EPIDEMIOLOGICAL STUDY FOR THE PRESENCE OF PESTICIDE RESIDUES IN HUMAN POPULATION OF PUNJAB

Thesis

Submitted to the Guru Angad Dev Veterinary and Animal Sciences University in partial fulfillment of the requirements for the degree of

MASTER OF VETERINARY SCIENCE
in
VETERINARY PUBLIC HEALTH
(Minor Subject: Veterinary Pharmacology and Toxicology)

By

Anupama Sharma
(L-2010-V-39-M)

Department of Veterinary Public Health and Epidemiology
School of Public Health and Zoonoses
College of Veterinary Science

GURU ANGAD DEV VETERINARY AND ANIMAL SCIENCES UNIVERSITY
LUDHIANA - 141004

2012

CERTIFICATE I

This is to certify that the thesis entitled **"Epidemiological Study for the Presence of Pesticide Residues in Human Population of Punjab"** submitted for the degree of **M.V.Sc.,** in the subject of **Veterinary Public Health** (Minor subject: **Veterinary Pharmacology & Toxicology**) of the Guru Angad Dev Veterinary and Animal Science University, Ludhiana is a bonafide research work carried out by **Dr. Anupama Sharma (L-2010-V-39-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

_____

(Dr. J. P. S. Gill)
Major Advisor
Director,
School of Public Health
and Zoonoses,
GADVASU, Ludhiana- 141004

CERTIFICATE II

This is to certify that the thesis entitled **"Epidemiological Study for the Presence of Pesticide Residues in Human Population of Punjab"** submitted by **Dr. Anupama Sharma (L-2010-V-39-M)** to the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, in partial fulfillment of the requirements for the degree of **M.V.Sc.,** in the subject of **Veterinary Public Health** (Minor subject: **Veterinary Pharmacology and Toxicology**) has been approved by the Student's Advisory Committee along with the Head of the Department after an oral examination on the same, in collaboration with an external examiner.

(Dr. J. P. S. Gill)
Major advisor

(Dr. J. K. Sharma)
External Examiner
Professor Retired
School of Public Health and Zoonoses
GADVASU, Ludhiana

(Dr. J. P. S. Gill)
Director

(Dr. S. N. S. Randhawa)
Dean, Postgraduate Studies

ACKNOWLEDGEMENTS

Foremost of all, I bow my head to the Almighty God, the merciful and compassionate with whose grace and blessings I have been able to accomplish this research successfully. His benign omnipresence in my life is the main key for my success.

The precious gift of learning is a debt that is difficult to pay; only gratitude can be left. Indeed the words at my command are inadequate in form or in spirit to express my heart-felt deep sense of unbound gratitude and indebtedness to my esteemed Major Advisor, ***Dr. J. P. S. Gill,*** *Director, School of Public Health and Zoonoses, for his altruistic guidance, constructive criticism, unceasing encouragement, whole hearted ever willing and untiring help and sense of forgiveness throughout my association with him.*

I owe my profound thanks to my advisory committee members ***Dr. Satyavan Rampal*** *(Minor Advisor), Professor-cum-head, Department of Veterinary Pharmacology and Toxicology,* ***Dr. R. S. Aulakh*** *(Dean PGs Nominee), Professor, School of Public Health and Zoonoses, and* ***Dr. Simranpreet Kaur****, Assistant Professor, School of Public Health and Zoonoses,* ***Dr. John Gordon,*** *University of Saskatchewan, Canada, for their timely and proper guidance at every point throughout the preparation of this manuscript.*

The words will be least to express sincere debt of gratitude to ***Dr. Baljit Singh,*** *Associate Dean (Research), Western College of Veterinary Medicine, University of Saskatchewan, Canada, as my supervisor during my research work in Canada. He has enlightened me through his wide Knowledge of Intelligence.*

Words are deserting my thoughts to express my special thanks and gratitude to ***Dr. J. S. Bedi****, Assistant Professor, School of Public Health and Zoonoses****. I would like to express my deepest sense of gratitude and indebtness to him for his keen interest, unstinted and invaluable guidance and supervision, untiring help and persistent encouragement during the course of this study. I express my gratefulness to* ***Dr. Rajneesh Sharma****, Assistant Professor. School of Public Health and Zoonoses,* ***Dr. Randheer****, Assistant Professor, School of Public Health and Zoonoses,* ***Dr. Kamaljeet Kaur****, Assistant Professor, School of Public Health and Zoonoses,* ***Dr. J. K Sharma****, for their generous support and co-operation during the entire period of my M.V.Sc*

I heartly thank ***Dr. Puneet A Pooni****, from Dayanand Medical College and Hospital, Ludhiana for granting permission and rendering help to collect mother's milk samples. Thanks are also due to* ***Dr. Prabha****, Research fellow, School of Public Health, PGIMER, Chandigarh,* ***Dr. Sameer Malhotra****, School of*

Public Health, PGIMER, Chandigarh ***Dr. J. S. Thakur****, School of Public Health, PGIMER, Chandigarh,* ***Dr. D. S. Pooni****,* ***Dr. Anubhav Khattar****, for providing help during collection of human blood samples.*

Pen and paper meagerly fail to express emotions as far as the love is concerned. I thank whole heartedly my parents, ***Sh. O. P. Raina*** *and* ***Smt. Kavita Kumari****, whose sacrifices remains as a source of inspiration and whose blessings kept me running with full spirit during tough times. I take this opportunity to thank my dear brother* ***Gaurav Sharma*** *for standing by me during my thick and thin.*

My special thanks to ***Dr. Sachit Sharma*** *for his constant inspiration, timely help and advice to complete this task. His moral support and useful criticism can never be forgotten. I can never forget the excellent company, selfless help and moral support extended by* ***Miss Prabhjit Kaur****. And how can I forget the love rendered, constant inspiration, advice and timely help offered by my senior cum friend* ***(Dr.) Anita Tewari*** *and my lovable junior* ***(Dr.) Kritima kapoor.***

I would also like to cherish the wonderful moments shared with ***(Dr.) Heena, Nandani, Gagandeep, Vigneshwaran, Upasna, Runjhun, Ketki, Sushma, Abhishek sir, Harpreet mam, Nasir sir, Satish sir, Bhavna mam****. I would like to thank the laboratory staff* ***Mr. Makhan****,* ***Mr. Baljinder****,* ***Mr. Mohan****,* ***Ranjhna mam*** *and* ***Anju mam*** *for their untiring help and selfless attitude.*

Lastly I thank all those who care for me. All may not be mentioned but none is forgotten.

Place: Ludhiana **(Anupama Sharma)**
Date:

Title of the Thesis	**:**	Epidemiological Study for the Presence of Pesticide Residues in Human Population of Punjab
Name of the student	**:**	Anupama Sharma
Admission No.	**:**	L-2010-V-39-M
Major Subject	**:**	Veterinary Public Health
Minor Subject	**:**	Veterinary Pharmacology and Toxicology
Name and Designation of the Major Advisor	**:**	Dr. J. P. S. Gill Director School of Public Health and Zoonoses
Degree to be Awarded	**:**	M. V. Sc.
Year of award of Degree	**:**	2012
Total pages in Thesis	**:**	102 + VITA
Name of University	**:**	Guru Angad Dev Veterinary and Animal Science University, Ludhiana - 141004

ABSTRACT

The present study was undertaken to determine the current status of pesticide residues in milk and blood samples from human population of Punjab. A total of 127 mother's milk samples and 111 human blood samples were analyzed by gas chromatograph and pesticide residues were detected in 25% of the milk samples and 36% of the blood samples. In mother's milk samples, residues of β-HCH, γ-HCH, p,p' DDE, p,p' DDT, cyfluthrin, cypermethrin, fenvalerate, chlorpyriphos, phosalone, profenphos and monocrotophos were detected with mean levels of 2.29, 2.64, 0.56, 3.03, 63.04, 3.63, 11.69, 1.91, 0.29, 2.66 and 1.63 ng g^{-1}, respectively. Cyfluthrin was leading pesticide detected in mother's milk contributing 27.77% to the total residue load. Residue levels were decreasing with increase in parity and age of mother and cyfluthrin had highest mean concentration of first parity and in youngest age group. Residue levels of all the pesticides were higher in urban population than the rural population although, statistically non-significant difference was found between the two ($p>0.05$). In the human blood samples, residues of α-HCH, β-HCH, p,p' DDD, p,p' DDE, p,p' DDT, β-endosulfan, monocrotophos, profenophos and phosalone were found with mean levels of 1.11 and 5.89, 0.51, 3.88, 0.39, 34.90, 0.79, 0.39 and 6.76 ng ml^{-1}, respectively, with β-endosulfan as leading pesticide. Statistically non-significant difference was observed in the levels of these residues between different age-groups, dietary habit, occupation, gender, living background and health status ($p>0.05$) according to t-test. A change in the pattern of pesticide residue levels was observed with a shift from organochlorines to organophosphates and synthetic pyrethroids.

Key words: Pesticide residues, gas chromatograph, mother's milk, human blood, Punjab

Signature of Major Advisor

Signature of Student

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS USED

%	:	Percent
bw	:	Body weight
°C	:	Degree celsius
D	:	Day
eV	:	Electron volt
et al	:	Et alia (and others)
Fig.	:	Figure
g/ha	:	Gram/hectare
hr	:	Hour
i.d.	:	Internal diameter
KV	:	Kilovolt
m	:	Minute
Mw	:	Molecular weight
m/z	:	Mass to charge ratio
m Pa s	:	Milipascal second
ND	:	Not detected
ng	:	Nanogram
ppm	:	Parts per million
sec	:	Second
W	:	Watt
ADI	:	Acceptable Daily Intake
DDT	:	Dichlorodiphenyl trichloroethane
p,p' DDD	:	4,4 dichlorodiphenyl dichloroethane
p,p' DDE	:	4,4 dichlorodiphenyl dichloroethylene
p,p' DDT	:	4,4 dichlorodiphenyl trichloroethane

o,p' DDE	:	2,4 dichlorodiphenyl trichloroethylene
FAO	:	Food and Agriculture Organisation
GC	:	Gas chromatography
GCMS	:	Gas chromatography mass spectrophotometry
HCH	:	Hexachlorocyclohexane
OCPs	:	Organochlorine pesticides
OPs	:	Organophosphates
SPs	:	Synthetic pyrethroids
USEPA	:	United States Environmental Protection Agency
WHO	:	World Health Organisation

CHAPTER I

INTRODUCTION

Pesticides are the chemical substances used for preventing, destroying or repelling the pests. They are among the most widely used groups of chemicals in modern world for the purpose of enhancing food production and improving health by destroying the insects and pests of food crops and vectors of human and animal diseases like malaria, dengue, encephalitis, filariasis etc. (Rekha and Naik 2006). However, the indiscriminant use of pesticides in crop protection, food preservation and vector control has resulted into their persistence in the environment and hence accumulation of these pesticides residues in the food chain which finally make their way into the human body. With the rise in human population, the territory of these pests has increased too and therefore, there is a need for effective and stronger alternatives to meet food security and to combat disease causing vectors (Hodgson 2003). Most pesticide users have little knowledge about these chemicals and thus the pesticide usage has been abused unfortunately. Most of the farmers think that more often they apply pesticides, greater will be the chance of destroying crop pests (Ntow *et al* 2006).

There are several categories of pesticides such as insecticides, fungicides, herbicides etc. Out of these only the insecticides are of primary concern in terms of their persistence in the environment and their adverse effects on human health. Insecticides include organophosphorus insecticides (OPIs), organochlorine insecticides (OCIs), carbamates, pyrethrins and pyrethroid insecticides. Due to their lipophilicity, hydrophobicity and the low rates of chemical and biological degradation, OCPs have

wide spread accumulation in food chain (Gill 2000, John *et al* 2001, Bedi *et al* 2005, Aulakh *et al* 2006) and subsequent magnification of concentration in human, a final link in food chain (Surendernath *et al* 2001). The route of exposure of these pesticide residues is either through the inhalation of vapors or through the consumption of milk as well as fruits and vegetables sprayed with these pesticides. Organochlorine pesticide residues get accumulated in the superficial soils, volatilized from their site of deposition and thus become a source of exposure to inhabitants via their vapors (Algeria *et al* 2008, Wong *et al* 2008). Food consumption mainly of animal origin that previously accumulated organochlorine pesticides become another source of exposure. Dermal contact is another important route of exposure (Rekha and Naik 2006). Mostly the farmers use either moderately or highly hazardous pesticides for spraying without using any form of protection while handling pesticides and are thus directly exposed to pesticides (Chitra *et al* 2006). Sometimes the exposure to the pesticides also occurs through faulty spraying equipments, leakage or accidental spillage of these pesticides. The residues of these pesticides get accumulated in the lipid- rich tissue in the body. The determination of the levels in human tissues like blood or adipose reflects the magnitude of local environmental pollution (Walliszewski *et al* 2010).

In India there has been massive production and consumption of pesticides in the past. Even in 1990's India was still using more than 70% of the pesticides formulations which were banned or severely restricted in the east and west (Iyer 1993, Gupta 2004). India occupies fourth position in the world after US, Japan and China in terms of total pesticides production (Gupta 2010). Apart from US, India is the only country which has applied more than 100 000 tons of DDT since its formulation, mainly in its agricultural

and malarial control programs until it was banned in agricultural use in 1989 (Anon. 1991, Kannan *et al* 1995, Voldner and Li 1995). Approximately 3750 tons of DDT was used by India in the year 2001 under National Malarial Program (NMP) in rural and peri-urban areas for residual spraying (Gupta 2004). Li *et al* (2003) has stated that India is one of the greatest consumer of HCHs and the most contaminated nation in the world. India has the highest concentrations of HCHs in its environmental and biological samples than in several other countries (Kannan *et al* 1997). The findings of persistent organochlorine residues like DDTs, HCHs and PCBs in human breast milk of Indian women (Tanabe *et al* 1990, Kalra *et al* 1994, Gill *et al* 2000, Minh *et al* 2003, Sanghi *et al* 2003) is of primary concern because infants and children may be susceptible to the toxic effects of any toxicant than the adults.

Punjab, due to its success in agricultural green revolution is among India's most prosperous state. No doubt, green revolution has led to the far-reaching and irreversible effects in the commercialization of agriculture, but it has also led to the contamination of soils and water systems from the use of pesticides, chemical fertilizers, modern irrigation systems and dependency on modern machinery and technology (Kaur and Sinha 2011). Punjab comprises of 1.57% of country's geographical area, contributes nearly 40% wheat and 60% rice to the central pool and is a classic example of fastest growing economy with agricultural basis (Punjab State Council for Science and Technology, 2005) but at the same time it consumes approximately 17% of the total pesticides used in India. The per hectare pesticide use is highest in Punjab (923 g/ha) as compared to other agriculturally advanced states like Haryana, Andhra Pradesh, Tamil Nadu, Karnataka and Gujarat (Agnihotri 2000, Tiwana *et al* 2009). About 54% of the total pesticides used in

Indian agriculture are consumed on cotton alone, though it accounts for only 5% of the total cultivated area (Puri *et al* 1999). In Punjab, most of the pesticide is used in the cotton belt, mainly the Bhatinda district. Such a widespread use of pesticides leads to their persistence in the environment and subsequently their adverse effects. There are studies which link environmental degradation with various diseases in Punjab. Halder (2009) found the premature graying of hair in children as early as ten years old, ageing and predisposition to cancer in Jajjal village of Bathinda district of Punjab. Another study by Thakur *et al* (2008) found the prevalence of various pesticides above the permissible limits in tap water and vegetable samples in Talwandi Sabo area in Bathinda district of Punjab.

Organochlorine pesticides (OCPs) are highly lipophilic as well as persistent and thus can bioaccumulate through food chain. The exposure of these chemicals to humans occurs through intake of food (Ahlborg *et al* 1992, Liem and Theelan 1997). Increased accumulation of these chemicals in food chain leads to serious health hazards (Jayashree and Vasudevan 2007). For example, exposure to organochlorine compounds affects thyroid function in children. Other effects are birth defects, low sperm count in males, increase in testicular cancer and other reproductive and developmental defects (Weltman and Norback 1983).

Exposure to OCPs also cause disruption of endocrine system by altering the hormonal balance (Colborn *et al* 1993, 1996; Kelce *et al* 1994, 1997; Janssen *et al* 1997, Beard *et al* 2000). Accumulation of OCPs increases risk of various types of human cancer including breast, lung, cervix, prostate, endometriosis, hypospadias and cryptorchidias (Wolff *et al* 1993, 2000; Birnbaum 1994, Rier *et al* 1995, Hosie *et al*

2000, Ahmed *et al* 2002, Amaral Mendes 2002) and genotoxic effects (Ennaceur *et al* 2008a,b). It is also associated with reduced growth (Gladen *et al* 2000, 2003; Longnecker *et al* 2001, Nagayama *et al* 2007, Alvarez-Pedrerol *et al* 2008, Schell *et al* 2008), mental and psychomotor development (Dorner and Plagemann 2002, BBC 2006, Ribas-Fito *et al* 2006, Sagiv *et al* 2008). Neurological and immune system disorders and infection are also related with OCP exposure (Bernier *et al* 1995, Karmaus *et al* 2003, Ntow *et al* 2008).

The exposure to organophosphorus compounds causes impaired neurobehavioral performance and disrupt learning and memory processes (IPCS/WHO 2001). Exposure to Malathion causes dose-dependent increase in the frequency of chromosomal aberrations and sperm abnormalities without affecting total sperm count (Giri *et al* 2002). Exposure of pregnant women to organophosphates leads to decreased fetal growth (Eskenazi *et al* 2004, Whyatt *et al*, 2004) and short gestation length (Eskenazi *et al* 2004) as a result of maternal- fetal transfer of organophosphates pesticides (Whyatt *et al* 2003).

Residues of these chemicals get deposited continuously in the human blood and tissues especially the fat rich tissues. The breast tissue of women is the main site for deposition of these residues which is thus of great concern because of the excretion of these residues in the breast milk which in turn becomes a continuous route of exposure to infants. Keeping in view the importance of pesticides in humans and their subsequent health hazards, exposure to infants through mother's milk and increasing incidence of chronic diseases in the human population of the cotton belt of Punjab, the present study was undertaken to assess the current load of pesticides in the human population. Several

studies have been conducted in the past but the present status is not known regarding their consumption and accumulation pattern. So the objectives of our study are:-

1. Monitoring of pesticide residues in blood/fat/milk of the human population of Punjab

2. Epidemiological studies to find the correlation of the pesticide residues with the health status and health- associated parameters.

CHAPTER II

REVIEW OF LITERATURE

Punjab is the second highest user of pesticides in India after Uttar Pradesh (Dureja and Gupta 2009). No doubt the use of pesticides lead the country to a far- reaching stage but, at the same time, it is continuously adding to the deterioration of the quality of environment which has a direct bearing on the health of the human beings. Although the pesticides are being developed under very strict regulations in order to minimize their impact on human health still there are raising concerns about the health risks from occupational exposure as well as from residues in food and drinking water (Damalas and Eleftherohorinos 2011). The sprayer population involved in mixing, loading, transportation and application of formulated pesticides are exposed to the hazardous effects of these pesticides because of the inadequate means of protection and direct exposure to pesticides. Exposure to general population occurs mainly through eating food and drinking water contaminated with the pesticides but substantial exposure may also occurs through living close to a workplace that uses pesticides (Jaga and Dharmani 2003). There are the studies reporting the direct relationship between the extent of pesticides use and the signs and symptoms of the illnesses due to exposure of farmers to the pesticides. Chitra *et al* 2006 conducted a study on the farmers in the Thanjavur district (South India). Six hundred thirty one farmers were interviewed during the cross-sectional survey, 537 being men and 94 women. Four hundred thirty three (68.6%) farmers including 4 women sprayed pesticides by themselves and thus were directly exposed to pesticides. More than 75% farmers used either “moderately” or “highly

hazardous" pesticides. Eighty eight percent did not use any form of protection while handling pesticides. About 50% of the sprayers mixed different brands of pesticides, many of which were substitutable to each other. Fifty six percent of farmers obtained information on pesticides from retail shop owners. The acute signs and symptoms observed in farmers were: excessive sweating (36.5%), burning/stinging/itching of the eyes (35.7%), dry/sore throat (25.5%), excessive salivation (14.1%). These signs and symptoms had a higher prevalence among the sprayers. Among men, excessive sweating, burning/stinging/itching eyes, dry/sore throat were significantly associated with exposure to pesticides.

Whatever may be the source of exposure, the route of entry, the concentration of pesticides in the environment and the duration of exposure, the residues of these pesticides get accumulated in the various tissues of human beings viz., blood, breast tissue, adipose tissue and other fat-rich tissues.

2.1 Pesticides residues in human blood and human milk

2.1.1 Pesticide residues in human blood

Agarwal *et al* (1976) examined blood samples from 182 people in Delhi, India, for DDT residues. All except eight contained DDT and its metabolites. In the whole blood average total DDT concentration ranged from 0.177 to 0.683 mg l^{-1} in males and from 0.166 to 0.329 mg l^{-1} in females. p,p' DDE, p,p' DDD and o,p' DDT were the DDT metabolites detected. p,p' DDE accounted for most of the total DDT.

Saxena *et al* (1987) analyzed blood from 50 volunteers from residents of area surrounding a DDT manufacturing factory in Delhi, India. It was found that mean total DDT in male (34.4 µg l^{-1}) was higher than that of female (22.9 µg l^{-1}). A very high level of DDT and its metabolite was found in whole blood of occupationally unexposed population of that area. Total DDT ranged from 0.53 to 66.3 µg l^{-1} with a mean value of 30.1 µg l^{-1}. Mean blood concentrations of (µg l^{-1}) of p,p' DDE, o,p' DDT, p,p' DDD, p,p' DDT and total DDT were 12.9 ± 0.61, 0.66 ± 0.44, 0.00005 ± 0.00007, 10.2 ± 0.79 and 30.1 ± 16.9, respectively.

Kanja *et al* (1992) analyzed a total of 41 samples of maternal blood, milk, subcutaneous fat and umbilical cord from mothers giving birth by caesarean operation at Kenyatta National Hospital in Nairobi in 1986 for organochlorine pesticide residues. The main contaminants found in all samples were p,p' DDT(100%), p,p' DDE (100%) o,p' DDT(59%), dieldrin (27%), transnonachlor (15%), β-HCH (12%) and lindane (2%) of all the samples analyzed. The mean level (mg kg^{-1} of fat) of total DDT was 5.9 in subcutaneous fat, 4.86 in mother's milk, 2.75 in maternal serum and 1.9 in maternal cord serum. β-HCH was found in subcutaneous fat and milk fat with the mean levels of 0.03 and 0.26 mg kg^{-1} fat, respectively.

