for cross-contamination and to correct sample values if needed. The detection limit was 1 µg/L for all organochlorine pesticides and residues are expressed as µg/L. All the data were log transformed to get normal distribution. Difference between sex and between study groups was determined by student *t*' test. Significance level was set to 5%. All the statistical tests were performed using statistical software, SPSS student version 12.

Results and Discussion

Sixteen organochlorine pesticides were included in this study. Many of these compounds were detected in blood samples. Of the various pesticides detected, HCH and its isomers showed higher burden of exposure than other compounds. Among the four isomers (α-, *β*-, *δ*- and *γ*-) of HCH, *γ*-HCH (lindane) was detected (97%) in higher frequency (Table 2). Among the metabolites of DDT, *p,p'*-DDE was detected in 80% of samples. The cyclodiene insecticide endosulfan and heptachlor epoxide was detected in 63% and 73% of the samples analysed, respectively. While, other pesticide were detected in 17%–33% of the samples, dieldrin, endrin, endrin aldehyde were not detected in any of the samples tested.

Since there is no significant ($p > 0.05$) difference between sex and study group with total organochlorine residues, the data were treated together for further results. Among the various isomers of HCH, *γ*-HCH was the maximum (10.2 ± 2.94 µg/L) to the total HCH. The most stable

Table 2 Organochlorine pesticide residues (μg/L) in human blood samples collected from neighborhood of Bangalore City, Karnataka

Pesticide	N	No. of sample detected[a]	Mean	SE	Minimum	Maximum
α-HCH	30	10 (33)	3.54	0.15	BDL	3.59
β-HCH	30	10 (33)	9.55	2.83	BDL	45.9
γ-HCH	30	29 (97)	10.2	2.94	BDL	87.6
δ-HCH	30	19 (63)	5.93	1.95	BDL	45.2
∑HCH	30	30 (100)	26.7	3.87	9.81	86.5
p,p'-DDD	30	5 (17)	3.01	0.19	BDL	5.65
p,p'-DDE	30	24 (80)	5.67	1.21	BDL	32.1
p,p'-DDT	30	30 (63)	3.81	1.51	BDL	48.3
∑DDT	30	30 (100)	10.6	2.15	6.72	51.7
∑Endosulfan	30	29 (63)	7.12	2.78	BDL	43.2
Heptachlor epoxide	30	22 (73)	4.43	1.02	BDL	23.5
∑OCP	30	30 (100)	56.4	6.72	27.8	147

BDL below detectable levels (1 μg/L)

[a] Values in the parentheses indicate the percentage of samples detected residues

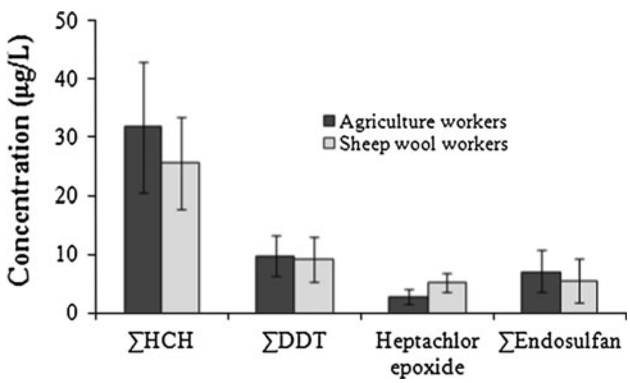

Fig. 1 Variation in organochlorine pesticide residues (μg/L, SE) in human blood samples collected from neighborhood of Bangalore City, Karnataka

isomer β–HCH was detected in 33% of the samples with a mean concentration of 9.55 ± 2.83 μg/L. This may be due to consumption of lindane for agriculture purpose in India. About 70% of the total pesticide consumption in India is γ-HCH, DDT and malathion (Abhilash and Singh 2009). The higher frequency of occurrence of γ-HCH indicating donors are subjected still to recent application of lindane. While γ-HCH (11.8 ± 2.57 μg/L) and β–HCH (10.76 ± 4.13 μg/L) concentrations were the maximum in Agricultural workers, heptachlor epoxide (5.14 ± 1.24 μg/L) was the maximum in sheep related workers (Fig. 1).