Dua *et al* (1996) monitored HCH and DDT contents in whole blood of general population of 37 males who were not involved in spraying of crops in Haridwar, India. Mean concentrations of HCH and DDT were 21.5 µg l^{-1} and 20.79 µg l^{-1}, respectively. Similarly 47 samples of occupationally exposed persons, involved in spraying operations of HCH and DDT during Ardh Kumbh Congregation at Haridwar in April, 1992 for the control of mosquitoes and flies, were screened for HCH and DDT contamination in

whole blood. Mean concentration of HCH was 68.01 μg l^{-1} and DDT 58.43 μg l^{-1} i.e., 3.1 times and 2.8 times more as compared to general population, respectively.

Nair *et al* (1996) conducted a study in Delhi, in which samples of maternal blood, breast milk and cord blood from 25 mothers and their newborn from Irwin Hospital, Delhi were screened for concentration of DDT and HCH. DDT was present at an average level of 1270, 27 and 14 μg l^{-1}, respectively. Breast milk contained four and a half times more DDT than the maternal serum. Levels of different metabolites of DDT in maternal serum were more than those in cord serum. HCH isomers were present in smaller amounts than those of DDT residues. The average value of HCH in the maternal blood, breast milk and cord blood was 32.7, 0.50 and 0.33 μg l^{-1} respectively. β-HCH isomer was the predominant isomer accounting for more than 60% of the various HCH isomers.

Dua *et al* (2001) analyzed serum samples of general human population in Nainital for DDT and HCH residues. The HCH residues were detected within range of 0.73-7.85 mg l^{-1}, while DDT was varying from 1.95-15.54 mg l^{-1} of blood. β-HCH isomer was reported highest in comparison to other 2 isomers (α-HCH and γ-HCH).

Bhatnagar *et al* (2004) analyzed eighteen serum samples for the residues of DDT, HCH and HCB. Serum levels of p,p' DDE, o,p' DDT, p,p' DDD and p,p' DDT ranged from 10.43-38.33, 0.42-2.41, 0.77-4.43 and 3.66-24.06 with a mean of 20.85, 1.15, 2.03 and 9.28 μg l^{-1} respectively. However, the total DDT content in serum samples had a mean of 32.61 μg l^{-1} ranging from 21.17-54.47 μg l^{-1}. p,p' DDE contributed about 64% of the total DDT. Ratio of DDE to ∑DDT ranged from 0.39 to 0.93. This ratio is an indicator of the extent of DDT degradation and its metabolite formation. Levels of α-

HCH, β-HCH and γ-HCH in serum samples had a mean of 4.49, 35.06 and 1.69 μg l^{-1} respectively. β-HCH contributed about 85% of the total HCH. HCB was identified in 7 samples in the range of 0.13-0.27 μg l^{-1} with an average of 0.2 μg l^{-1}.

Mathur *et al* (2005) analyzed blood samples from four Villages of Punjab for pesticides. 0.57 μg l^{-1} of HCH, 0.23 μg/l of lindane were detected in the blood samples which ranged from 0.14 to 0.57 μg l^{-1}. Similarly the levels of DDT, endosulphan were 0.65 μg l^{-1} and 0.45 μg l^{-1}, respectively. The levels of monocrotophos, chlorpyriphos, malathion and phosphamidon were detected at mean levels of 0.95, 0.66, 0.30 and 0.37 μg l^{-1}, respectively.

Lino and Silveria (2006) evaluated the contamination level of Portuguese population by estimating organochlorine pesticide residues in human serum from students of university of Coimbra, Portugal. Endosulphan sulphate, p,p' DDE, o,p' DDT and p,p' DDT were the most frequently identified residues. The highest concentration of endosulphan was 42.6 μg $l^{-1}$with range of undetected to 129.5 μg l^{-1} while for the o,p' DDT and p,p' DDT highest levels were 24.8 and 21.9 μg l^{-1}, respectively.

Subramaniam and Solomon *et al* (2006) studied the BHC and DDT levels in the blood samples from the human population of Madurai, India. People were divided into two groups, the group one people were directly exposed to pesticides like agriculturists and public health workers and the group two people were indirectly exposed to pesticides through food chain. High concentrations of BHC and DDT were observed in the serum samples of the people directly exposed to pesticides. The pesticide residue concentration in the serum ranged from 0.006 to 0.130 ppm for BHC and 0.002 to 0.033 ppm for DDE.

Pathak *et al* (2008) analyzed the levels of organochlorine pesticide residues in maternal and cord blood samples of North Indian normal healthy women with full time pregnancy and also studied the status of pesticide burden in newborns. Out of the total organochlorine residue, HCH was at highest level followed by endosulfan, p,p' DDE and p,p' DDT being the least. The data indicated a transfer rate of 60-70% of these pesticides from mother to newborn which is of great concern as it may adversely affect the growth and development of newborn.

Hayat *et al* (2010) analyzed 27 blood samples from field workers involved in pesticide spraying activities at three different farms in tehsil Mailsi in the district Vehari, Pakistan ranging from 16 to 50 years of age and having one to nine years of pesticide application experience for 383 pesticides by Gas Chromatograph Mass Spectrophotometer (GC-MS) multi-residue analytical technique. Only chlorpyrifos and pyributicarb were detected in the blood samples in the concentration of 0.009 mg l^{-1} and 0.001mg l^{-1} respectively.

Organochlorine pesticides being highly lipophilic get accumulated in lipid rich compartments and thus can easily pass through the placental barrier and appear in the cord blood. Herrero –Mercedo *et al* (2010) monitored the levels of organochlorine pesticides in 70 umbilical cord blood samples in Veracruz, Mexico. The levels of organochlorines detected were as: β-HCH (4%, 3.9 μg l^{-1} median concentration on wet weight, p,p' DDE (100%, 0.7 μg l^{-1}) and p,p' DDT (4%, 1.4 μg l^{-1}) .

Herrero-Mercado *et al* (2011) determined levels and calculated ratios of copartition coefficients among organochlorine pesticides β-HCH, p,p' DDE, o,p' DDT and p,p' DDT in maternal adipose tissue, maternal blood serum and umbilical blood

serum of mother-infant pairs from Veracruz, Mexico. Organochlorine pesticides were analyzed in 70 binomials: maternal adipose tissue, maternal serum and umbilical cord serum samples. The results were expressed as mg/kg on fat basis. p,p' DDE was the major organochlorine component, detected in every maternal adipose tissue (0.770 mg kg^{-1}), maternal serum sample (5.8 mg kg-1 on fat basis) and umbilical cord blood sample (6.9 mg kg^{-1} on fat basis). p,p' DDT was detected at 0.101 mg kg^{-1}, 2.2 mg kg^{-1} and 5.9 mg kg^{-1} respectively in maternal adipose tissue, maternal serum and umbilical cord serum samples. β-HCH was detected at 0.027 mg kg^{-1}, 4.2 mg kg^{-1} and 28.0 mg kg^{-1} respectively. o,p' DDT was detected only in maternal adipose tissue at 0.011 mg kg^{-1}. The copartition coefficients among samples identify significant increases in concentrations from adipose tissue to maternal blood serum and to umbilical blood serum. The increase indicated that maternal adipose tissue released organochlorine pesticides to blood serum and that they are carried over to umbilical cord blood.

2.1.1 Pesticides residues in human milk

Al-saleh *et al* (2003) designed a cross sectional study to measure DDT residues and its metabolites in breast milk samples collected randomly from Saudi lactating mothers living in Al-Ehssa region; which was under leishmania control until 1995. The samples were compared to samples from mothers living in Riyadh region where no spraying activities were involved. p,p' DDE, p,p' DDD and p,p' DDT residues were measured in 878 breast milk samples. Variation in the DDT and its metabolites levels were investigated with respect to regional distribution. Wilcoxon rank sum tests showed that the average ranks of p,p' DDE, p,p' DDD, p,p' DDT and ∑DDT in lactating mothers from Al-Ehssa region were significantly higher than those living in Riyadh region. It was

estimated that 99.2% of infants of lactating mothers living in Al-Ehssa region had ∑DDT daily intakes that exceeded 20 μg/Kg-day of body weight, the WHO/UNEP Acceptable Daily Intakes for a 5-Kg infant. Exposure of infants to these chemicals through breast-feeding is clearly a public health concern.

Kumar *et al* (2006a) analyzed the levels of the DDT and HCH residues in human milk collected from remote rural part of Agra. Out of total HCH excreted in breast milk, δ-HCH was not detected in the samples, β and γ isomers of HCH accounts for the major residue. A total of 95% of the samples were found to be contaminated with DDT and its metabolites. DDD was found in 88% of the samples analyzed. The total concentration of DDT and HCH were found lower than the previous studies carried out in India. The study shows the decreasing levels of these organochlorine pesticides from the environment. The concentration of total DDT is higher than total HCH.

Kumar *et al* (2006b) analyzed blood and milk samples collected from lactating women in Anupgarh, Rajasthan who were divided into four groups on the basis of different living standards viz residence area, dietary habits, working conditions and addiction to tobacco. The level of total organochlorine pesticides in blood ranged from 3.319-6.253 mg l^{-1} while in milk samples it ranged from 3.209–4.608 mg l^{-1}.

Subramanian *et al* (2007) analyzed mothers' milk samples from Chennai (formerly Madras), India and three other places Perungudi, Chidambaram, and Parangipettai. Measurable concentrations of HCHs, DDTs, PCBs, CHLs and HCB were reported. It was also observed that the levels of the two organochlorine pesticides (HCHs and DDTs) increased in Chennai mothers' milk in the last decade. Levels of the two classical organochlorines (DDTs and HCHs) have declined in many of the food items

when compared with data collected two decades before in the same locations, showing the effectiveness of the ban on both these chemicals in the country

Mueller *et al* (2008) analyzed 157 human milk samples collected during 2002 and 2003 as well as 24 samples collected in 1993 were analysed as 20 regional pools for 17 OCPs. OCPs were detected in all pooled human milk samples from 2002-03 typically with highest concentrations of p,p' dichlorodiphenyldichloroethylene (p,p' DDE) (mean ± standard deviation; median concentration 311 ± 174; 279 ng g^{-1} lipid) followed by β-hexachlorocyclohexane (β-HCH) (80 ± 173; 21 ng g^{-1} lipid). Other OCPs consistently detected included dieldrin (16 ± 6; 17 ng g^{-1} lipid), hexachlorobenzene (HCB) (18 ± 16; 14 ng g^{-1} lipid), transnonachlor (11 ± 5; 9 ng g^{-1} lipid) and p,p' dichlorodiphenyltrichloroethane (p,p' DDT) (9 ± 6; 7 ng/g lipid).

A free analysis of milk was offered to lactating mothers residing in the state of Lower Saxony, Germany by Zeitz *et al* (2008). The human milk was analyzed over a period of 8 years for 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT), hexachlorobenzene (HCB) and b-hexachlorocyclohexane (b-HCH). In total, 4314 samples were collected in the years 1999–2006 and analyzed for their content of these persistent organic pollutants (POPs). A clear downward trend of median total, DDT, β-HCH and HCB values in all participants and also in different selected subgroups could be observed. The median values of calculated total DDT was 0.0815 mg kg^{-1} lipid, that of β-HCH 0.0116 mg kg^{-1} lipid and of HCB 0.0229 mg kg^{-1} lipid. There were reductions between 40.9% and 47.1% compared to the year 1999. Among other influencing factors, median concentrations of total DDT, β-HCH and HCB showed a clear rise with increasing age of mothers whereas an increasing number of breastfed infants per mother

led to a decrease. The proportions of other measured substances exceeding limits of quantification were as follows: dieldrin 68.6%, α-HCH 1.3%, γ-HCH 60.1%, heptachlor epoxide 41.5%.

Polder *et al* (2009) determined polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) levels in 423 breast milk samples from women living in Norway. Median concentrations (range) of PCBs, p,p' DDE, HCB, β-HCH and oxychlordane in breast milk of the Norwegian women, all parities included, were 103 (34–450), 41 (5.4–492),11 (3.6–24), 4.7 (0.9–37) and 2.8 (0.5–16) ng g^{-1} lipid weight, respectively. Results indicated that sum of 18 PCBs, p,p' DDE and β-HCH are good predictors for monitoring of PCB, DDT and HCH levels in Norwegian breast milk. Age was strongly associated with increasing OC levels (Pb0.001), whereas parity was associated with decreasing OC levels (Pb0.001). Smoking was associated with higher levels of PCBs, p,p' DDE and β-HCH.

The level of some organochlorine pesticides keeps on varying with the different periods of lactation. Waliszewski *et al* (2009) conducted a study to determine organochlorine pesticide levels in breast milk samples from 4^{th} to 30^{th} day of lactation and the trend in their concentration time to forecast the time tendency of residue levels and the pesticides excretion pattern. Fourty samples were analyzed by GLC- ECD. The organochlorine pesticides residues determined in the breast milk samples during lactation decreased. β-HCH decreased from 0.095 to 0.066 mg kg^{-1}, p,p' DDE from 1.807 to 1.423 mg kg^{-1} and p,p' DDT from 0.528 to 0.405 mg kg^{-1}.

Mishra and Sharma (2011) demonstrated the concentrations of organochlorine contaminants, DDT and HCH in human breast milk from Dibrugarh and Nagaon districts

of Assam state, North-East India. Mean levels of total DDT were 3210 ng g^{-1} lipid weight and 2870 ng g^{-1} lipid weight in Nagaon and Dibrugarh respectively. Likewise the mean levels of total HCH were 2720 ng g^{-1} lipid weight and 2330 ng g^{-1} lipid weight in Nagaon and Dibrugarh respectively. The levels of investigated pollutants between the two districts did not differ significantly. There was a significant difference in ADI (Average daily intake) for DDT between the two districts. A positive correlation was observed between OCP levels in breast milk and age of mothers. The ADI by the infants has been estimated on the basis of OCP levels in human breast milk. High daily intake of DDTs and HCHs by the infants exceeded the TDI (Tolerable daily intake) which implied the high risk to the infants of the region by these contaminants.

Tsang *et al* (2011) determined the body burdens of persistent organic pollutants in 29 human milk samples and 21 human blood including cord blood samples collected from residents of Hong Kong. The samples were analyzed for OCPs levels. Higher levels of DDTs were detected in human milk samples when compared to maternal serum and cord serum (Breast milk: 3099 ng g^{-1} fat, maternal serum: 1934, cord serum: 1556). p,p' DDE and p,p' DDT were the only metabolites of DDT detected in the three types of human tissues. High detection rate of the p,p' DDE and p,p' DDT (90%) were noted in the three types of human samples. The higher levels of DDTs in the three types of human tissues were correlated together with fish consumption, maternal age and tissue fat. It was found that more the consumption of freshwater and marine fishes, higher are the ΣDDTs and p,p' DDE levels in human milk. Further it was found that more the maternal age higher are the residues levels of these pesticides. Seven out of 29 human samples exceeded the tolerable daily intake (TDI) which is 20 ng g^{-1} day^{-1} as proposed by the

Health Canada Guidelines in terms of DDTs levels. The high intake of DDTs by infants may be of concern as infants are more susceptible to the adverse effects imposed by various environmental contaminants.

Tutu *et al* (2011) conducted a study to determine the types and levels of organochlorine pesticide residues in the breast milk of 21 primipara mothers in La, a suburb of Accra an urban community in the Greater Accra region of Ghana. Fourteen different organochlorine pesticides residues namely p,p' DDT, p,p' DDE, gamma-HCH, delta-HCH, heptachlor, aldrin, endrin, endrin-aldehyde, endrin-ketone, alpha-endosulphan, endosulphan-sulphate, gamma-chlordane, dieldrin, and methoxychlor were identified and quantified in the individual breast milk samples using a Gas Chromatograph (GC) with an Electron Capture detector. p,p'- DDE recorded 100% incidence ratio. Also p,p' DDT, delta-HCH, gamma-HCH, and endosulfan sulfate recorded incidence ratios of 76.79, 95.25, 80.95 and 85.71%, respectively for the breast milk samples. The concentrations of organochlorine pesticide residues in the human breast milk samples ranged from 1.839 to 99.05 μg kg^{-1} fats. With the exception of Endosulphan Sulphate whose mean concentration (99.052 μg kg^{-1}) was above the Australian Maximum Residue Limit (MRL) of 20 μg kg^{-1} for milk, the mean concentrations for all the other organochlorines detected were below their respective limits.

Zhou *et al* (2011) investigated the occurrence of persistent organochlorine pesticides (OCPs) in breast milk samples collected from mothers from twelve provinces in mainland China. Dichlorodiphenyltrichloroethanes (DDTs) were the most prevalent agent, followed by HCHs and HCB, whereas levels of chlordane compounds, drins and

mirex were lower. The relatively lower DDE/DDT ratio in the Fujian rural area suggested more recent exposure to DDT than in other areas. The mean level of DDTs in breast milk from the southern China was higher than those from northern China ($p < 0.05$). There was observed a positive correlation between concentration of DDTs in human milk and consumption of animal-origin food, suggesting that this parameter could play an important part in influencing OCPs burdens in lactating women. The mean estimated daily intakes of different OCPs for breastfed infants were lower than the tolerable daily intake.

CHAPTER III

MATERIAL AND METHOD

3.1 Preparation and purification of chemicals and glassware

Purification of reagents and solvents is of utmost importance to avoid the interferences from reagents and solvents. Reagent blank sample was run for reduction of any kind of contamination of post purification. All the chemicals of analytical reagent (AR) grade were obtained from E. Merck and Sd-fine (India) Ltd.

3.1.1 Glassware

All the glassware was thoroughly washed with extran and after proper rinsing with distilled water, dried in hot air oven. Before use of these glasswares they were again rinsed with solvents to make them free from residual contamination, if any.

3.1.2 Solvents

All the solvents viz., acetone, acetonitrile, chloroform, dichloromethane (DCM), diethyl ether, hexane and methanol were of analytical grade and were re-distilled using all glass apparatus. n-hexane and acetone of HPLC grade were used for making of different dilutions of pesticide standards and for the reconstitution of the final evaporated sample extract**.**

3.1.3 Florisil

It was procured from Merck with 60-100 mesh size, the powder was washed with distilled water twice and later on dried in hot air oven at 130°C for one hour. The dried

florisil was then transferred to silica bowls and activated at 400°C for 3hours in muffle furnace and stored in desicator at room temperature**.**

3.1.4 Silica gel

Silica gel of 60-120 mesh size weighing 200 g was taken in one litre Erlenmeyer flask and to it 500 ml of methanol was added. The contents of flask were shaken for 30 mins and filtered through filter paper. The washed silica gel was transferred to another one litre Erlenmeyer flask containing 500 ml acetone. After stirring again for 30 minutes the contents were filtered through filter paper. Silica gel was then dried and activated by heating at 400°C for 2 hours in muffle furnace and stored in desicator at room temperature in air tight glass bottles.

3.1.5 Sodium sulphate and sodium chloride

Anhydrous granulated reagent grade sodium sulphate and sodium chloride from from E.Merck were used for analysis. These may contain phthalate esters as impurities which may interfere in analysis of pesticide while using ECD, therefore to remove these esters the compounds were heated at 600°C for 3 hours in muffle furnace and then stored at room temperature in desicator.

3.1.6 Glass wool/cotton

To avoid any contamination from glass wool or cotton, these were used after washing with hexane or acetone and then air dried.

3.1.7 Activated charcoal

Activated charcoal was used as such without any treatment.

3.1.8 Water

Double distilled water was used in all experiments.

3.2 Preparation of pesticide standard solution

Analytical standards of OCPs, OPs and SPs (Table 1) were purchased from Sigma-Aldrich and Rankem Ltd. Purities of pesticide standards were in between 93-99%.

Table 1: List of the pesticides standards used in present study

Organochlorine pesticides		**Organophosphorus pesticides**	**Synthetic pyrethroids**
α-HCH	Endrin	Monocrotophos	L-Cyhalothrin
β-HCH	Endosulphan	Dimethoate	Permethrin
γ-HCH	pp-DDD	Parathion	Cyfluthrin
δ-HCH	op-DDD	Carbaryl	Cypermethrin
Heptachlor	Endosulfan sulphate	Malathion	Fenvalerate
Fenitrothion	pp-DDT	Chlorpyrifos	Deltamethrin
Aldrin		Fenamiphos	
Fipronil		Profenophos	
Butachlor		Ethion	
Dieldrin		Triazophos	
pp-DDE		Phosalone	
op-DDD			

3.3 Collection of samples

3.3.1 Sampling

Around 111 samples of human blood and 127 samples of human milk were collected from Punjab state. All the samples were collected after taking consent from the donors. Venous blood (10 ml) of 111 people from two districts of Punjab- Bhatinda and

Muktsar were collected in residue free heparinised 20 ml glass vials containing 200 USP units of heparin in 0.2 ml solution with the help of sterilized syringe. These samples were collected with the help of researchers from PGIMER, Chandigarh under an existing research project in collaboration with our laboratory. Blood samples were transported in dry ice in the laboratory and stored at -20°C until analyzed. Similarly mother's milk samples were collected from local Medical College and Hospital of Ludhiana. Around 10 ml of milk was expressed by mothers which was collected in sterilized glass bottle and brought to laboratory under chilled conditions. The mother's milk samples were collected after taking the prior approval of ethical committee of the hospital and signed consent were taken from mothers on consent letter countersigned by the samples collector. All donors of blood and milk samples were to answer a questionnaire, to provide their socio-demographic data, food consumption habits, age, weight, height, place of birth, place of residence, years of residence etc. as mentioned on the performas.

3.4 Extraction and cleaning of pesticides residues

3.4.1 Extraction of residues in human blood samples.

For extraction of pesticide residues from human blood samples, blood (5 ml) was diluted with 25 ml distilled water and 2 ml of saturated brine solution added and transferred to 125 ml capacity separatory funnel and extracted by hexane: acetone (1:1) 20 ml thrice by shaking the separatory funnel vigorously for 2-3 minutes, releasing the pressure intermittently. The layers were allowed to separate. The three combined extracts were passed through anhydrous sodium sulfate and concentrated to about 1-2 ml using rotary vacuum evaporator.

Consent Form

I confirm that

- I have read/understand/carefully considered the study
- I had a chance to ask questions and have been fully answered
- My participation in this study and providing milk sample is voluntary
- I agree to allow all study procedures as required
- I understand that I am free to discontinue at any stage
- I have received a copy of information and consent
- I am aware that the data and records are kept confidentially

My address is:

Name:

Signature: Date:

Name of person taking consent:

Signature: Date:

Proforma for collection of mother's milk samples

<table>
<tr><td colspan="2">Name of mother:</td></tr>
<tr><td colspan="2">Address:

Phone no</td></tr>
<tr><td>Place of birth:</td><td>Age: _____ (Years)</td></tr>
<tr><td>Weight: _____ (Kg)</td><td>Height: ____ (feet) ____ (inch)</td></tr>
<tr><td>Occupation: Working/Housewife (Tick any option)</td><td>Dietary Habits: Vegetarian / Non-Vegetarian</td></tr>
<tr><td>Source of drinking water:
Municipality / Submersible pump / Hand pump</td><td>Spraying of pesticides around house for mosquito control:
Yes/No</td></tr>
<tr><td rowspan="2">Spraying of pesticides inside of house for mosquito control: Yes/No</td><td>Parity: Ist 2nd 3rd</td></tr>
<tr><td>Number of breastfed children:
One/Two/Three</td></tr>
<tr><td>Previous History of:
Abortion/Miscarriage/Still birth/None</td><td>Reproductive/respiratory/neurologicalproblem: tick if any</td></tr>
<tr><td>Gestational period: _____ (days)</td><td>Av. daily intake of milk and milk products: _____ (gm)</td></tr>
<tr><td>Sex of child: Male/Female</td><td>Weight of child at time of birth:
_____ (kg)</td></tr>
<tr><td>Length of child at time of birth: _____ (cms)</td><td>Age of child at time of sampling:
_____ (days)</td></tr>
<tr><td>Circumference of head: _____(cms)</td><td>Any genetic/pathological/physiological problem: Yes/No
If yes: nature of problem</td></tr>
</table>

Proforma for blood collection

Name of the Person	
Complete Address	
Age	
Sex	
Height	
Weight	
Food habits	
History of any disease condition Unhealed ulcers in mouth/oral cavity Persistent coughing Weight loss Urogenital problems Reproductive problems Skin problems Hypersensitivity Any other chronic disease Nervous systems/Neurotoxicity Hand numbness Dizziness Red Eye Muscle pain Stomach Pain Breathing Difficulties Headache Nail infection or decay Vomiting Visual or hearing problems	
Tobacco chewing/smoking	
Protective measures during spraying	

3.4.2 Clean- up

Clean up was done by USEPA method 3620B- Florisil clean up by column chromatography. Florisil was activated at 130°C overnight and cooled in a dessicator before use. Weight of florisil taken was predetermined by calibration using lauric acid. 1g florisil was packed in the 20 cm length and 12 mm i.d. glass chromatographic column, anhydrous sodium sulfate was added to the top of the florisil column (0.5 cm) and the column was pre-eluted with hexane and discarded. The extract was transferred to the column and eluted with 10 ml of hexane, 6% diethyl ether in hexane (10 ml), 15% diethyl ether in hexane (10 ml), 50% diethyl ether in hexane (10 ml) and finally with diethyl ether (10 ml). Eluent was collected and evaporated to dryness. Final samples were prepared in 2 ml n-hexane (HPLC grade) and analyzed by GC-ECD for organochlorines and GC-FTD for organophosphorus pesticides.

3.4.3 Extraction and cleaning up of residues in mother's milk samples

Pesticides residues from milk samples were extracted by following a method of Battu *et al* (2004). Samples were brought to room temperature and were thoroughly mixed before the start of analysis. 5 g of sample was taken and thoroughly mixed with 20 g of prewashed and freshly activated silica gel and 20 g of anhydrous sodium sulfate to form a free flowing powder in a pestle and mortar. The free flowing powder was packed into extraction glass column (60 x 2 cm i.d.) containing 40 ml of dichloromethane over a plug of glass wool. The mixture was allowed to stand for 90 minutes. The air bubbles formed in packed column were frequently eliminated by tapping the glass column. Then the solvent was eluted drop wise firstly with dichloromethane present in column and then further with 150 ml of dichloromethane: acetone (1:2 v/v) mixture. To remove the

turbidity, anhydrous sodium sulfate was added to the eluate. The extracted sample was concentrated to around 2 ml under vacuum by using rotary evaporator at 40°C. Then 15 ml of n-hexane was added and concentrated to around 5 ml. This step was repeated to eliminate dichloromethane completely. Finally the volume was made with n-hexane: acetone (1:1) mixture for analyzing on GC equipped with ECD and FTD.

3.5 Estimation of residues

The residues in cleaned up extracts were quantified using gas chromatography (GC). The Electron Capture Detector (ECD) and Flame thermionic detector (FTD) were used for organochlorine and organophosphorus compounds, respectively.The cleaned up extract measuring 2 µl was injected in gas chromatography (GC).GC solution software on PC was used for integration and computation of signals. The compounds were identified and quantified by comparison of the retention time and peak heights/area of the sample chromatograph with those of standards run under the same operating conditions. The formula used for the quantification of residues was:

$$\text{Residues(mg/kg)} = \frac{\text{Peak area of the sample x ng of insecticide standard injected x final volume of the sample extract(ml)}}{\text{Peak area of the standard x volume of the sample (µl) injected x weight of the sample(g)}}$$

In general, the volumes of the sample extract for injection was so chosen that it gave approximately the same area or that of the same peak height obtained with the standards

3.5.1 GC column characteristics

Before analysis, column was pre-conditioned for 10 hr at 350°C to remove solvents and other volatile materials that may cause interference during analysis. The carrier gas flow was kept unchanged. We condition the column at weekly intervals due to use of large sample analysis. Column temperature is an important variable for desired resolution and minimum retention. Here, we use Rxi- 5MS (low polarity phase, 5% diphenyl-95% dimethyl polysiloxane) capillary column with temperature range 60-350°C.

3.5.2 Detectors

ECD contains Ni^{63} as radioactive source for analysis of OCP's. ECD works on the principle that a flux of β- particles generated by the radioactive source collides with the carrier gas molecules causing them to ionize by ejecting electrons. These electrons migrate to an anode and generates current signal. When a sample contains compound it captures and remove electrons resulting in reduction in baseline current as some of the electrons are prevented from reaching the anode. The change in current provides signal response for the electron capturing compounds.

The estimation of organophosphorus was done by using the column RTX-5 (55 biphenyl- 95% methylpolysiloxane). For the estimation of organophosphorus we use FTD which is highly sensitive but a specific detector that gives a strong response to

Table 2: GC condition for the method used for OCPs, SPs and OPs detection

		OCPs, SPs			OPs		
Auto injector	Injection volume	2 µl			1 µl		
	No. of rinses with solvent (pre run)	5			5		
	No. of rinses with solvent (post run)	10			10		
	No. of rinses with sample	2			2		
	Plunger	High			High		
	Viscosity comp. time	0.2 sec.			0.2 sec.		
	Syringe insertion speed	High			High		
	Injection mode	Normal			Normal		
Injection port	Temperature	280 ° C			280 ° C		
	Injection mode	Split			Split		
	Carrier gas	N_2			N_2, H_2, zero air		
	Flow control mode	Pressure			Pressure		
	Pressure	117.4 kPa			117.4 kPa		
	Total Flow	14.0 ml/min.			5.2 ml/min.		
	Column flow	1.00 ml/min.			1.08 ml/min.		
	Linear velocity	30.7 cm/sec.			31.7 cm/sec.		
	Purge flow	3.0 ml/min.			3.0 ml/min.		
	Split ratio	10.0			1.0		
Column	Type	RTX-5			RTX-5		
	Length	30.0 m			30.0 m		
	Inner diameter	0.25 mm ID			0.25 mm		
	Film thickness	0.25 µm			0.25 µm		
	Equilibration time	0.5 min.			0.5 min.		
	Temperature (programmed)	Rate	Temp.° C	HT (min.)	Rate	Temp.° C	HT (min.)
			170	13 .0		180	2 .0
		3.0	270	20.0	10.0	270	3.0
		0.0	0.0	0.0	5.0	280	5.0
Detector	Type	ECD			FTD		
	Temperature	310 ° C			300 ° C		
	Sampling rate	40 msec.			40 msec.		

	Current	1.00 pA	1.30 pA
Gas	Hydrogen		3.0 ml/min.
	Air		145 ml/min.

.

compounds that contain nitrogen and/ or phosphorus. The essential component is rubidium or cesium bead which is present inside a small heater coil. Nitrogen is the carrier gas used which after leaving the column, mixes with hydrogen and then passes into the detector through a small jet. The cesium or rubidium bead is situated in a wire coil that is heated by a current passing through it. A potential is applied between the bead and the anode. During normal operation, when no solute is being eluted from the column, the heated alkali bead emits electrons by thermionic emissions that are collected at the anode and thus, produce a constant ion current. When a nitrogen or phosphorus containing solute is eluted from the column, the partially combusted nitrogen and phosphorus are adsorbed on the surface of the bead. The absorbed material on the bead surface reduces the work function of the surface and as a result the electron emission is increased and the current collected at the anode is increased (Table 2).

3.5.3 Sample introduction in GC

The clean up extract measuring one to two µl gets injected into GC through auto injector Shimadzu AOC 20i. For auto injections, sample extract and standard solutions were placed in glass vials with vapor tight septum caps. GC solution software on PC was used for integration and computation of signals. Calibration of standard curves were created and residues were quantitatively determined by comparison of the retention time and peak heights/ areas of the sample chromatogram with those of standard solutions run

under the same operating conditions. The concentrations of various residues in each sample were reported as ng/g on a fresh weight basis. In general, the volumes of the sample extract for injection was chosen that it gave approximately the same area or peak height obtained with the standards.

Table 3: Retention time of various organochlorine pesticide standards

S.no.	Name of pesticide	Retention time (minutes)
1.	α-HCH	13.177
2.	β-HCH	14.939
3.	γ-HCH	15.400
4.	δ-HCH	17.019
5.	Heptachlor	20.349
6.	Fenitothion	21.784
7.	Aldrin	22.784
8.	Fipronil	26.268
9.	Butachlor	28.325
10.	Dieldrin	29.802-29.898
11.	p,p'-DDE	29.802-29.898
12.	o,p'-DDD	30.390
13.	Endrin	31.383
14.	Endosulfan	32.012
15.	p,p'-DDD	32.627
16.	o,p'-DDT	32.841
17.	Endosulfan sulphate	34.863
18.	p,p'-DDT	35.093
19.	L-Cyhalothrin	42.054
20.	Permethrin	44.638-45.128
21.	Cyfluthrin	47.085-48.024
22.	Cypermethrin	48.506-49.542
23.	Fenvalerate	53.479-54.717
24.	Deltamethrin	56.617-57.751

Table 4: Retention time of various organophosphrous pesticide standards

S. No.	Name of pesticide	Retention time (min.)
1.	Monocrotophos	10.36
2.	Dimethoate	10.77
3.	Parathion	13.01
4.	Chlorpyrifos	14.53
5.	Profenophos	17.86
6.	Fenamiphos	18.02
7.	Carbaryl	19.30
8.	Malathion	19.70
9.	Ethion	19.85
10.	Phosalone	19.93
11.	Triazophos	20.48
12.	Amitraz	28.13

3.6 Confirmation of results

The confirmation of pesticide residues were done on GCMS (Gas Chromatography-Mass spectrometry). GC-MS is a very specific and very sensitive technique. The principle of GCMs is that when a molecule is ionized in vacuum, a characteristic group of ions of different masses are formed. A mass spectrum is produced

by separating these ions and recording a plot of ion abundance versus ionic mass. Mass spectrometry use difference in mass to charge ratio (m/z) of ionized molecules to separate them from each other. Generally mass spectrometry consists of an ion source, a mass analyzer of ions, an ion detector and a vacuum system.

We use Shimadzu GCMS 2010 plus model operated by auto sampler AOC 20i. Electron ionization was used as ion source which were produced by thermionic emission from tungsten. These electrons leave filament and accelerate towards the ion source chamber which was held at positive potential and typically 70 electron volts (70 eV) energy was provided for this action. The electron trap is held at fixed positive potential, electron strikes this trap and produces current which is used as a feedback circuit to stabilize electron beam. Magnetic field is created in ion chamber for production of magnetic flux parallel to the electron beam which causes electron beam to spiral from the filament to the trap and thus increases the chance and efficiency of analyte ionization.

Gaseous analyte when introduced into the ion source gets ionized by the electronic interactions with electron beam. The positive ion repeller voltage and the negative excitation voltage work together to produce an electric field in the source chamber such that ions will leave the source through the ion exit slit and enter the mass analyzer.

Quadrupole mass analyzer used in our GCMS consists of 4 parallel metal rods. Two opposite rods have an applied potential of U+Vcos (wt) and other two rods have a potential of –U+Vcos(wt), where U is the DC voltage and Vcos (wt) is ac voltage. The applied voltage therefore affects the trajectory of ions traveling down the flight path

centered among four rods. For a given dc and ac voltages, only ions of a certain mass to charge ratio can pass through the quadrupole filter and all other ions are thrown out of their original path. A mass spectrum is obtained by monitoring ions passing through the quadrupole filter as the voltage on the rods are varied (Table 5)

Table 5: GC-MS parameters used for pesticide detection

Auto injector	Injection volume	0.8 µl		
	No. of rinses with solvent (pre run)	5		
	No. of rinses with solvent (post run)	10		
	o. of rinses with sample	2		
	Plunger speed (suction and injection)	High		
	Viscosity comp. time	0.2 sec.		
	Syringe insertion speed	High		
	Injection mode	Normal		
Injection port	Temperature	285 ° C		
	Injection mode	Splitless		
	Carrier gas	He		
	Flow control mode	Pressure		
	Pressure	60.1 kPa		
	Total Flow	13.3 ml/min.		
	Column flow	0.94 ml/min.		
	Linear velocity	35.7 cm/sec.		
	Purge flow	3.0 ml/min.		
	Split ratio	10.0		
Column	Type	RTX-5		
	Length	30.0 m		
	Inner diameter	0.25 mm		
	Film thickness	0.25 µm		
	Equilibration time	0.5 min.		
	Temperature (programmed) Total run time = 32 min.	Rate	Temp.°C	Hold time (min.)
			80	3
		20.0	180	2.0
		2.0	190	2.0
		5.0	280	10.0

MS	Ion source	Electron Ionization
	Ion source temperature	200° C
	Interface temperature	290° C
	Solvent cut time	5.0 min.
	Mass analyzer	Quadrupole
	Voltage	0.70 KV

3.7 Method validation

During pesticide residue analysis studies it is utmost important that the method used for analysis ensures the recovery of the pesticide to the maximum possible extent that reflects the acceptance of the method employed for analysis. In order to validate the multiple residue analysis method for different commodities in present study, recovery experiments were carried out at specified fortification levels. The various parameters evaluated in our study for validation of methods worth trueness, repeatability, sensitivity and the limit of detection (LOD). The trueness was estimated by calculating the attained recovery from fortification experiments.

The spiking of samples was done at the concentration of 50 and 100 ng/g to the different matrices before extraction. Blank matrices were simultaneously extracted and analysed as control while each set of the experiment was replicated three times. According to AOAC guidance document, mean recoveries should be in the range of 70-120 % (AOAC/ FAO/ IAEA/IUPAC 1999).

Table 6: Retention times and characteristic fragments of organochlorine pesticides

Pesticides	Retention times (min)	m/z
α-HCH	5.66	219,183,217,181
β-HCH	5.80	181,183,219,217
γ-HCH	6.98	181,183,111,219
δ-HCH	7.11	181,219,183,111
Heptachlor	11.30	86,57,51,56
Fenitothion	13.03	91,69,125,135
Aldrin	13.60	56,57,66,83
Fipronil	17.40	367,369,213,77
Butachlor	19.90	176,91,160,73
Dieldrin	20.69	79,81,82,77
p,p'-DDE	21.04	71,55,248,246
o,p'-DDD	21.57	23,165,56,84
Endrin	22.10	57,91,81,67
Endosulfan	22.79	91,237,209,195
p,p'-DDD	23.80	235,165,237,57
o,p'-DDT	23.96	235,237,165,199
Endosulfan sulphate	25.70	83,86,281,55
p,p'-DDT	26.29	235,237,165,82
L-Cyhalothrin	33.97	181,207,135,197
Permethrin	36.13	183,163,165,185
Cyfluthrin	38.89	163,165,206,51
Cypermethrin	39.60	163,165,181,91
Fenvalerate	42.22	125,167,225,281
Deltamethrin	44.80	181,253,77,251

Table 7: Retention times and characteristic fragments of organophosphrous pesticides

Pesticides	**Retention times (min)**	**m/z**
Monocrotophos	12.36	127,67,97,58
Dimethoate	12.77	87,93,125,47
Parathion	17.01	109,125,47,263
Carbaryl	22.75	144,115,116,57
Malathion	22.95	125,93,127,173
Chlorpyrifos	14.23	97,197,199,65
Profenophos	21.20	43,97,139,208
Ethion	24.35	206,234,116,148
Phosalone	24.10	182,121,97,184
Triazophos	25.19	161,162,77,172
Amitraz	33.25	121,132,137,162

Fig. 1: Flow chart for extraction of pesticide residues in human milk

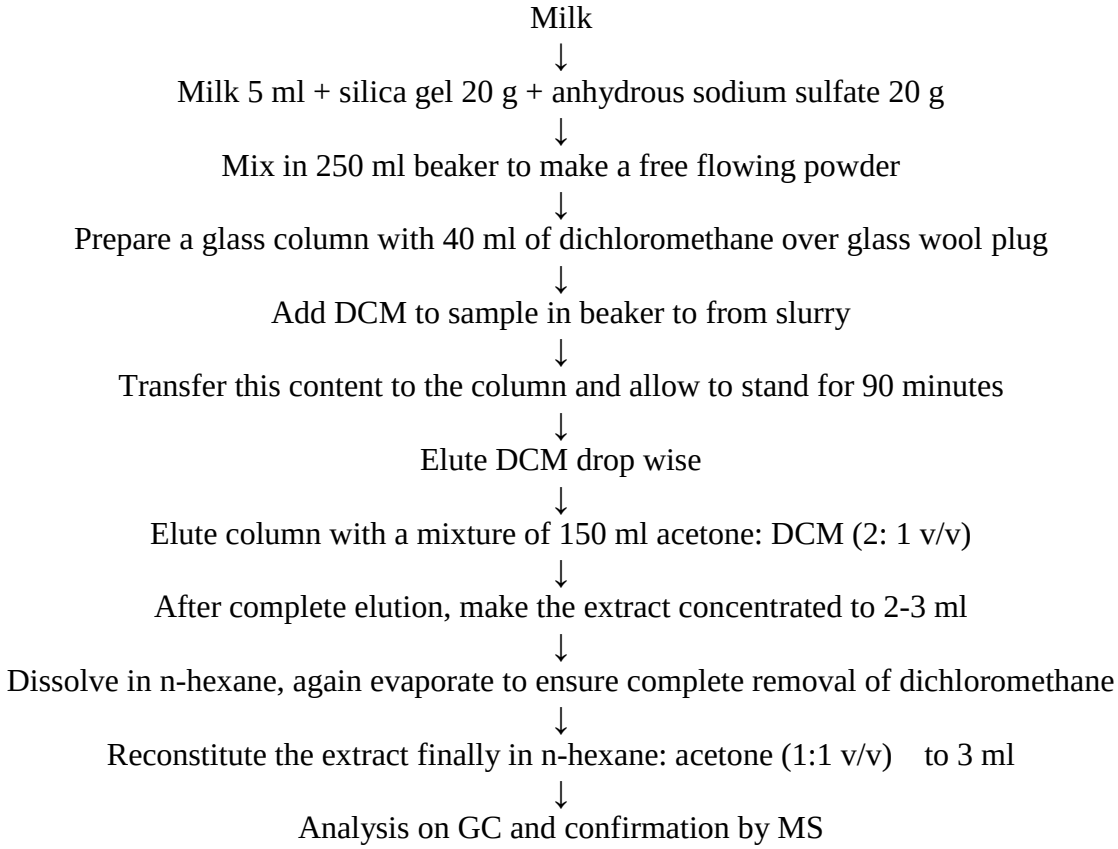
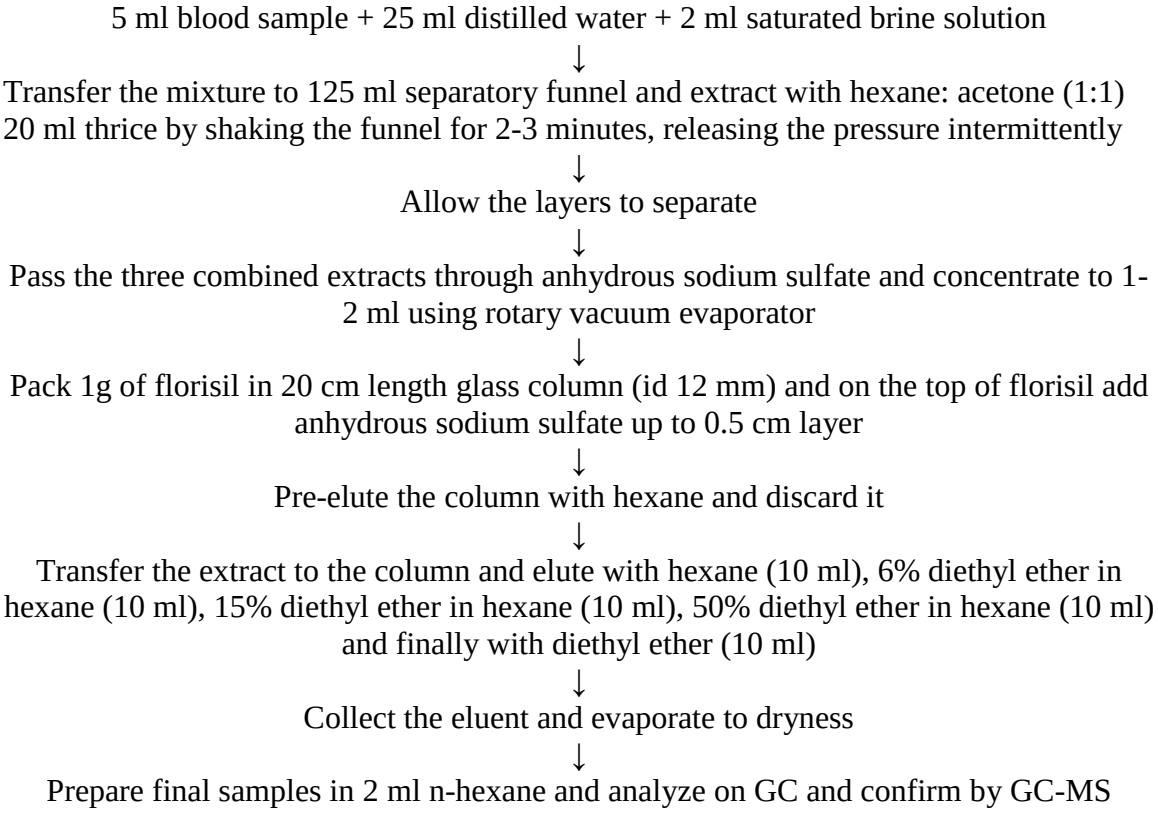


Fig. 2: Flow diagram for extraction of pesticide residues in human blood



CHAPTER IV

RESULTS AND DISCUSSION

Pesticides are the widely used chemical substances throughout the world in agriculture and public health. Because of their high biological activity and persistent nature, the use of pesticides causes undesirable effects to human health and to the environment. Agricultural development continues to be a major objective of Indian planning and policy. Thus, the pesticides have become an important tool as plant protection agent for increasing food production. Also they play an important role in improving human health by keeping away many dreadful diseases. The exposure to these pesticides cause a range of human health problems like immune suppression, hormone disruption, diminished intelligence, reproductive abnormalities and cancer (Abhilash and Singh 2009). Thus it is of utmost importance to monitor the current levels of pesticide residues in human population.