The present study revealed that the work population in the area studied is widely exposed to these contaminants. HCH concentrations recorded in the present study are

Table 3 Comparison of organochlorine pesticide residues (μg/L) in human blood collected from neighborhood of Bangalore City with the earlier studies in India

Location	α-HCH	β-HCH	γ-HCH	∑HCH	*p,p'*-DDE	*p,p'*-DDD	*p,p'*-DDT	∑DDT	∑endosulfan	Source
Lucknow	–	–	–	75	–	–	–	28	–	Kaphalia and Seth (1983)
Delhi	–	–	–	490	–	–	–	710	–	Ramachandra et al. (1984)
Delhi	–	–	–	–	–	–	–	301	–	Saxena et al. (1987)
Ahmedabad (rural)	–	–	–	148	37.2	1.33	8.83	47.7	–	Bhatnagar et al. (1992)
Ahmedabad	4.49	35.1	1.69	41.2	20.9	2.03	9.28	32.6[$]	–	Bhatnagar et al. (2004)
Punjab	–	–	–	57	–	–	–	65.2	–	Mathur et al. (2005)
Madurai	–	–	–	6.0–61	–	–	–	8.0–26	–	Subramaniam and Solomon (2006)
Bangalore (rural)	3.54	9.55	10.2	26.7[@]	5.67	3.01	3.81	10.6	7.12	Present study

$—Total DDT (*p,p'*-DDE, *p,p'*-DDD, *p,p'*-DDT, *o,p'*-DDT) in serum

@—∑HCH (α-HCH, β-HCH, γ-HCH, δ-HCH)

–Not available

comparatively less than the concentration reported in blood samples of the general population of the different cities of India (Kaphalia and Seth 1983; Ramachandra et al. 1984; Bhatnagar et al. 1992; Mathur et al. 2005) (Table 3). In this study, 2 samples (Case No. 8 and 23) had maximum levels of 394 µg/L and 318 µg/L of total HCH, respectively, which are 10-12 folds higher than the mean values reported for the rest of the population. It is worth mentioning that both participants reported chest pain for the last 3 years, which needs to be considered for further discussion. About 30% of the sample exceeded the tolerance limits of 10 µg/L for HCH set out under the prevention of food adulteration Act (Dudani 1994).

Levels of DDTs in the present study were similar to those in Madurai (8–26 µg/L) (Subramaniam and Solomon 2006), but levels of DDTs were lower than those reported in Lucknow (Kaphalia and Seth 1983), Delhi (710 µg/L) (Ramachandra et al. 1984; Saxena et al. 1987) and Ahmedabad (Bhatnagar et al. 2004). Elevated level of DDTs have been found in human breast milk samples collected from major cities in India (Devanathan et al. 2009), and other countries (Minh et al. 2004). However, total DDT concentration was less than levels reported in earlier studies (Table 3). Presence of *p,p'*-DDE, metabolites of *p,p'*-DDT suggesting wide usage in the past and long-term accumulation of DDTs in humans.

The cyclodiene group of insecticides, endosulfan and heptachlor epoxide recorded in the range of BDL-43.2 µg/L and BDL to 23.5 µg/L, respectively. Generally persons participating in pesticide exposure studies are in small numbers due to apprehension of giving blood samples. In our present study also it reflects the same. The voluntarily participation was only 30 numbers and the results might indicate the presence of pesticides in the environment as well as their food in these areas. Since these people are also handling pesticides for their crop protection and pest infection of their sheep and other domestic animals, unsystematic way of pesticide usage will affect their health as well as consumers. Therefore, awareness program on these issues is very much essential among the agricultural workers.

Acknowledgments We thank the Director, NIOH, Ahmedabad for extending constant support. We wish to express our sincere gratitude to the DG, ICMR, Delhi for his encouragement and continued guidance. We would like to acknowledge all staff of ROCH (S), for their help. The support and participation of the villagers is greatly appreciated.

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