4.1 Pesticide residues in mother's milk

The persistence and lipophilicity of pesticides leads to the deposition of their residues in fat rich tissues. Under certain conditions such as lactation, mobilization of these residues occurs which lead to their excretion in the mother's milk (Kumar *et al* 2006b). Therefore burden of these residues in mother's milk can indicate the risks of exposure to breast-fed infants. Moreover mother's milk with a relatively high content can be a best indicator to study long term exposure to these OCP's as it is easy to obtain and can be collected non-invasively (Devanathan *et al* 2009).

Table 8: Detailed characteristics of donor mothers

Sample No.	Background	Age (yrs)	W (kg)	Occupation	Parity	Dietary habits	History
1	Rural	30	60	Housewife	1st	Non-vegetarian	No
2	Urban	33	45	Housewife	3rd	Vegetarian	No
3	Rural	29	59	Housewife	2nd	Non-vegetarian	Abortion
4	Rural	30	65	Housewife	2nd	Vegetarian	Abortion
5	Rural	26	50	Housewife	1st	Vegetarian	No
6	Urban	30	45	Housewife	1st	Vegetarian	No
7	Rural	20	50	Housewife	2nd	Non-vegetarian	No
8	Urban	30	60	Housewife	1st	Vegetarian	No
9	Urban	27	55	Housewife	1st	Vegetarian	No
10	Urban	31	60	Shopkeeper	2nd	Vegetarian	No
11	Rural	27	55	Housewife	2nd	Vegetarian	No
12	Urban	31	90	Teacher	3rd	Vegetarian	Abortion
13	Rural	28	65	Housewife	3rd	Vegetarian	Abortion
14	Urban	23	45	Housewife	2nd	Vegetarian	No
15	Urban	29	46	Housewife	1st	Non-vegetarian	No
16	Rural	26	80	Housewife	2nd	Vegetarian	Abortion
17	Rural	21	57	Housewife	2nd	Vegetarian	Abortion
18	Urban	21	55	Housewife	1st	Non-vegetarian	No
19	Rural	25	55	Housewife	2nd	Non-vegetarian	Still birth
20	Urban	33	50	Housewife	1st	Vegetarian	No
21	Rural	27	58	Computer operator	1st	Vegetarian	No
22	Rural	27	68	Teacher	1st	Non-vegetarian	No
23	Urban	26	50	Housewife	1st	Vegetarian	No
24	Urban	31	55	Housewife	3rd	Non-vegetarian	Abortion
25	Urban	27	50	Housewife	2nd	Non-vegetarian	No
26	Rural	25	35	Housewife	3rd	Non-vegetarian	Abortion
27	Rural	29	100	Teacher	2nd	Vegetarian	No

Sample No.	Background	Age (yrs)	W (kg)	Occupation	Parity	Dietary habits	History
28	Rural	28	45	Housewife	3rd	Non-vegetarian	No
29	Rural	28	78	Housewife	1st	Vegetarian	No
30	Urban	26	51	Teacher	1st	Vegetarian	No
31	Rural	38	108	Housewife	1st	Vegetarian	No
32	Urban	25	52	Housewife	1st	Vegetarian	No
33	Rural	33	55	Housewife	1st	Non-vegetarian	No
34	Rural	29	40	Teacher	1st	Vegetarian	No
35	Rural	30	50	Housewife	3rd	Non-vegetarian	No
36	Rural	24	60	Clerk	3rd	Vegetarian	Abortion
37	Urban	28	85	Principal	1st	Vegetarian	No
38	Rural	30	57	Nurse	1st	Non-vegetarian	No
39	Rural	32	70	Housewife	2nd	Vegetarian	No
40	Rural	29	40	Housewife	1st	Non-vegetarian	No
41	Urban	23	55	Housewife	1st	Vegetarian	No
42	Urban	27	50	Doctor	1st	Vegetarian	No
43	Urban	37	71	Teacher	2nd	Vegetarian	Abortion
44	Rural	29	75	Housewife	1st	Vegetarian	No
45	Urban	20	74	Housewife	1st	Vegetarian	No
46	Rural	21	51	Housewife	2nd	Non-vegetarian	Miscarriage
47	Urban	23	45	Housewife	2nd	Vegetarian	Miscarriage
48	Urban	29	90	Housewife	2nd	Vegetarian	Abortion
49	Rural	30	60	Teacher	1st	Vegetarian	No
50	Rural	34	62	Housewife	1st	Vegetarian	No
51	Urban	23	60	Housewife	1st	Non-vegetarian	No
52	Rural	21	80	Housewife	1st	Vegetarian	No
53	Rural	25	63	Housewife	2nd	Vegetarian	Abortion
54	Urban	22	65	Housewife	2nd	Vegetarian	Abortion
55	Rural	24	65	Housewife	1st	Vegetarian	No
56	Urban	33	60	Housewife	1st	Vegetarian	No
57	Rural	27	66	Housewife	2nd	Vegetarian	No
58	Rural	29	82	Housewife	3rd	Vegetarian	Abortion
59	Rural	28	59	Housewife	1st	Vegetarian	No
60	Urban	20	45	Housewife	1st	Non-vegetarian	No
61	Rural	22	44	Housewife	1st	Non-vegetarian	No

Sample No.	Background	Age (yrs)	W (kg)	Occupation	Parity	Dietary habits	History
62	Urban	30	75	Housewife	1st	Vegetarian	No
63	Rural	25	60	Housewife	1st	Vegetarian	No
66	Rural	26	50	Housewife	3rd	Vegetarian	Abortion & Miscarriage
67	Rural	27	80	Housewife	2nd	Vegetarian	No
68	Rural	31	54	Teacher	2nd	Vegetarian	Miscarriage
69	Urban	26	48	Housewife	2nd	Non-vegetarian	No
70	Rural	27	60	Housewife	3rd	Non-vegetarian	No
71	Urban	26	50	Housewife	3rd	Vegetarian	Abortion & Miscarriage
72	Rural	22	60	Housewife	2nd	Vegetarian	Abortion
73	Urban	24	60	Housewife	1st	Vegetarian	No
74	Rural	25	56	Housewife	1st	Vegetarian	No
75	Urban	26	66	Housewife	2nd	Vegetarian	Miscarriage
76	Urban	26	69	Housewife	1st	Vegetarian	No
77	Rural	20	55	Housewife	1st	Vegetarian	No
78	Rural	27	60	Housewife	1st	Housewife	No
79	Urban	31	68	Housewife	3rd	Vegetarian	No
80	Rural	28	49	Housewife	1st	Non-vegetarian	Abortion
81	Rural	23	69.5	Housewife	1st	Vegetarian	No
82	Urban	29	107	Housewife	3rd	Non-vegetarian	Abortion
83	Rural	27	71.7	Teacher	1st	Vegetarian	No
84	Rural	22	45	Housewife	2nd	Non-vegetarian	Abortion
85	Rural	30	72	Housewife	2nd	Non-vegetarian	No
86	Rural	20	66	Housewife	1st	Non-vegetarian	No
87	Rural	22	55	Housewife	3rd	Non-vegetarian	No
88	Rural	29	50	Housewife	2nd	Non-vegetarian	Miscarriage
89	Rural	29	57	Housewife	2nd	Non-vegetarian	No
90	Rural	30	61	Housewife	2nd	Non-vegetarian	No
91	Rural	26	54	Housewife	1st	Non-vegetarian	No
92	Urban	27	59	Housewife	1st	Non-vegetarian	No
93	Rural	25	47	Housewife	1st	Vegetarian	No
94	Rural	27	63	Housewife	2nd	Non-vegetarian	No

Sample No.	Background	Age (yrs)	W (kg)	Occupation	Parity	Dietary habits	History
95	Urban	30	72	Assistant prof.	1st	Vegetarian	No
96	Urban	28	77.5	Housewife	2nd	Non-vegetarian	Abortion
97	Urban	34	80	Housewife	4th	Vegetarian	Abortion
98	Rural	26	63	Housewife	1st	Vegetarian	No
99	Urban	26	46	Housewife	2nd	Non-vegetarian	Abortion
100	Rural	27	59	Teacher	1st	Non-vegetarian	No
101	Urban	29	61	Housewife	1st	Vegetarian	No
102	Rural	31	63	Housewife	1st	Vegetarian	No
103	Rural	30	59	Housewife	2nd	Vegetarian	No
104	Urban	27	62	Teacher	1st	Non-vegetarian	No
105	Rural	29	59	Housewife	2nd	Vegetarian	No
106	Urban	22	53	Housewife	1st	Vegetarian	No
107	Rural	27	62	Housewife	2nd	Vegetarian	No
108	Rural	26	58	Housewife	1st	Vegetarian	No
109	Rural	29	61	Teacher	2nd	Non-vegetarian	Abortion
110	Urban	28	59	Housewife	2nd	Vegetarian	No
111	Rural	23	60	Housewife	2nd	Vegetarian	No
112	Urban	26	59	Housewife	1st	Vegetarian	No
113	Rural	28	60	Housewife	2nd	Vegetarian	No
114	Rural	30	59	Housewife	2nd	Vegetarian	Abortion
115	Rural	27	55	Housewife	1st	Vegetarian	No
116	Urban	24	57	Housewife	1st	Vegetarian	No
117	Urban	25	59	Teacher	1st	Vegetarian	No
118	Urban	25	61	Housewife	2nd	Non-vegetarian	No
119	Rural	28	59	Housewife	1st	Non-vegetarian	No
120	Rural	23	60	Housewife	2nd	Vegetarian	Miscarriage
121	Rural	24	59	Housewife	2nd	Vegetarian	No
122	Rural	24	58	Housewife	1st	Vegetarian	No
123	Urban	27	60	Housewife	1st	Non-vegetarian	No
124	Urban	29	61	Teacher	2nd	Vegetarian	No
125	Urban	30	63	Housewife	1st	Vegetarian	No
126	Urban	28	59	Housewife	2nd	Vegetarian	Stillbirth
127	Rural	21	58	Housewife	1st	Non-vegetarian	No

Table 9: General demographic characteristics of donor mothers

	Characteristics	Mean	Standard deviation	Median	Minimum	Maximum
MATERNAL	Age(years)	26.98	3.54	27	20	38
	Height(inches)	62.37	2.41	62	56	69
	Weight(kg)	60.54	12.21	59	40	108
	BMI[a] (kg m^{-2})	24.11	4.63	23.82	16.12	43.54
	Milk fat(%)	3.01	0.57	2.94	1.82	5.00
	Gestation Period(days)	256	17.48	260	195	295
INFANT	Weight(kg)	2.64	0.66	2.9	0.8	3.8
	Age at sampling(days)	5.92	4.14	6	4	37
Male infants- 77 (59.68 %) *Female infants-52(40.31%)*						

[a] *BMI: body mass index (weight(kg)/[height(m)^2]).*

In the present study, a total of 127 mother's milk samples were collected to monitor the level of pesticide residues in mother's milk. The average age of mothers was 26.98 years with a range from 20 to 38 years. Body mass index of mothers was calculated on the basis of their height and weight and ranged from 16.12 to 43.54 kg m^{-2} with a mean value of 24.11 kg m^{-2}. Average weight of the mothers came out to be 60.54 kg with a range from 40 to 108 kg. The milk fat ranged from 1.82 to 5 percent with a mean value of 3.01 percent. Gestation period of mothers ranged from 195 to 295 days with a mean of 256 days. Weight of infants of respective mothers ranged from 0.8 to 3.8 kg with a mean of 2.64 kg and the age of infants on the day of sampling ranged from 4 to 37 days with an average of 5.92 days (Table 9).

On analyzing the milk samples it was observed that 25% of the samples were found positive for pesticide residues. Residues of β-HCH, γ-HCH, p,p' DDE, p,p' DDT, cyfluthrin, cypermethrin, fenvalerate, chlorpyriphos, phosalone, profenphos and monocrotophos were detected in mother's milk samples (Table 10). Out of these, cyfluthrin being the dominant pesticide was detected in 11.81% of the samples followed by γ-HCH, p,p' DDT, chlorpyriphos and β-HCH which were detected in 8.66, 4.72, 3.93 and 3.14% of the mother's milk samples, respectively.

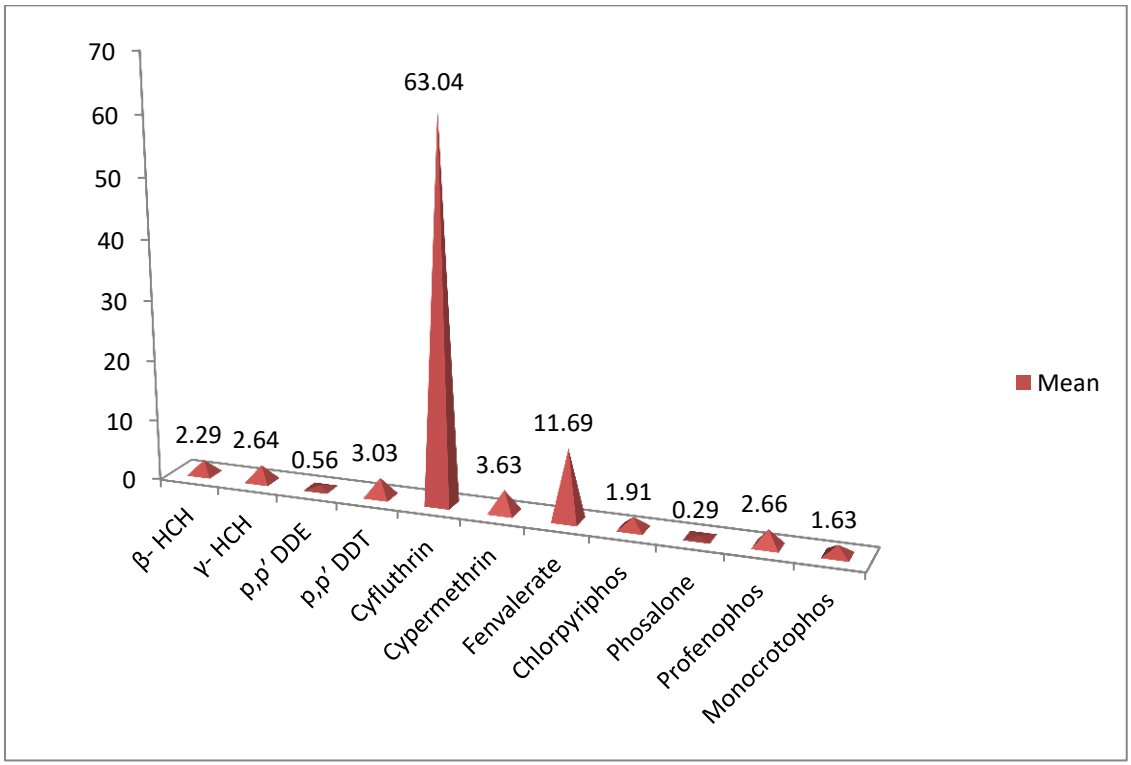
Table 10: Levels of pesticide residues ng g^{-1} in mother's milk

Pesticides	Mean	Standard deviation	Median	Range	Positive percentage	Percentage contribution
β- HCH	2.29	14.39	62.46	ND-133.88	3.14	7.4
γ- HCH	2.64	10.35	34.1	ND-61.13	8.66	20.37
p,p' DDE	0.56	4.22	17.16	ND-43.19	2.36	5.55
p,p' DDT	3.03	17.39	58.56	ND-150.3	4.72	11.16
Cyfluthrin	63.04	385.50	189.81	ND-4100.39	11.81	27.77
Cypermethrin	3.63	27.06	105.9	ND-276.35	2.36	5.55
Fenvalerate	11.69	107.48	742.54	ND-1172.87	1.57	3.70
Chlorpyriphos	1.91	14.63	21.91	ND-160.48	3.93	9.25
Phosalone	0.29	3.28	36.13	ND-37.02	1.57	3.70
Profenophos	2.66	26.60	169.45	ND-297.25	1.57	3.70
Monocrotophos	1.63	18.44	207.81	ND-207.81	0.78	1.85

Residues of each p,p' DDE and cypermethrin were detected in 2.36% of the samples. Fenvalerate, phosalone and profenophos residues each were detected in 1.57% of the samples whereas monocrotophos residues were detected only in 0.78% of the samples. The mean concentration level of β-HCH was 2.29 ng g^{-1} with a range of ND-133.88 ng g^{-1}. Similarly the mean concentration level of γ-HCH was 2.64 ng g^{-1} with the maximum level of 61.13 ng g^{-1} in the samples. p,p' DDE residues were observed with

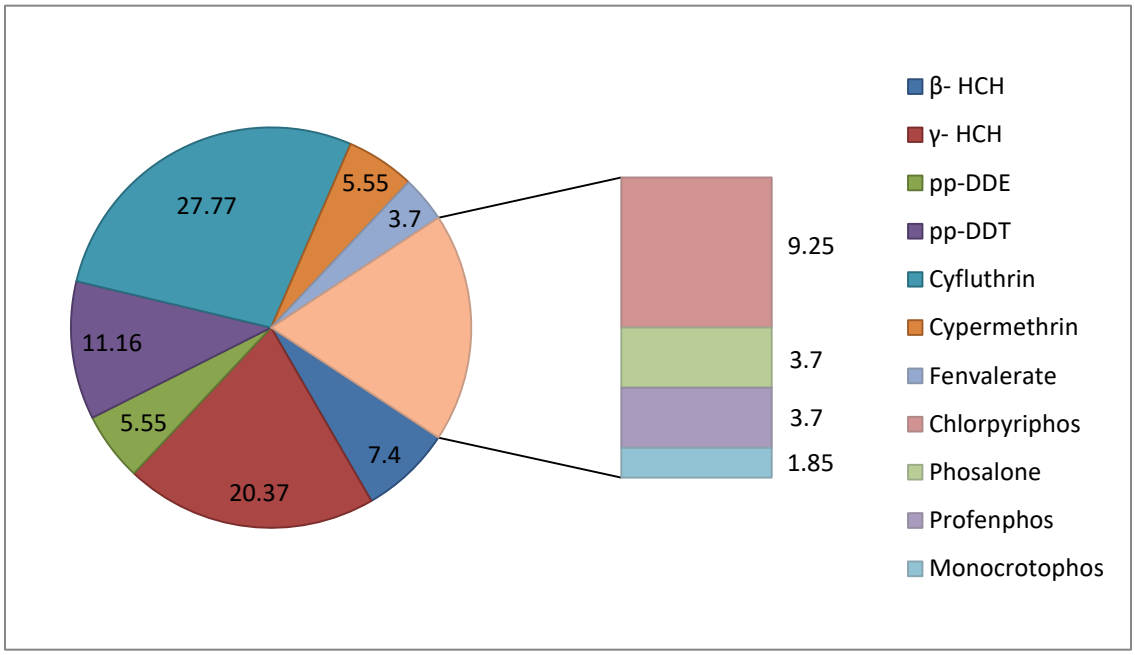
mean levels of 0.56 ng g^{-1} with a range of ND-43.19 ng g^{-1} in the samples. The residue levels of p,p' DDT were found with mean concentration level of 3.03 ng g^{-1} and their maximum concentration found out to be 150.3 ng g^{-1} in the milk samples.

Fig. 3: Mean residue level of pesticides detected in mother's milk



Cyfluthrin was detected with highest mean concentration level of 63.04 ng g^{-1} with a range from ND-4100.39 ng g^{-1} (Fig. 3). Among other pyrethroids, cypermethrin and fenvalerate were detected with a mean concentration level of 3.63 and 11.69 ng g^{-1}, respectively. Maximum concentration was 276.35 and 1172.87 ng g^{-1} for cypermethrin and fenvalerate, respectively. Among OPs, the mean concentration level was maximum for chlorpyriphos i.e. 1.91 ng g^{-1} with a range of ND-160.48 ng g^{-1} followed by profenophos, monocrotophos and phosalone with mean concentration level of 2.66, 1.63 and 0.29 ng g^{-1}, respectively.

Fig. 4: Percentage contribution of different pesticides in mother's milk



The residues of cyfluthrin contributed 27.77% to the total residues detected in mother's milk followed by γ-HCH residues as 20.37% of the total residues detected. Among DDT metabolites, p,p' DDE and p,p' DDT contributed 5.55 and 11.16%, respectively. Among HCH isomers, β-HCH contributed 7.4% of the total residues while cypermethrin contributed 5.55% to the total pesticide residues in mother's milk. Fenvalerate, phosalone and profenophos contributed 3.7% each to the total pesticide residues. The percentage contribution of chlorpyriphos and monocrotophos to the total pesticide load in mother's milk was 9.25 and 1.85%, respectively (Fig. 4).

Various factors affect the levels of pesticide residues in mother's milk. These factors include maternal age, parity, living background, dietary habits, occupation, health status, household chemical usage, length of previous lactation, duration of breastfeeding time of feeding and time within a feed (Cohen 2007). Age, parity, smoking and intake of

highly contaminated food are most important among them (Skaare *et al* 1988). Thus the occurrence of major persistent pesticide residues were analyzed according to the parity of mothers, their age, living background and dietary habits (Table 11-14).

Out of 127 mothers, 65 were in their first parity, 46 were in second parity, 15 were in third parity and only one of the mothers was in her fourth parity. The percentage of positive samples in 1^{st}, 2^{nd} and 3^{rd} parity were 26.15, 19.57 and 86.66%, respectively. The sample from fourth parity was found negative. In the first parity, β-HCH and γ-HCH residues were found with mean value of 3.47 and 2.83 ng g^{-1} in 4.61 and 7.69% of the samples, respectively, leading the total HCH residues to the mean value of 6.29 ng g^{-1} with 12.30% frequency of occurrence. Similarly residues of p,p' DDE and p,p' DDT were found in 3.07 and 6.15% of the samples with mean concentration of 0.44 and 4.39 ng g^{-1}, respectively. However, total DDT residues were found in 9.23% of the samples with a mean value of 4.83 ng g^{-1}. Cyfluthrin is found in maximum concentration among all residues in first parity with a mean value of 90.63 ng $g^{-1}$and the frequency of occurrence was 12.31% which was also highest among others found in first parity. Cypermethrin was detected in 1.53% of the samples with mean concentration of 4.14 ng g^{-1} while chlorpyriphos was found with a mean value of 0.97 ng g^{-1} with 4.61% frequency of occurrence.

In the second parity, only γ-HCH was detected among HCH metabolites with a mean value of 2.77 ng g^{-1} and 8.69% frequency of occurrence. Among DDT metabolites p,p' DDT was the only detected residue in the mean concentration of 0.39 ng g^{-1} with 2.17% frequency of occurrence. Cyfluthrin, cypermethrin and chlorpyriphos were detected in the mean concentration of 41.38, 3.60 and 3.48 ng g^{-1} with 10.87, 2.17 and

Table 11: Correlation between pesticide residue levels (ng g^{-1}) in mother's milk and the parity of mother

Pesticides	Parity					
	First (n=65)		Second (n=46)		Third (n=15)	
	Mean ± S.D.	Frequency (%)	Mean ± S.D.	Frequency (%)	Mean ± S.D.	Frequency (%)
β-HCH	3.47±18.4	4.61	ND	-	4.11±16.43	6.66
γ-HCH	2.83±11.65	7.69	2.77±9.83	8.69	1.74±5.67	13.33
∑HCH	6.29±21.32	12.30	2.77±9.83	8.69	5.63±16.88	20
p,p' DDE	0.44±2.53	3.07	ND	-	2.87±11.15	6.66
p,p' DDT	4.39±22.04	6.15	0.39±2.65	2.17	5.43±21.01	6.66
∑DDT	4.83±22.09	9.23	0.39±2.65	2.17	7.78 ±22.39	13.33
Cypermethrin	4.14±34.28	1.53	3.60±24.46	2.17	4.99±19.95	6.66
Cyfluthrin	90.63±512.24	12.31	41.38±198.85	10.87	13.23±52.91	6.66
Chlorpyriphos	0.97±4.47	4.61	3.48±23.66	2.17	1.16±4.64	6.66

Table 12: Correlation between pesticide residue levels (ng g^{-1}) in mother's milk and the age of mother

Pesticides	Age (years)					
	20-25 (n=30)		26-30 (n=66)		31-35 (n=27)	
	Mean ± S.D.	Frequency (%)	Mean ± S.D.	Frequency (%)	Mean ± S.D.	Frequency (%)
β-HCH	ND	_	4.41±19.81	6.06	ND	_
γ-HCH	3.31±11.81	13.33	2.72±10.97	7.57	2.16±7.90	7.40
∑HCH	3.31±11.81	12.90	7.13±22.10	13.63	2.16±7.91	7.40
p,p' DDE	ND	_	1.08±5.83	4.54	ND	_
p,p' DDT	4.84±17.91	10	3.62 ±20.90	4.54	ND	_
∑DDT	4.84±17.91	10	4.71±21.52	9.09	ND	_
Cypermethrin	ND	_	7.91±40.40	4.54	ND	_
Cyfluthrin	21.11±56.09	13.33	106.25±530.79	13.63	13.84±49.72	7.40
Chlorpyriphos	5.95±29.37	6.66	0.96±4.46	4.54	ND	_

Table 13: Correlation between pesticide residue levels (ng g^{-1}) in mother's milk and residential background of mother

Pesticides	Residential background			
	Rural (n=75)		Urban (n=52)	
	Mean ± S.D.	Frequency (%)	Mean ± S.D.	Frequency (%)
β-HCH	3.00±17.16	4	1.26±9.12	1.92
γ-HCH	2.48±10.84	8	2.88±9.69	9.61
∑HCH	5.48±22.23	12	4.14±14.08	11.53
p,p' DDE	0.72±5.14	2.66	0.33±2.38	1.92
p,p' DDT	2.82±17.88	5.33	3.33±16.84	3.85
∑DDT	3.55±22.79	8	3.65±18.49	5.77
Cypermethrin	3.28±21.15	2.67	5.31±38.32	1.92
Cyfluthrin	40.18±169.80	12	96.01±568.72	11.54
Chlorpyriphos	0.85±4.19	4	3.43±22.35	3.85

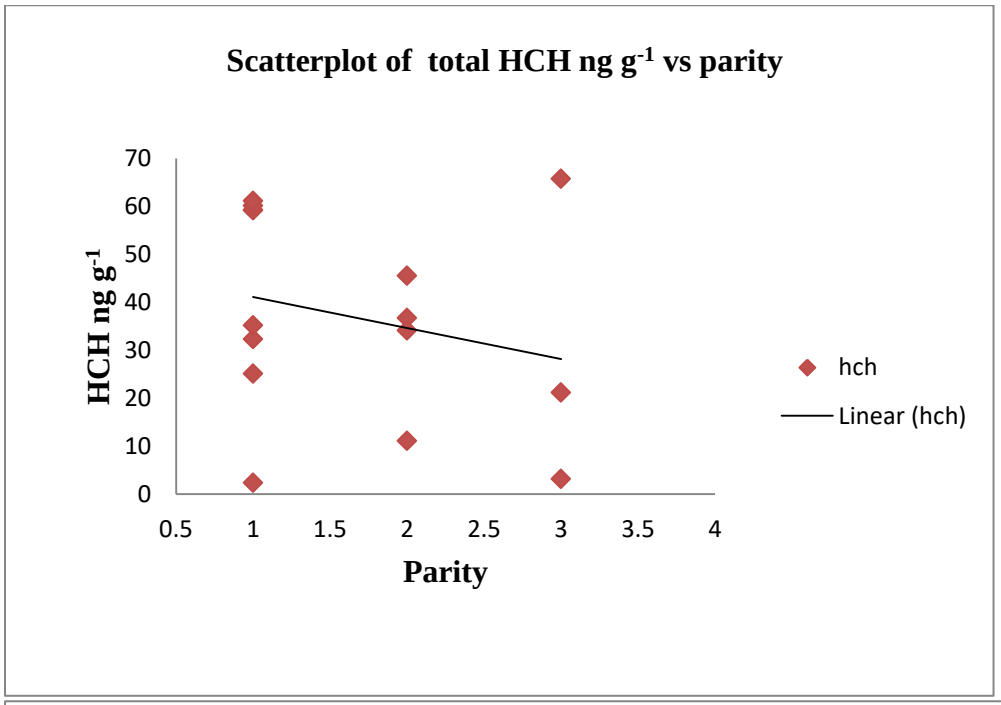
Table 14: Correlation between pesticide residue levels (ng g^{-1}) in mother's milk and dietary habit of mother

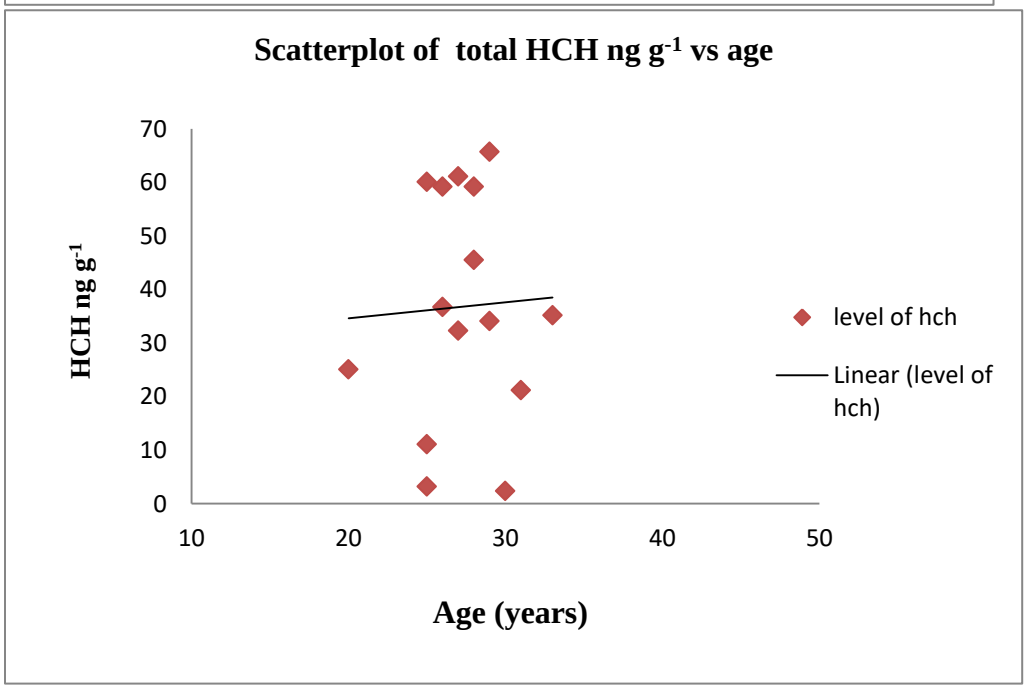
Pesticides	Dietary habits			
	Vegetarian (n=85)		Non-vegetarian (n=42)	
	Mean ± S.D.	Frequency (%)	Mean ± S.D.	Frequency (%)
β-HCH	2.27±15.81	2.35	2.35±11.19	4.76
γ-HCH	3.45±11.96	9.41	1.01±5.67	7.14
∑HCH	5.72±22.46	11.76	12.85±3.34	11.90
p,p' DDE	0.84±5.15	3.43	ND	_
p,p' DDT	4.44±21.14	5.88	0.19±1.21	2.38
∑DDT	5.28±25.66	9.41	0.19±1.21	2.38
Cypermethrin	6.14±35.70	3.43	ND	_
Cyfluthrin	81.93±466.90	12.94	24.81±88.53	9.52
Chlorpyriphos	1.89±17.41	1.17	1.94±6.10	9.52

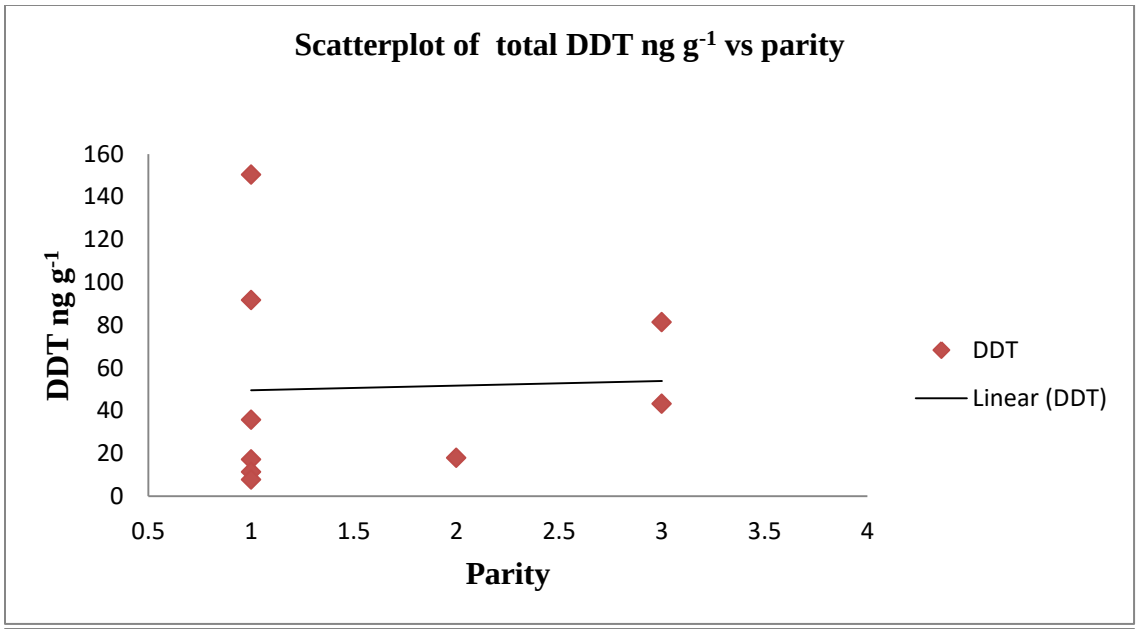
2.17% frequency of occurrence, respectively. In third parity, β-HCH and γ-HCH were detected in 6.66 and 13.33% of the samples in the mean concentration of 4.11 and 1.74 ng g^{-1}, respectively, thus leading to the total HCH concentration to the mean value of 5.63 ng g^{-1} with 20% frequency of occurrence. Similarly p,p' DDE and p,p' DDT were the two DDT metabolite detected in the mean concentration of 2.87 and 5.43 ng g^{-1}, respectively, with 6.66% frequency of occurrence each thereby leading the total DDT concentration to the mean value of 7.78 ng g^{-1} with 13.33% frequency of occurrence. The mean concentration of cyfluthrin, cypermethrin and chlorpyriphos were 13.23, 4.99 and 1.16 ng g^{-1}respectively each with 6.66% frequency of occurrence. There was a single mother on fourth parity with no pesticide residue being detected in her milk sample. The occurrence of total HCH, DDT, cyfluthrin and cypermethrin are more in first parity followed by second parity (Fig. 5).

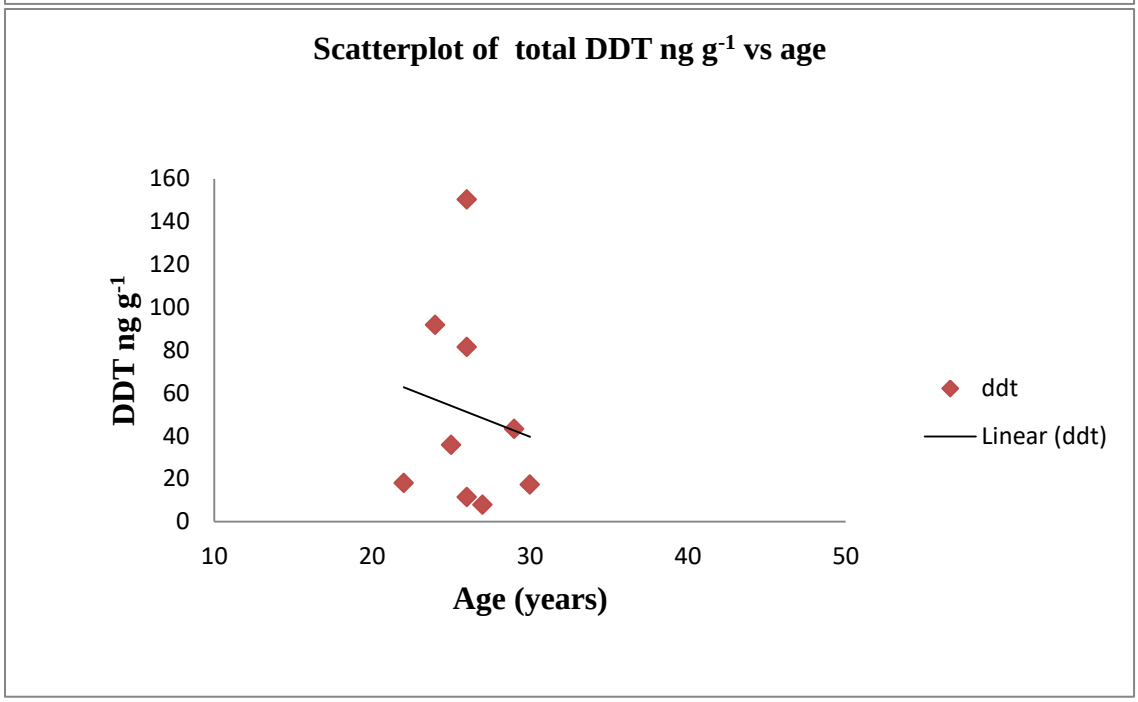
Thus it is clear that there is a decline in the concentration of residues in relation to the parity of mother. Various studies reported in the past have also shown a declining trend in the concentration of pesticide residues in relation to the parity. Minh *et al* 2004 observed higher mean levels of DDT residues in breast milk of primiparous mothers as compared to the levels in multiparous mothers. As the duration of lactation increases, the levels of residue decrease because of the excretion of these residues through breast milk. During each period of lactation there is a continuous loss of OCPs from mother's body due to breast feeding as the residues are mobilized to the mother's milk (Kumar *et al* 2006b, Albers *et al* 1996). Indeed, the present result provides another evidence for the influence of lactation on OC burden of nursing women. Declining levels of POPs with parity were also reported by Czaja *et al* 2001. Polder *et al* 2009 also observed same

Fig. 5 Scatter plot of HCH and DDT in relation to the age and parity of mother






Scatterplot of total DDT ng g-1 vs parity
DDT ng g-1
160
140
120
100
80
60
40
20
0
0.5
1
1.5
2
2.5
3
3.5
4
Parity
DDT
Linear (DDT)


Scatterplot of total DDT ng g-1 vs age
DDT ng g-1
160
140
120
100
80
60
40
20
0
10
20
30
40
50
Age (years)
ddt
Linear (ddt)

declining trend in the residue levels with the parity of mother. Thus our results are in accordance to the reported studies in the past.

All of the donor mothers were divided into four age groups viz, 20-25, 26-30, 31-35 and 36-40 years. Among the HCH metabolites, only γ-HCH residues were detected in the first age group at mean level of 3.31 ng g^{-1}. The mean residue concentration of total HCH was 3.31 ng g^{-1} with a 12.90% frequency of occurrence. Similarly the total DDT mean residue concentration was 4.84 ng g^{-1} with 10% frequency of occurrence. Residues of cyfluthrin and chlorpyriphos were detected among 13.33 and 6.66% of the samples with mean concentration of 21.11 and 5.95 ng g^{-1}, respectively. In the second age group, mean concentration of HCH was 7.13 ng g^{-1} with 13.63% frequency of occurrence and that of total DDT was 7.91 ng g^{-1} with 9.09% frequency of occurrence. Cyfluthrin has mean concentration of 106.25 ng g^{-1} with 13.63% frequency of occurrence. Cypermethrin and chlorpyriphos were found in mean levels of 7.91 and 0.96 ng g^{-1}, respectively, each with 4.54% frequency of occurrence. In the third age group only γ-HCH and cyfluthrin residues were detected in the mean concentration of 2.16 and 13.84 ng g^{-1}, respectively, with 7.40% frequency of occurrence each. The fourth age group has four mothers and no pesticide residues were detected in their milk samples.

On comparing second and third age groups, a characteristic decline in the concentration was observed in the mean concentration of pesticide residues which may be due to the fact that more the breast feeding mother is older, less is the concentration of pesticide residues due to transfer via breast milk to the mother (Mishra and Sharma 2011). In the developing countries, women breast feed their infants for relatively longer periods as compared to those in developed countries. This may be one of the reasons for

negative correlation between age and concentration of pesticides residues in milk in our study.

The mothers were divided into two groups according to their residential background as rural and urban. Analysis of data indicates that mean residue levels of total HCH in rural population was 5.48 ng g^{-1} with 12% frequency of occurrence while that in urban population was 4.14 ng g^{-1} with 11.53% frequency of occurrence. The mean residue level of total DDT in rural population was 3.55 ng g^{-1} while in urban population it was 3.65 ng g^{-1} with 8 and 5.77% frequency of occurrence, respectively. The mean residues levels for cyfluthrin, cypermethrin and chlorpyriphos were 40.18, 3.28 and 0.85 ng g^{-1} with 12, 2.67 and 4% frequency of occurrence, respectively, in rural population and 96.01, 5.31 and 3.43 ng g^{-1} with 11.54, 1.92 and 3.85% frequency of occurrence, respectively, in urban population. Except for total HCH levels, the mean residue levels of all other pesticides were high in urban population as compared to rural population.

According to the food habits, mean residue levels of total HCH in vegetarian population were 5.72 ng g^{-1} with 11.76% frequency of occurrence while in non-vegetarian population it was found to be at mean concentration of 12.85 ng g^{-1} with 11.90% frequency of occurrence. Mean residue levels for total DDT in vegetarian and non-vegetarian population was observed to be 5.28 and 0.19 ng g^{-1} with 9.41 and 2.38% frequency of occurrence, respectively. Cypermethrin was detected only in vegetarian population with mean levels of 6.14 ng g^{-1} and 3.43% frequency of occurrence. Cyfluthrin and chlorpyriphos were detected in mean residue levels of 81.93 and 1.89 ng g^{-1} in vegetarian population with 12.94 and 1.17% frequency of occurrence, respectively, and in non-vegetarian population, the cyfluthrin and chlorpyriphos residues were found in

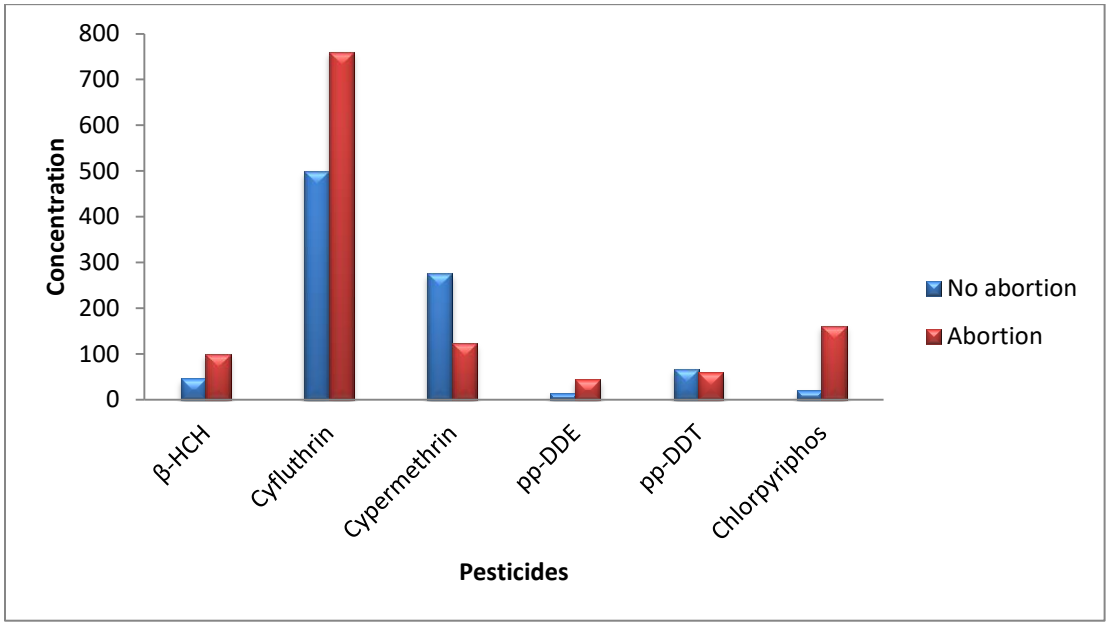
the mean level of 24.81 and 1.94 ng g^{-1} respectively with 9.52% frequency of occurrence each. The residues of total HCH and chlorpyriphos were higher in non-vegetarian population. On the contrary, residues of total DDT, cypermethrin and cyfluthrin were high in vegetarian population.

Statistically non-significant difference (P= 0.9174) between rural and urban population was observed for total HCH and for DDT also a non-significant difference was observed between rural and urban population (P= 0.578). Similarly a non-significant difference was observed between vegetarian and non vegetarian for total HCH and total DDT at p values 0.4987 and 0.1803, respectively.

The mean residue levels of pesticide residues were compared between the mothers having a history of abortion, miscarriage and still birth and the mothers without any such history. It was found that the mean residue levels of these pesticides varied among the two groups. The mean residue levels of almost all the pesticides were higher in the samples from the mothers with previous history of abortion, miscarriage or stillbirth than those with no such history. Mean residue levels of β-HCH were 99.81 and 45.75 ng g^{-1}, respectively in samples from mothers with history of abortion and samples from mothers with no history of abortion. Similarly mean levels of Cyfluthrin and Cypermethrin were 498.86 and 276.35 in ng g^{-1}, respectively, in mothers without abortion history and 760.29 & 122.86 ng g-1, respectively, in mothers with abortion history. Likewise, mean levels of p.p' DDE and p,p' DDT were 14.26 and 66.96 ng g^{-1}, respectively, in normal mothers and it was 43.19 and 58.56 ng g^{-1}, respectively, in mothers with abortion history. Chlorpyriphos levels were eight times higher in mothers with a history of abortion as compared to mothers with no such history (Fig. 6).

Statistically there was non-significant difference between mean residue levels of pesticides in mothers with an abortion history and mother without any history of abortion (p=0.70)

Fig.6: Comparison of pesticides residue levels in mothers with an abortion history and without abortion history



4.4.1 Risk assessment for infants

Estimated daily intake (EDI) is calculated to estimate the magnitude of exposure to pesticides in infants (table 15). It is calculated by assuming that a 5 kg infant ingests approximately 700 g milk per day (Oostdam *et al* 1999). The average daily intake values of HCH and DDT by an infant from her mother's milk by the equation**:-**

$$EDI = Cmilk \times 700g / 5$$

where, EDI is estimated daily intake (ng/kg bw/day), Cmilk is the concentration of HCH and DDT residues in milk (ng g^{-1}). The individual intake for residues was compared with tolerable daily intake (TDI) guidelines proposed by FAO/WHO (FAO 2008). According

Table 15: Estimated daily intake (ng/kg bw/day) of total HCH and DDT residues by infants based on residues in mother's milk

Sample no.	EDI for HCH	EDI for DDT	Sample no.	EDI for HCH	EDI for DDT
1	3080	8820	42	0	0
2	0	0	43	0	0
3	0	0	44	0	0
4	0	0	45	0	0
5	0	21042	46	0	0
6	4823	0	47	0	4795
7	0	0	48	0	0
8	0	0	49	0	0
9	0	0	50	0	0
10	0	0	51	0	0
11	12972	0	52	0	0
12	0	0	53	0	0
13	0	22582	54	0	0
14	0	0	55	0	4666
15	0	0	56	4922	0
16	0	0	57	0	0
17	0	0	58	0	6046
18	8093	17094	59	8285	0
19	0	0	60	0	0
20	0	0	61	0	0
21	0	0	62	0	2402
22	0	1094	63	0	0
23	0	0	64	0	0
24	10780	0	65	0	0
25	0	0	66	0	0
26	446	0	67	0	0
27	0	0	68	0	0
28	0	0	69	5139	0
29	0	11137	70	0	0
30	0	0	71	0	11394
31	0	0	72	0	2517
32	1555	0	73	0	12843
33	0	0	74	0	5003
34	0	0	75	0	0
35	0	0	76	0	0
36	0	0	77	3514	0
37	0	14490	78	0	0
38	0	0	79	2969	0
39	0	0	80	0	3575
40	0	0	81	0	0
41	0	0	82	9205	0

Sample no.	EDI for HCH	EDI for DDT	Sample no.	EDI for HCH	EDI for DDT
83	0	0	106	0	0
84	0	0	107	0	0
85	4949	0	108	0	0
86	0	0	109	0	0
87	0	8398	110	6371	0
88	0	0	111	0	0
89	0	0	112	0	0
90	0	0	113	0	0
91	0	0	114	0	0
92	0	0	115	8558	0
93	8414	0	116	0	0
94	0	0	117	0	0
95	0	0	118	0	0
96	0	0	119	0	0
97	0	0	120	0	0
98	18743	1590	121	4760	12012
99	0	0	122	0	0
100	4524	0	123	4340	0
101	0	0	124	0	0
102	0	0	125	0	0
103	0	0	126	3220	10682
104	0	0	127	0	0
105	4774	0			

to the guidelines, the tolerable daily intake for DDT and HCH residues is 10000 and 300 ng/kg bw/day, respectively. In the present study it was found that daily intake of 9 samples of DDT and all the positive samples for HCH (i.e. 23 samples) exceeded the tolerable daily intake (Table 15). Occurrence of DDTs, which are the predominant contaminants in developing countries, is a matter of concern with respect to exposure in mother and children. Some earlier studies suggest that p,p'-DDE is associated with adverse effects such as premature birth, lower weight of infants and reduced baby size at birth (Longnecker *et al* 2001). The estimated infant daily intake of OCs shows that the intake of HCHs and DDTs through lactation exceeded the TDI, which is of concern on infant health. Nursing infants are considered a risk group for several reasons. Their only

food intake is breast milk. Also their metabolism could not be mature enough to eliminate the pollutants they are exposed to. Their ratio food ingested per body weight is the highest in humans. Lastly they are at a life stage of great growth and development, so they may be more affected by the presence of various pollutants

Table 16: Comparison of DDT and HCH residues in mother's milk in present study with earlier studies

Country	Survey year	HCH	DDT	Reference
Developed nations				
Australia	1995	645	1185	Quinsey *et al* (1995)
Canada	1996	23	470	Newsome and Ryan (1999)
UK	1997-98	103	470	Harris *et al* (1999)
Japan	2001-2004	110	340	Kunisue *et al* (2006)
Russia	2003-2004	810	660	Tsydenova *et al* (2007)
Norway	-	18	136	Polder *et al* (2009)
Developing nations				
Brazil	1992	280	1700	Paumgartten *et al* (2000)
Mexico	1997-98	60	4700	Waliszewski *et al* (2001)
China	1999-2000	1110	3550	Wong *et al* (2002)
Indonesia	-	90	830	Burke *et al* (2003)
China	2002	1400	2100	Kunisue *et al* (2004)
Vietnam	2000-2001	57.5	2260	Minh *et al* (2004)
Malaysia	20003	230	1600	Sudaryanto *et al* (2005)
South Africa	-	-	4797.8	Bouwman *et al* (2006)
Tunisia	2002-2005	65.0	1931	Ennaceur *et al* (2008b)
China	-	640.1	773	Leng *et al* (2009)
Iran	2006	5740	3560	Behrooz *et al* (2009)
India				
Nagaon	2009-2010	2717	3206	Mishra and Sharma (2011)
Delhi	2005-2006	340	1500	Devanathan *et al* (2009)
Kolkata	2004-2005	670	1100	Devanathan *et al* (2009)
Chennai	2002-2003	4500	1200	Subramanian *et al* (2007)
Agra	-	127.5*	175.5*	Kumar *et al* (2006a)
Punjab	1999-2000	121.1*	132.9*	Gill (2000)
Punjab	**2011-2012**	**8.12***	**10.25***	**Present study***
		270.66	**341.66**	**Present study**

**- on fresh milk weight basis, rest on milk lipid weight basis*

The residues of DDT and HCH in present study were compared with previous studies in the in India and other developed and developing nations (Table 16). It was observed that the levels of HCH are higher as compared to many other countries but levels of DDT were low as compared to the previous studies. In the present study the results were expressed on fresh milk weight basis whereas results in other studies were calculated on milk lipid weight basis. The residues levels for HCH and DDT in the present study were 8.12 and 10.25 ng g^{-1}, respectively. So assuming the average lipid content of mother's milk as 3% we calculated our results on milk lipid weight basis and observed that the residues of HCH were relatively high in this study as compared to previous studies from most of the developed nations. These residues are relatively less in mother's milk samples of Punjab when compared to the studies from other parts of the country in the past (Kumar *et al* 2006a, Subramanian *et al* 2007, Devanathan *et al* 2009, Mishra and Sharma 2011). Also the residue levels were less on comparison to other developing nations (Wong *et al* 2002, Kunisue *et al* 2004, Behrooz *et al* 2009). On the other hand the levels are relatively high when compared to the developed countries.

On comparison to the previous study in Punjab a decade ago (Gill 2000), the levels are quite lower in present study. The levels of HCH and DDT residues in Punjab in 2000 were 121.1 ng g^{-1} and 132.9 ng g^{-1}, respectively while in present study the levels are 8.12 and 10.25 ng g^{-1}, respectively. Hence, levels of HCH and DDT have declined by 15 and 12 times, respectively during the last 10 years which can be attributed to the ban on the use of DDT and HCH in the agriculture and public health sector. The use of DDT has been banned in agricultural sectors and its usage has been restricted in public health programmes since 1991. Since DDT is known to undergo metabolic conversion and

dehydrochlorination, presence of metabolites of DDT i.e. DDD and DDE encountered in this study might be due to such metabolic processes. Likewise technical grade HCH has been also banned since 1997 and among various isomers of HCH, only γ-HCH is permitted for use in agriculture. Thus, the higher mean concentration of γ-HCH in present study may be due to its permitted use as compared to the β-HCH. Commercial formulations of HCHs used in India are technical mixture of HCH and Lindane. Technical HCH is composed of a mixture of many isomers, of which α, β, γ and δ are common while lindane is composed entirely of γ-HCH insecticidal form. As the application of technical mixture of HCH has been banned, Government of India has now encouraged the use of Lindane (γ-HCH) comprising all the insecticidal properties of technical HCH (Gupta 2004). The presence of β-HCH in the present study can be attributed to the fact that it is the the most persistent and prevalent isomer of HCH.

The consumption of SPs has increased significantly with the decline in the use of OCP's (Mukherjee *et al* 2007). Thus the results of this study regarding use of SPs can be related with the increasing use of SPs in replacement of persistent organochlorines. Cyfluthrin, is the new pesticide detected in our study as compared to previous study from India in the past. However the presence of pyrethrins in the breast milk from other nations has been reported (Bouwman *et al* 2006, Corcellas *et al* 2012).

4.2 Pesticide residues in human blood samples

To study the levels of pesticide residues in blood samples of human population, samples were collected from Muktsar and Bhatinda districts of Punjab which is the cotton belt of Punjab and the highest user of pesticides in Punjab. After analyzing samples, it was

Table 17: General characteristics of blood samples donors from Bhatinda & Muktsar districts

Sample	Age (yr)	Sex	Height	Weight (kg)	Dietary habits	Any ailment	Spray of pesticides	Tobacco chewing/ smoking
1	50	Male	5’9”	77	Vegetarian	No	No	No
2	58	Male	5’10”	75	Vegetarian	Joint pain	No	No
3	24	Male	5’7”	60	Non-vegetarian	No	No	No
4	27	Male	5’10”	60	Non-vegetarian	No	Yes	No
5	38	Male	5’7”	78	Non-vegetarian	Skin irritation	Yes	No
6	20	Male	5’7”	65	Non-vegetarian	No	No	No
7	21	Male	5’10”	55	Non-vegetarian	No	No	No
8	33	Male	6’2”	95	Non-vegetarian	No	Yes	No
9	55	Male	5’6”	50	Non-vegetarian	No	No	No
10	25	Male	5’5”	60	Vegetarian	No	Yes	No
11	21	Male	6’0”	70	Non-vegetarian	No	Yes	No
12	30	Male	5’11”	76	Non-vegetarian	No	No	No
13	25	Male	5’4”	73	Non-vegetarian	No	No	No
14	25	Male	5’8”	60	Non-vegetarian	No	No	No
15	45	Male	5’8”	75	Non-vegetarian	No	No	No
16	33	Male	5’4”	70	Non-vegetarian	No	No	Yes
17	21	Male	6’0”	75	Non-vegetarian	No	No	No
18	47	Male	5’4”	50	Non-vegetarian	No	No	No
19	26	Male	5’7”	60	Non-vegetarian	No	No	Yes
20	34	Male	5’4”	61	Non-vegetarian	No	No	Yes
21	36	Male	5’7”	78	Non-vegetarian	No	No	Yes
22	15	Male	5’0”	45	Non-vegetarian	No	No	No
23	30	Male	6’0”	50	Non-vegetarian	No	No	Yes
24	29	Male	5’0”	57	Non-vegetarian	No	No	Yes
30	30	Male	5’5”	60	Vegetarian	Head injury	No	No
31	28	Male	5’6”	72	Non-vegetarian	No	No	Yes
32	17	Male	5’4”	56	Non-vegetarian	No	No	No
33	24	Male	5’11”	24	Vegetarian	No	No	No
34	39	Male	5’9”	95	Non-vegetarian	No	No	No
35	19	Male	5’8”	75	Non-vegetarian	No	Yes	No
36	24	Male	5’6”	48	Vegetarian	No	Yes	No
37	21	Male	5’8”	65	Vegetarian	No	Yes	No
38	35	Male	5’5”	65	Vegetarian	No	No	No
39	28	Male	5’9”	84	Vegetarian	Itchy skin	Yes	No
40	34	Male	5’10”	83	Non-vegetarian	No	No	No

Sample	Age (yr)	Sex	Height	Weight (kg)	Dietary habits	Any ailment	Spray of pesticides	Tobacco chewing/ smoking
41	40	Male	5’0”	86	Non-vegetarian	No	No	No
42	37	Male	5’6”	68	Non-vegetarian	No	No	No
43	45	Male	4’8”	50	Non-vegetarian	No	No	Yes
44	25	Male	5’4”	73	Non-vegetarian	No	No	No
45	26	Male	5’7”	60	Non-vegetarian	No	No	Yes
46	29	Male	5’0”	57	Non-vegetarian	No	No	Yes
47	27	Male	5’8”	80	Non-vegetarian	No	No	Yes
48	39	Male	5’9”	95	Non-vegetarian	No	No	No
49	24	Female	5’2”	64	Non-vegetarian	No	No	No
50	34	Male	5’6”	75	Vegetarian	Allergy	No	Yes
51	34	Male	5’4”	72	Non-vegetarian	Allergy	No	No
52	30	Male	5’11”	78	Vegetarian	No	No	No
53	36	Male	5’9”	69	Vegetarian	Asthma	No	No
54	21	Male	5’8”	70	Vegetarian	Allergy	No	No
55	35	Male	5’9”	80	Vegetarian	No	No	Yes
56	35	Male	5’10”	78	Vegetarian	No	No	No
57	24	Male	5’7”	65	Non-vegetarian	Allergy	No	No
58	47	Male	5’10”	77	Vegetarian	No	No	Yes
59	44	Male	5’7”	72	Vegetarian	Allergy	No	Yes
60	50	Male	5’7”	68	Vegetarian	Allergy	No	Yes
61	62	Male	5’10”	65	Non-vegetarian	No	Yes	No
62	32	Male	6’2”	82	Non-vegetarian	Allergy	Yes	No
63	32	Male	5’6”	76	Non-vegetarian	No	No	No
64	35	Male	5’5”	57	Non-vegetarian	No	No	No
65	35	Male	6’0”	50	Non-vegetarian	Allergy	No	Yes
66	48	Male	5’11”	55	Non-vegetarian	No	No	Yes
67	60	Female	5’4”	60	Vegetarian	No	Yes	No
68	60	Male	5’8”	80	Vegetarian	Allergy, Asthma	No	Yes
69	60	Male	5’8”	74	Vegetarian	Allergy	No	No
70	55	Male	5’4”	60	Non-vegetarian	Allergy	No	No
71	34	Male	6’0”	68	Vegetarian	Allergy	No	Yes
72	45	Male	5’4”	72	Vegetarian	No	No	Yes
73	29	Male	5’9”	55	Non-vegetarian	No	No	No
74	16	Male	5’10”	60	Non-vegetarian	Asthma	No	No
75	45	Male	5’7”	65	Vegetarian	Allergy	No	No
76	47	Male	5’10”	72	Non-vegetarian	No	No	No
77	60	Male	5’7”	80	Vegetarian	Allergy	No	No
78	60	Male	5’7”	78	Non-vegetarian	Asthma	No	Yes
79	55	Male	5’10”	88	Non-vegetarian	Asthma	No	Yes

Sample	Age (yr)	Sex	Height	Weight (kg)	Dietary habits	Any ailment	Spray of pesticides	Tobacco chewing/ smoking
80	48	Male	6’2”	57	Non-vegetarian	No	No	Yes
81	40	Male	5’6”	50	Non-vegetarian	No	No	Yes
82	55	Male	5’5”	55	Non-vegetarian	No	No	Yes
83	42	Male	6’0”	50	Non-vegetarian	Allergy	No	No
84	39	Male	5’11”	80	Non-vegetarian	Asthma	No	No
85	42	Male	5’4”	74	Vegetarian	No	No	Yes
86	58	Male	5’8”	60	Vegetarian	No	Yes	Yes
87	39	Male	5’8”	65	Vegetarian	Allergy	No	No
88	21	Male	5’4”	60	Vegetarian	No	Yes	No
89	55	Male	6’0”	74	Vegetarian	Allergy	No	No
90	55	Male	5’4”	78	Vegetarian	No	Yes	Yes
91	34	Male	5’7”	65	Non-vegetarian	No	Yes	Yes
92	30	Male	5’4”	65	Vegetarian	No	Yes	No
93	38	Male	5’7”	84	Vegetarian	No	No	Yes
94	28	Male	5’0”	83	Vegetarian	Allergy	No	No
95	57	Male	6’0”	86	Vegetarian	No	Yes	No
96	40	Female	5’0”	68	Vegetarian	No	Yes	No
97	34	Male	4’8”	50	Vegetarian	Allergy	No	Yes
98	28	Male	5’6”	73	Vegetarian	No	Yes	No
99	45	Male	5’6”	60	Vegetarian	No	No	No
100	41	Male	5’8”	57	Vegetarian	No	No	No
101	30	Male	5’10”	80	Non-vegetarian	No	Yes	Yes
102	52	Male	5’5”	95	Non-vegetarian	No	Yes	No
103	27	Female	5’4”	64	Vegetarian	Allergy	Yes	No
104	40	Female	5’4”	75	Vegetarian	No	No	No
105	30	Female	5’2”	72	Vegetarian	Allergy, Asthma	No	No
106	20	Male	5’9”	78	Vegetarian	No	No	No
107	55	Female	5’4”	69	Vegetarian	Asthma	No	No
108	40	Male	5’6”	70	Vegetarian	No	No	Yes
109	50	Male	5’9”	68	Vegetarian	No	No	Yes
110	52	Female	5’2”	68	Vegetarian	No	Yes	Yes
111	55	Female	5’4”	66	Vegetarian	No	Yes	Yes

TABLE 18: Levels of pesticide residues (ng $ml^{-1)}$ in human blood

Pesticides	Mean	Standard deviation	Median	Range	Positive percentage	Percentage contribution
α-HCH	1.11	10.38	7.57	ND-109	2.70	6
β-HCH	5.89	21.57	84.56	ND-102.63	9.00	20
p,p' DDD	0.51	5.38	56.71	ND-56.71	0.90	2
p,p' DDE	3.88	12.81	13.22	ND-102.12	16.21	36
p,p' DDT	0.39	4.07	42.87	ND-42.87	0.90	2
β-Endosulfan	34.90	193.64	248.68	ND-1823.18	8.11	18
Monocrotophos	0.79	4.23	23.045	ND-27.11	3.60	8
Phosalone	0.39	3.29	21.46	ND-33.51	1.80	4
Profenophos	6.76	54.19	375.47	ND-527.29	1.80	4

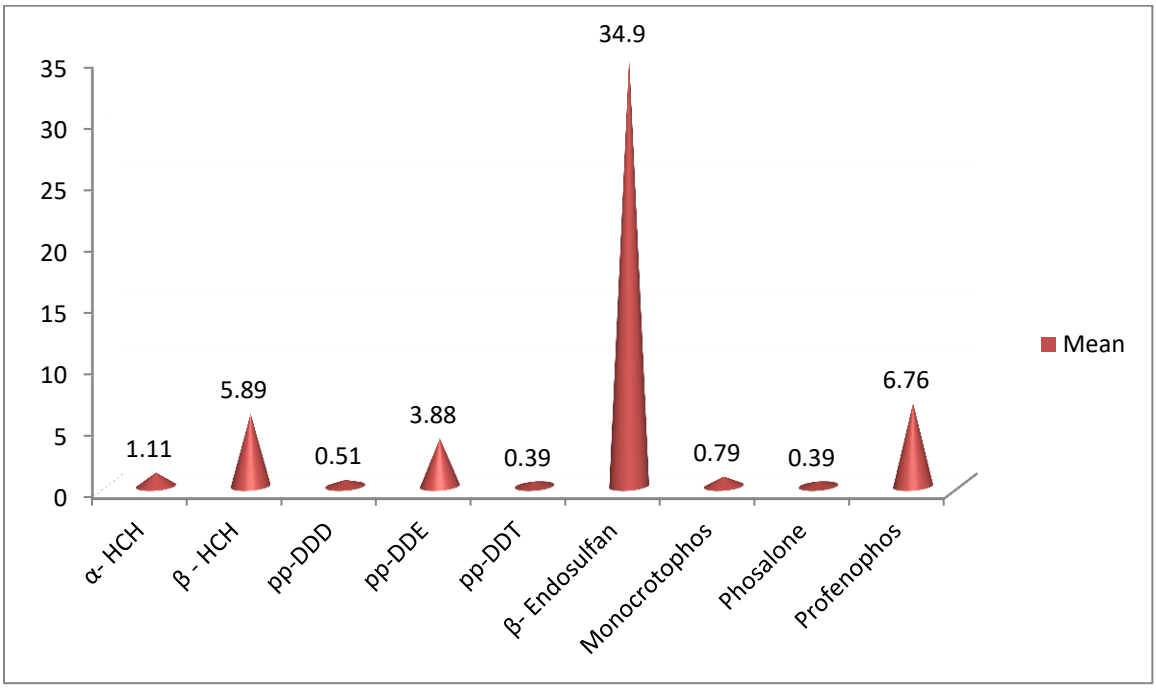
observed that 35% of the total samples collected were contaminated with different pesticide residues. Residues of α-HCH, β-HCH, p,p' DDD, p,p' DDE, p,p' DDT, β-endosulfan, monocrotophos, phosalone and profenophos were found in blood samples. The highest mean residue concentration was found for β-endosulfan and the lowest mean level was found for p,p' DDT and phosalone (Fig 7).

Among the isomers of HCH, α-HCH and β-HCH were the only two found in the samples with mean residue level of 1.11 and 5.89 ng ml^{-1} with a range of ND-109 ng ml^{-1} and ND-102.63 ng ml^{-1}, respectively. The frequency of occurrence was 6 and 20%, respectively, for α-HCH and β-HCH. Residues of p,p' DDD, p,p' DDE and p,p' DDT were found in 2, 36 and 2% of the blood samples, respectively. The mean residue levels of p,p' DDD, p,p' DDE and p,p' DDT were 0.51, 3.88, 0.39 ng ml^{-1}, respectively (Table 18).

Mean residue level of β-endosulfan was 34.90 ng ml^{-1} with 18% frequency of occurrence and a range of ND-1823.18 ng ml^{-1}. Monocrotophos, phosalone and profenophos were the only OPs detected in 8, 4 and 4% of the blood samples at mean residue levels of 0.79, 0.39 and 6.76 ng ml^{-1}, respectively. Out of the total HCH residues β-

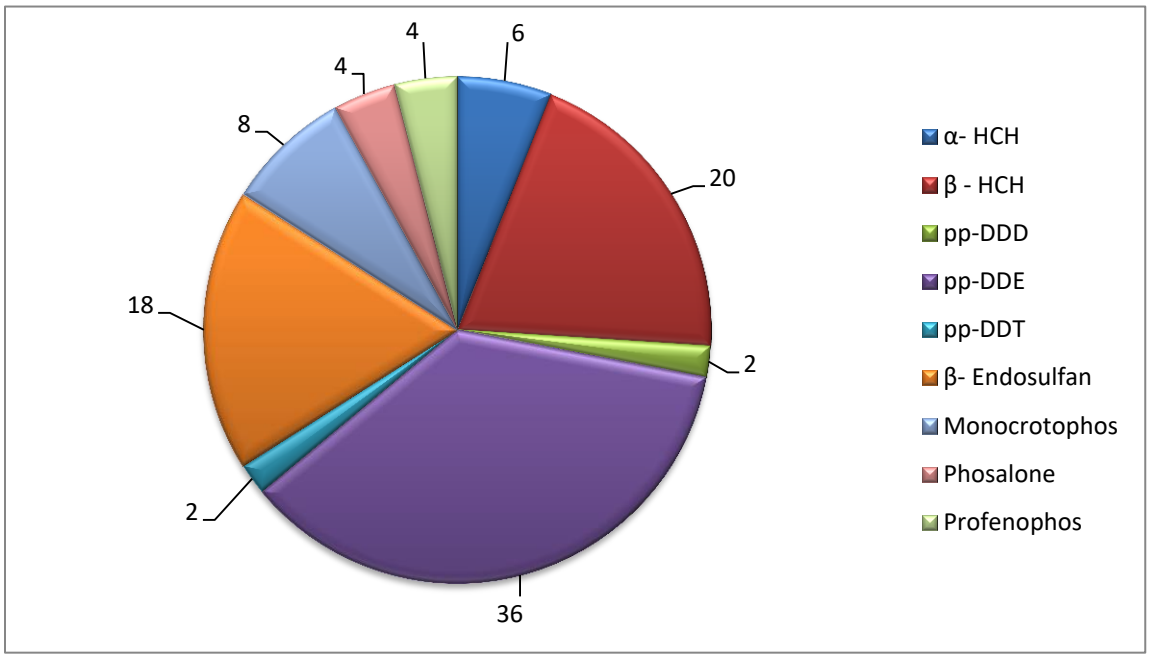
HCH was at highest mean residual concentration followed by α-HCH. Similarly among DDT metabolites, p,p' DDE was found in highest mean concentration followed by p,p' DDD and p,p' DDT.

Fig 7: Mean residue levels of different pesticides in human blood (ng ml^{-1}).



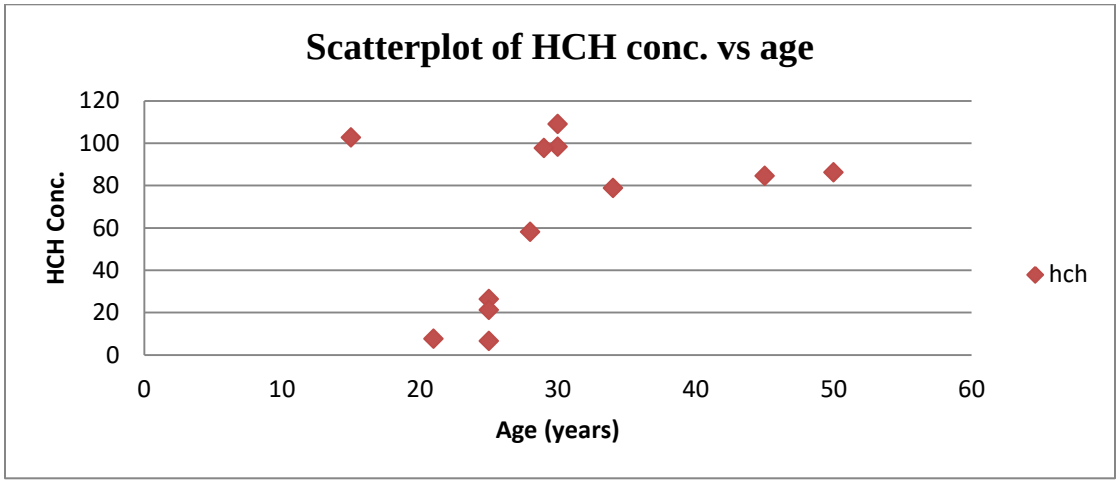
Of the total pesticide residues found in human blood, the maximum contribution was observed for p,p' DDE which was 36% followed by β-HCH as 20% (Fig 8). Likewise the percentage proportion for α-HCH was 6%. p,p' DDD and p,p' DDT contributed 2% each to the total. β-endosulfan contributed around 18% and monocrotophos contributed around 8%. Phosalone and profenophos contributed 4% each to the total pesticide load.

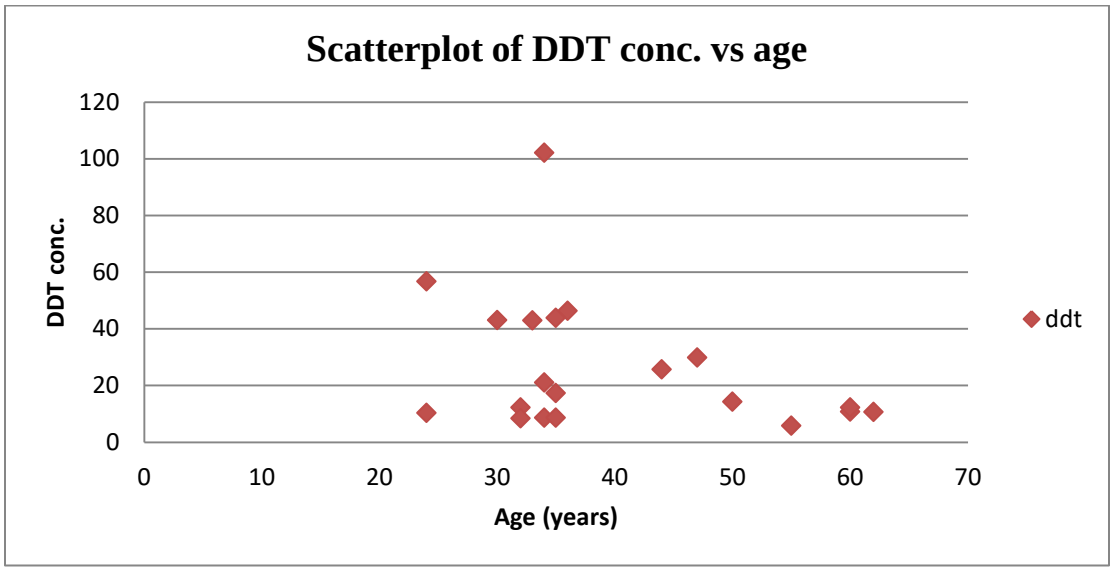
Fig 8: Percentage contribution of different pesticides found in human blood samples



These pesticides levels are studied in relation to the age, dietary habits, spraying activity as well as gender of the donor to find out whether these parameters are positively or negatively correlated with residue levels in human blood (Tables 19-22). Scatterplot of residues level in relation to age (Fig. 9) clearly indicates that HCH and β-endosulfan residues are positively correlated to age whereas DDT residues are negatively correlated to age. Blood sample donors were divided into four age groups viz, <20-30, 31-40, 41-50 and >51-60 years. Mean residue concentration of HCH in first age group was 12.26 ng ml^{-1} 18.60% frequency of occurrence. Likewise 9.30% of the donors contained DDT residues in their samples at mean residue level of 3.55 ng ml^{-1} in the first age group. β-endosulfan is found in 11.62% of the samples at mean level of 27.31 ng ml^{-1}.

Fig 9: Scatter plot of HCH, DDT and β-endosulfan residues in human blood in relation to their age





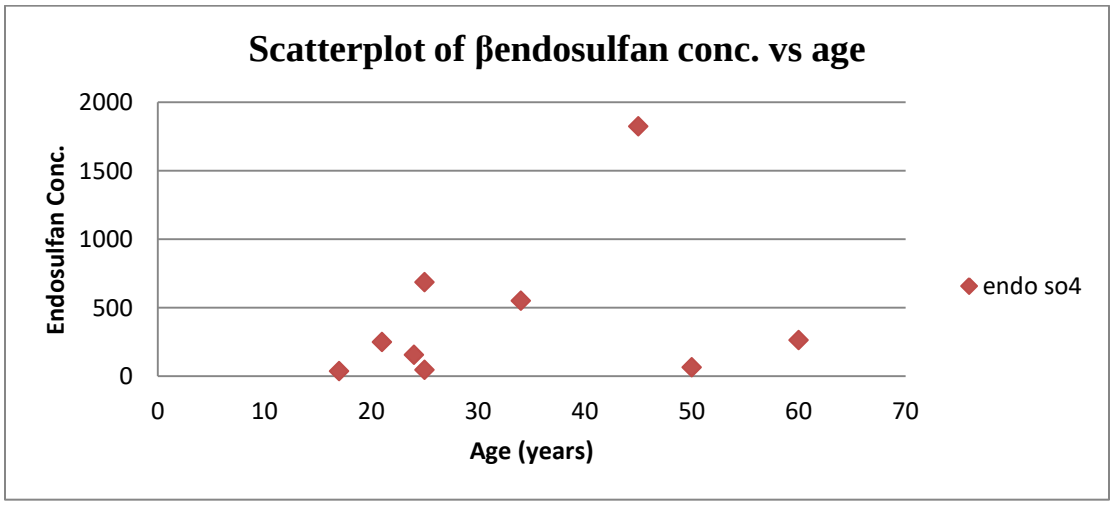


Table 19: Mean residue levels of pesticides (ng ml^{-1}) in human blood in relation to different age groups

Age group (years)	HCH	DDT	β-endosulfan	Monocrotophos	Phosalone	Profenophos
<20-30 (n=43)	12.26±30.87	3.55±12.40	27.31±112.12	0.86±3.89	ND	5.20±34.11
Frequency of occurrence (%)	18.60	9.30	11.62	4.65	-	2.32
31-40 (n=29)	2.72±14.63	9.24±21.64	18.33±100.41	0.93±5.03	ND	ND
Frequency of occurrence (%)	3.44	31.03	3.44	3.44	-	-
41-50 (n=20)	8.54±26.27	3.48±8.90	94.351±407.17	1.26±5.63	1.67±7.49	26.36±117.90
Frequency of occurrence (%)	10	15	10	5.00	5.00	5.00
>51-60 (n=19)	ND	2.06±4.27	13.87±60.46	ND	0.49±2.16	ND
Frequency of occurrence (%)	-	21.05	5.26	-	5.26	-

Table 20: Mean residue levels of pesticides (ng ml^{-1}) in human blood in relation to dietary habits

Dietary habits	HCH	DDT	β-endosulfan	Monocrotophos	Phosalone	Profenophos
Non-vegetarian (n=63)	12.33±31.63	2.80±9.46	7.75±37.23	0.83±4.62	ND	ND
Frequency of occurrence (%)	17.46	14.28	63.49	3.17	_	_
Vegetarian (n=48)	ND	7.37±18.52	34.91±18.52	0.79±4.23	0.39±3.29	6.76±54.19
Frequency of occurrence (%)	_	22.92	10.42	41.67	41.67	41.67

Table 21: Mean residue levels of pesticides (ng ml^{-1}) in human blood in relation to gender

Gender	HCH	DDT	β-endosulfan	Monocrotophos	Phosalone	Profenophos
Male (102)	7.61±24.59	4.53±16.68	34.91±193.64	0.79±4.23	0.39±3.29	6.76±54.18
Frequency of occurrence (%)	10.78	17.64	8.25	27.52	1.83	0.91
Female (n=9)	ND	7.49±18.79	ND	1.68±5.05	ND	24.85±74.55
Frequency of occurrence(%)	-	22.22	-	11.11	-	11.11

Table 22: Mean residue levels of pesticides (ng ml^{-1}) in human blood in relation to their occcupation

Occupation	HCH	DDT	β-endosulfan	Monocrotophos	Phosalone	Profenophos
Pesticide sprayer(n=24)	0.31±1.54	3.19±9.29	28.57±139.99	0.63±3.09	ND	9.32±45.65
Frequency of occurrence (%)	41.66	16.66	4.2	4.2	-	4.2
Pesticide non-sprayer (n=87)	8.84±27.51	6.06±56.53	36.65±206.66	0.84±4.50	4.93±3.72	6.06±56.53
Frequency of occurrence (%)	11.49	18.39	9.19	3.44	22.99	1.14

Monocrotophos and profenophos were detected in 4.65 and 2.32% of the samples with a mean concentration of 0.86 and 5.20 ng ml^{-1}, respectively.

In the second age group, HCH and DDT residues were found in 3.44 and 31.03% of the population with mean residue levels at 2.72 and 9.24 ng ml^{-1}, respectively. No residues of phosalone and profenophos were detected in second age group. β-endosulfan and monocrotophos were detected at mean levels of 18.33 and 0.93 ng ml^{-1}, respectively in 4% of the blood samples each. In the third age group, highest level of β-endosulfan was found at mean level of 94.35 ng ml^{-1} in 10% of the population. In the fourth age group only DDT, β-endosulfan and phosalone were detected in 21.05, 5.26 and 5.26% of the samples with 2.06, 13.87 and 0.49 ng ml^{-1} mean residue levels, respectively. Statistical analysis of the samples shows that age has positive correlation with HCH and β-endosulfan with a correlation coefficient of 0.34 and 0.27, respectively. Also there is a significant difference between different age groups and HCH levels (p value 0.01) but non-significant difference between age groups and β-endosulfan levels (p= 0.06). There is a negative correlation between residual level of DDT and age (r=-0.36) and a significant difference between age groups and residue levels (p= 0.03). Increasing age in relation to higher pesticide residue levels have been reported earlier by many researchers (Bates *et al* 2004, Dirtu *et al* 2006).

The study of pesticide residue levels in relation to dietary habit of the humans suggest that there is a non significant difference between vegetarian and non-vegetarian population for the residue levels of DDT and β-endosulfan (p=0.23 and 0.15

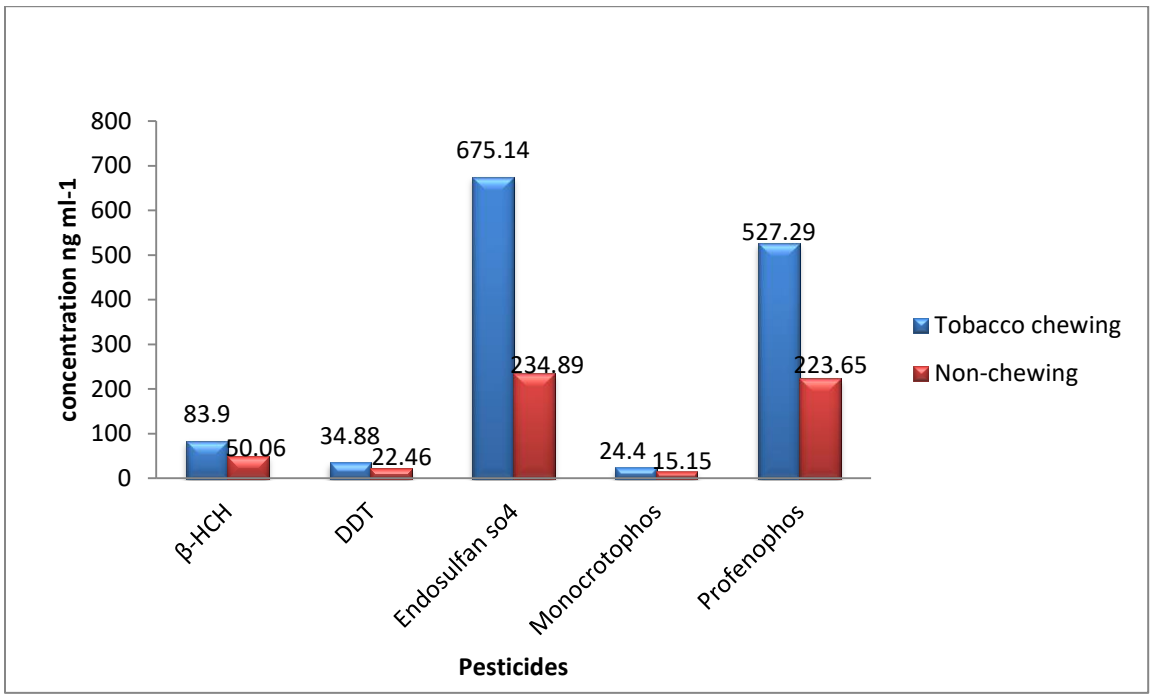
HCH residues are found only in vegetarian population at mean levels of 12.33 ng ml^{-1} in 17.46% of the samples. Whereas DDT, β-endosulfan and monocrotophos were detected in both vegetarian and non vegetarian population. In vegetarian population the mean concentration level of DDT, β-endosulfan and monocrotophos were found as 2.80, 7.75 and 0.83 ng ml^{-1} in 14.28, 63.49 and 3.17 percent of the samples, respectively. In the non-vegetarian population, mean levels DDT, β-endosulfan and monocrotophos were found to be 7.37, 34.91 and 0.79 ng ml^{-1}, respectively, in 22.92, 10.42 and 41.67% of the non-vegetarian population. Phosalone and profenophos residue were detected in non-vegetarian population only at mean residue levels of 0.39 and 6.76 ng ml^{-1} with 41.6% frequency of occurrence each.

In relation to gender, no significant difference was found between male and female in relation to levels of pesticide residues. In this study total samples were collected from 102 males and 9 females. HCH, β-endosulfan and phosalone are found only in males at mean residue levels of 7.61, 34.91 and 0.39 ng ml^{-1}, respectively, with 10.78, 8.25 and 1.83% frequency of occurrence. DDT, monocrotophos and profenophos are found in both male and female population. In males DDT, monocrotophos and profenophos were found at mean residual concentration of 4.53, 0.79 and 6.76 ng ml^{-1} respectively and respective frequency of occurrence was 17.64, 27.52 and 0.91%, respectively. In females, the levels of DDT, monocrotophos and profenophos were relatively high viz, 7.49, 1.68 and 24.85 ng ml^{-1} and the frequency of occurrence was 22.22, 11.11 and 11.11%, respectively. There are previous studies from various countries which have also shown higher prevalence of pesticide residues in females as compared to males (Charlier and Plomteux 2002, Lino and Silveria 2006, Luzardo *et al* 2006).

Similarly on comparing the mean residue levels between pesticide sprayer and non-sprayer population, it was observed that the mean residue levels of HCH and DDT were 0.31 and 3.19 ng ml^{-1} , respectively, in sprayer population and 8.84 and 6.06 ng ml^{-1} respectively, in non-sprayer population. The frequency of occurrence for HCH and DDT was 41.66 and 16.66%, respectively, in sprayer population and 11.49 and 18.39, respectively, in non-sprayer population. Hence it is clear that although the mean value of HCH is less in sprayer population but the prevalence is more in sprayer population which may be because of their direct exposure to pesticides during their handling and usage. Similarly monocrotophos and profenophos have higher prevalence in sprayer population as compared to the non-sprayer population. Mean residue levels of β-endosulfan in sprayer population was 28.57 ng ml^{-1} with 4.2% frequency of occurrence. The residue levels of phosalone are detected only in sprayer population at 4.93 ng ml^{-1} with 22.99% frequency of occurrence. Stastically there was no significance difference between residue levels in sprayer and non sprayer population.

A comparison was made for the pesticide residue levels in blood of humans with tobacco chewing and smoking habit and with no chewing and smoking habit. It was observed that the mean residue levels of pesticides were higher in the people with chewing and smoking habit as compared to those with no habit of tobacco chewing and smoking. Levels of β-endosulfan was around 3 times higher in tobacco chewing population than non-chewing population with mean residue levels of 675.14 and 234.89 ng ml^{-1} in chewing and non-chewing population, respectively. Similarly mean levels of profenophos was around two times higher in people with chewing and smoking habit as

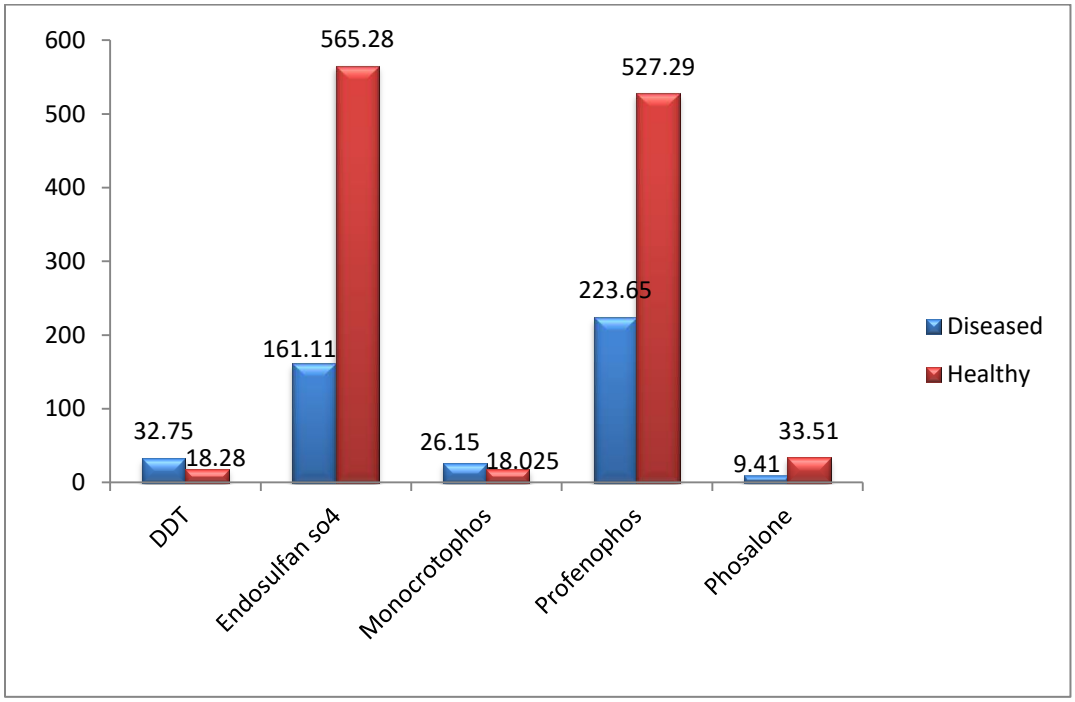
Fig. 10: **Comparison of mean pesticide residue levels (ng ml^{-1}) in blood samples of tobacco chewing/ smoking and non-chewing population**



compared to non-chewing population. Levels of β-HCH, DDT and monocrotophos were also higher in tobacco-chewing population as compared to non-chewing population. This may be correlated to the fact that people with tobacco-chewing habits may have direct oral exposure to the pesticides during their spraying on the crops and in the fields (Fig. 10). Statistically there was a non significant difference between residue levels the blood samples of tobacco chewing and non-tobacco chewing population ($p=0.32$). A comparison was also made between the mean pesticide residues levels in blood samples of healthy and diseased humans (Fig. 11). It was observed that the mean residue levels of

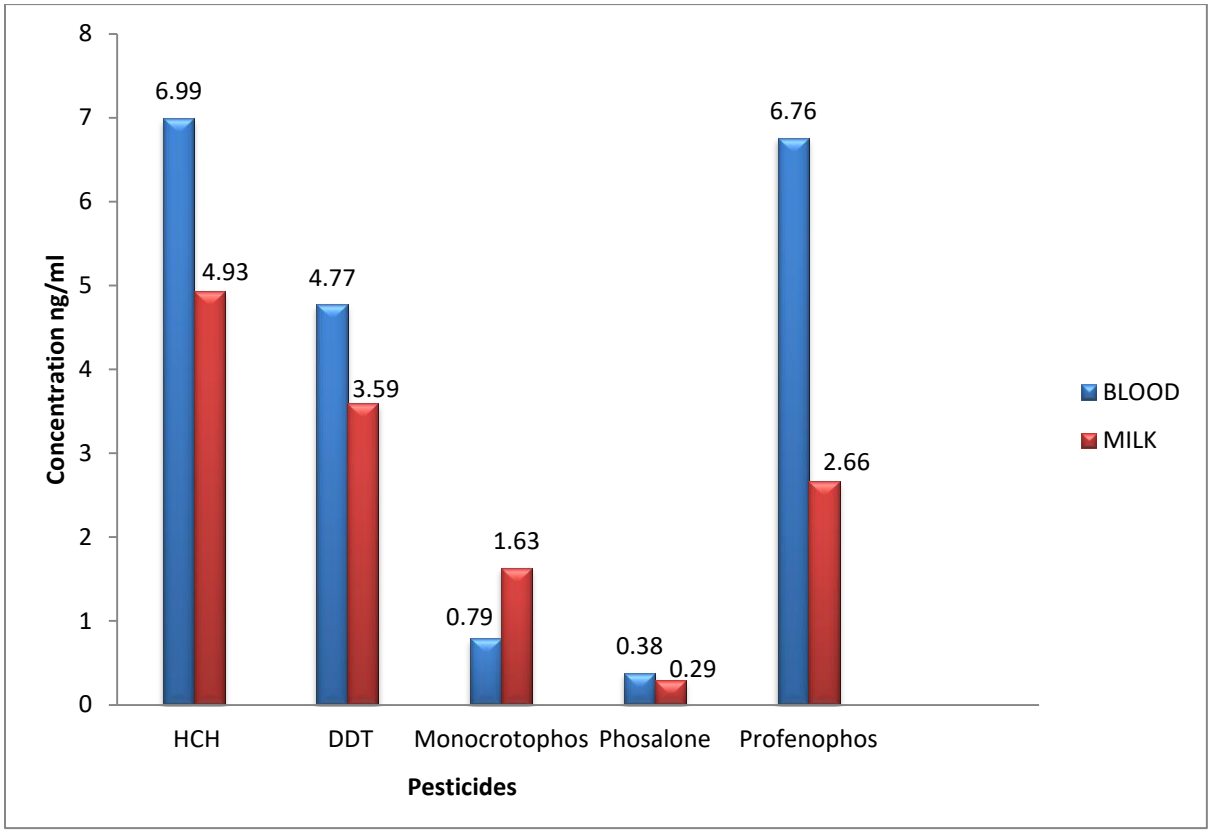
DDT and monocrotophos was higher in blood samples from diseased population than the samples from healthy population with the mean values of 32.75 and 26.15 ng ml^{-1} in

Fig. 11: Comparison of mean pesticide residue levels in blood samples of Disease and Healthy population



diseased population for DDT and monocrotophos, respectively. Whereas the mean residue levels of DDT and monocrotophos in healthy population were 18.28 and 18.025 ng ml^{-1} respectively. Further it was found that the mean residue levels of β-endosulfan, profenophos and phosalone were rather higher in samples from healthy population as compared to those from diseased population. Stastically there was a non significant difference between mean levels of pesticide residues in human blood from diseased and healthy population (p=0.34)

Fig. 12: Overall comparison of pesticide residue levels in human blood and human milk.



On comparing levels of pesticides residues in human milk and blood samples it was found that the levels of HCH, DDT, phosalone and profenophos were higher in human blood as compared to the levels in human milk (Fig. 12). Only monocrotophos was in higher concentration in human milk than in human blood. Stastically there was a non significant difference between mean levels of pesticide residues in human blood and human milk (p=0.44)

Table 23: Comparison of HCH and DDT residues (ng ml^{-1}) in human blood sample in present study with earlier studies

World	Survey year	HCH	DDT	Refrences
Brazil	-	32.4	76.9	Minelli and Ribeiro (1996)
Germany	-	6.36	15.4	DeVoto *et al* (1998)
Mexico	-	1.6	16.4	Waliszewski *et a*l (1999)
Sweden	-	51.6	836.1	Glynn *et al* (2000)
Spain	1997-1998	1.06	2.24	Sala *et al* (2001)
Japan	-	0.54	6.3	Hanaoka *et al* (2002)
Belgium	-	-	8.2	Charlier and Plomteux (2002)
Portugal	2001-2002	13	93.5	Cruz *et al* (2003)
Spain	1998-2000	13.77	49.4	Falcon *et al* (2004)
Spain	1997-1998	-	370	Zumbado *et al* (2005)
Portugal	1997-2001	24.9	74.7	Lino and Silveria (2006)
Romania	2005	1114	2420	Dirtu *et al* (2006)
Poland	2004	3.9	401	Jaraczewska *et al* (2006)
United Kingdom	2003	15	100	Thomas *et al* (2006)
Spain	1992-1995	1291.4	4895.8	Porta *et al* (2008)
India				
Dibrugarh	2009-2010	348	417	Mishra *et al* (2011)
Nagaon	2009-2010	627	743	Mishra *et al* (2011)
Delhi	1988-89	1600	7170	Nair and Pillai (1992)
Delhi	-	50	271	Dua *et al* (2001)
Nainital	-	3120	6920	Dua *et al* (2001)
Ahmedabad	-	41.23	32.61	Bhatnagar *et al* (2004)
Rajasthan	-	750	950	Kumar *et al* (2006b)
Delhi	-	23.50	1.71	Pathak *et al* (2009)
Punjab	**2011-12**	**6.99**	**4.77**	**Present study**

The occurrence of residues levels in present study was compared with those of other studies from India and abroad (Table 23). It is observed that the levels of HCH and DDT detected were several times low in present study than previous studies from India. Such a declining trend may be related to the ban imposed on the use of technical grade HCH and DDT in agriculture and public health programmes. In order to differentiate between current and historical exposure to DDT, DDT/DDE ratio has been used frequently (Jaga and Dharmani 2003). A low DDT/DDE ratio indicates high persistence in the environment and ongoing biomagnifications (Jaga and Dharmani 2003), whereas a high DDT/DDE ratio implies continuous exposure to DDT. In our study, the DDT to DDE ratio is quite lower which may be attributed to the fact that although ban on the usage of DDT has been imposed, but the persistent nature of this pesticide has been responsible for its occurrence in the human blood and tissue. The levels of DDT in this study are slightly higher as compared to the last study from Delhi (Pathak *et al* 2009). However the levels of HCH are four times lower in the present study when compared with the same in 2009. The mean residual levels of β-endosulfan are quite higher in our study on comparison to the previous study from Punjab carried out by Mathur *et al* (2005) reporting 4.6 ng ml^{-1} of endosulfan in agricultural population in Punjab villages. The higher values found is possibly a result of occupational exposure of organochlorines among cultivators and sprayers.

When compared to the levels of these residues with the earlier studies from the developed nations like Romania, Brazil, Spain and Sweden a declining trend of HCH and DDT residues in human body is observed in this study which is due to the decline in the use of these two pesticides. However the presence of organophosphate pesticide residues

in this study is due to the increase in the usage of these pesticides from last few years. Profenophos is found in highest mean concentration in both blood and milk samples among all the OP's found.

Thus in the present study the change in the pattern of consumption of pesticides was observed. There has been a shift from the usage of organochlorines towards the organophosphates and synthetic pyrethroids which may be due to restriction in the use of certain organochlorines. Among synthetic pyrethroids, cypermethrin is most commonly used pesticide in India to control various pests, including moth pest of cotton, fruit and vegetable crops (Ullah *et al* 2006).

CHAPTER V

SUMMARY

The environmental persistence and lipophilicity of pesticides leads to the deposition of their residues in fat rich tissues. Under certain conditions such as lactation, mobilization of these residues occurs which lead to their excretion in the mother's milk. Therefore burden of these residues in mother's milk can indicate the risks of exposure to breast-fed infants. Moreover mother's milk with a relatively high content can be a best indicator to study long term exposure to these OCP's as it is easy to obtain and can be collected non-invasively. In the present study, total 127 mother's milk samples were collected to monitor the level of pesticide residues in mother's milk. Residues of β-HCH, γ-HCH, p,p' DDE, p,p' DDT, cyfluthrin, cypermethrin, fenvalerate, chlorpyriphos, phosalone, profenphos and monocrotophos were detected in mother's milk samples. Out of these, cyfluthrin being the dominant pesticide was detected in 11.81% of the samples followed by γ-HCH, p,p' DDT, chlorpyriphos and β- HCH which were detected in 8.66, 4.72, 3.93 and 3.14% of the mother's milk samples respectively. Residues of each p,p' DDE and cypermethrin were detected in 2.36% of the samples. Fenvalerate, phosalone and profenophos residues each were detected in 1.57% of the samples whereas monocrotophos residues were detected only in 0.78% of the samples.

Cyfluthrin was detected with maximum mean concentration level of 63.04 ng g^{-1} in the samples. The mean concentration level of β-HCH was 2.29 ng g^{-1} with a range of ND-133.88 ng g^{-1}. Similarly the mean concentration level of γ-HCH was 2.64 ng g^{-1} with the maximum level of 61.13 ng g^{-1} in the samples. p,p' DDE residues were observed with mean levels of 0.56 ng g^{-1} with a range of ND-43.19 ng g^{-1} in the samples. The residue

levels of p,p' DDT were found with mean concentration level of 3.03 ng g^{-1} and their maximum concentration found out to be 150.3 ng g^{-1} in the milk samples. Thus the occurrence of major persistent pesticide residues were analyzed according to the parity of mothers, their age, living background and dietary habits. It was observed that the occurrence of total HCH, DDT, cyfluthrin and cypermethrin are more in first parity followed by second parity. Thus it is clear that there is a decline in the concentration of residues in relation to the parity of mother. During each period of lactation there is a continuous loss of OCPs from mother's body due to breast feeding as the residues are mobilized to the mother's milk.

On comparison of the pesticide residue levels with different age groups, a characteristic decline in the concentration was observed which may be due to the fact that more the breast feeding mother is older, less is the concentration of pesticide residues due to transfer via breast milk to the infants. In the developing countries, women breast feed their infants for relatively longer periods as compared to those in developed countries. This may be one of the reasons for negative correlation between age and concentration of pesticides residues in milk in our study. When the residue levels of pesticides were compared among rural and urban population it was observed that higher levels were found in urban population than the rural population. Similarly residues of total HCH and chlorpyriphos were higher in non-vegetarian population. On the contrary, residues of total DDT, cypermethrin and cyfluthrin were high in vegetarian population. Estimated daily intake (EDI) is calculated to estimate the magnitude of exposure to pesticides in infants. It was found that daily intake of nine samples of DDT and all the positive samples for HCH exceeded the tolerable daily intake.

The residues of DDT and HCH in present study were compared with previous studies in India and other developed and developing nations. It was observed that the levels of levels of HCH are higher as compared to many other countries but levels of DDT were low as compared to the previous studies. On comparison to the previous study in Punjab it was found that levels of HCH and DDT have declined by 15 and 12 times during the last 10 years.

For the study of pesticide residues in blood samples of human population, samples were collected from Muktsar and Bhatinda districts of Punjab which is the cotton belt of Punjab and the highest user of pesticides in Punjab. In our study, residues of α-HCH, β-HCH, p,p' DDD, p,p' DDE, p,p' DDT, β-endosulfan, monocrotophos, phosalone and profenophos were found in human blood samples. The highest mean residue concentration was found for β-endosulfan and the lowest men level was found for p,p' DDT and phosalone. α-HCH and β-HCH were the only two isomers of HCH found in the samples with mean residue level of 1.11 and 5.89 ng ml^{-1} with a range of ND-109 ng ml^{-1} and ND-102.63 ng ml^{-1} respectively. Of the total pesticide residues found in human blood, the maximum contribution was observed for p,p' DDE which was 36% followed by β-HCH as 20%.These pesticides levels are studied in relation to the age, dietary habits, spraying activity as well as gender of the donor to find out whether these parameters are positively or negatively correlated with residue levels in human blood. It was observed that that HCH and β-endosulfan residues are positively correlated to age whereas DDT residues are negatively correlated to age. Similarly on comparing the mean residue levels between pesticide sprayer and non-sprayer population, it was observed that

monocrotophos and profenophos have higher prevalence in sprayer population as compared to the non-sprayer population.

Although the mean value of HCH is less in sprayer population but the prevalence is more in sprayer population which may be because of their direct exposure to pesticides during their handling and usage. A comparison was made for the pesticide residue levels in blood of humans with tobacco chewing and smoking habit and with no chewing and smoking habit. It was observed that the mean residue levels of pesticides were higher in the people with chewing and smoking habit as compared to those with no habit of tobacco chewing and smoking. Levels of β-endosulfan was around 3 times higher in tobacco chewing population than non-chewing population with mean residue levels of 675.14 and 234.89 ng ml^{-1} in chewing and non-chewing population respectively. Similarly mean levels of profenophos was around two times higher in people with chewing and smoking habit as compared to non-chewing population. Levels of β-HCH, DDT and monocrotophos were also higher in tobacco-chewing population as compared to non-chewing population. This may be correlated to the fact that people with tobacco-chewing habits may have direct oral exposure to the pesticides during their spraying on the crops and in the fields. Statistically there was a non significant difference between residue levels the blood samples of tobacco chewing and non-tobacco chewing population ($p=0.32$). A comparison was made between the mean pesticide residues levels in blood samples of healthy and diseased humans. It was observed that the mean residue levels of DDT and monocrotophos was higher in blood samples from diseased population than the samples from healthy population with the mean values of 32.75 and 26.15 ng ml^{-1} in diseased population for DDT and monocrotophos, respectively. On the other hand the

mean residue levels of DDT and monocrotophos in healthy population were 18.28 and 18.025 ng ml^{-1} respectively. Further it was found that the mean residue levels of β-endosulfan, profenophos and phosalone were rather higher in samples from healthy population as compared to those from diseased population. Statistically there was a non significant difference between mean levels of pesticide residues in human blood from diseased and healthy population ($p=0.34$). On comparing levels of pesticides residues in human milk and blood samples it was found that the levels of HCH, DDT, phosalone and profenophos were higher in human blood as compared to the levels in human. Only monocrotophos was in higher concentration in human milk than in human blood. Statistically there was a non significant difference between mean levels of pesticide residues in human blood and human milk ($p=0.44$). The occurrence of residues levels in present study was compared with those of other studies from India and abroad. It is observed that the levels of HCH and DDT detected were several times low in present study than previous studies from India and some of the developed nations like Romania, Spain and Sweden suggesting a declining trend of HCH and DDT residues in human body.

Overall cyfluthrin and profenophos were the two new pesticides detected in present study while the residues of HCH and DDT were quite lower than the previous study which may suggest the slow phasing out of these pesticides from the environment as well as the human body.

CONCLUSIONS

1. Present study revealed that 25% of the mother's milk samples were positive for pesticide residues with cyfluthrin as the leading pesticide. Other pesticide residues being found were β-HCH, γ-HCH, p,p' DDE, p,p' DDT, cypermethrin, fenvalerate, chlorpyriphos, phosalone, profenphos and monocrotophos.
2. Among human blood samples, 36% of the samples were positive for pesticide residues with β-endosulfan as leading pesticide and the other pesticides found were α-HCH, β-HCH, p,p' DDD, p,p' DDE, p,p' DDT, monocrotophos, profenophos and phosalone.
3. HCH and DDT levels were reduced by 15 and 12 times respectively as compared to previous study from Punjab which may be due to restrictions on their use in agriculture.
4. HCH and DDT residues exceeded Estimated Daily Intake for infants which is a matter of concern regarding the health of infants
5. There was a negative correlation between pesticide residue levels in mother's milk and age and parity of the mother because of excretion of pesticide residues from mother's body via breast milk.
6. Presence of synthetic pyrethroids and organophosphate residues in the samples indicate a shift in consumption pattern of pesticides from organochlorines to these pesticides.

REFRENCES

Abhilash P C and Singh N. 2009. Pesticide use and application: An Indian scenario. *Journal of Hazardous Materials* **165**: 1-12.

Agarwal H C, Pillai M K K, Yadav D V, Menon K B and Gupta R K 1976. Residues of DDT and its metabolites in human blood samples in Delhi, India. *Bulletin of the World Health Organisation* **54**: 349-51

Agnihotri N P. 2000. Pesticide consumption in agriculture in India: An update. *Pesticide research journal* **12**(1): 150-55.

Ahlborg U, Hanberg A and Kenne K. 1992. Risk assessment of polychlorinated biphenyls (PCBs). Report NORD. Copenhagen: Nordic Council of Ministers: 26.

Ahmed M T, Loutfy N and El Shieke E. 2002. Residue levels of DDE and PCBs in blood serum of women in Port Said in Egypt. *Journal of Hazardous Material.* **89**: 41–8.

Albers J M C, Kries I A, Liem A K D and Van Zoonen P. 1996. factors that influence the level of contamination of human milk with polychlorinated organic compounds. *Archives of Environmental Contamination and Toxicology* **30**: 285-91.

Algeria H, Wong F, Jantunen L M, Bidleman TF, Salvador Figueroa M, Gold Bouchot G, Ceja Moreno V, Waliszewski S M and Infanzon R. 2008. Organochlorine Pesticides and PCBs in air of southern Mexico, (2002-2004). *Atmospheric Environment* **42**: 8810-18.

Al-Saleh, Iman, Shinwari N, Basile P, El-Doush I, Al-Zahrani M, Al-Shanshoury M, El-Din Mohammed G. 2003. DDT and Its Metabolites in Breast Milk From Two Regions in Saudi Arabia. *Journal of Occupational & Environmental Medicine* **45(4)**: 410-27.

Alvarez-Pedrerol M, Ribas-Fito N, Torrent M, Carrizo D, Grimalt J O and Sunyer J. 2008. Effects of PCBs, p, p′-DDT, p, p′-DDE, HCB and beta-HCH on thyroid function in preschoolers. *Occupational and Environmental Medicine* **65**(7): 452-57.

Amaral Mendes J J. 2002. The endocrine disruptors: a major medical challenge. *Food and Chemical Toxicology* **40**: 781–88.

Anonymous. 1991. Survey of the Environment, The Hindu.

Aulakh R S, Gill J P S, Bedi J S, Sharma J K, Joia, B S and Ockerman H W. 2006. Organochlorine pesticide residues in poultry feed, chicken muscle and egg at a poultry farm in Punjab, India. *Journal of the Science of Food and Agriculture* **86**: 741-44.

Bates M N, Buckland S J,Garret N,Ellis H, Neddham, L L And Patterson D G. 2004. Persistent organochlorines in the serum of non-occupationally exposed New Zealand population *Chemosphere* **54**: 1431-43

BBC. DDT 'link' to slow child progress. http://news.bbc.co.uk/2/hi/health/5145450.stm 2006.

Beard J, Marshall S, Jong K, Newton R, Triplett-McBride T and Humphries B. 2000. 1,1,1 Trichloro-2,2-bis(p-chlorophenyl)-ethane (DDT) and reduced bone mineral density. *Archives of Environmental Health* **55**: 177–80.

Bedi J S, Gill J P S, Aulakh R S, Joia B S and Sharma J K. 2005. Contamination levels of DDT and HCH residues in different caprine tissues in Punjab, India. *Indian Journal of Animal Sciences.* **75**: 11-13.

Behrooz R D, Sari A E, Bahramifar N and Ghasempouri S M. 2009. Organochlorine pesticide and polychlorinated biphenyl residues in human milk from the Southern Coast of Caspian Sea, Iran. *Chemosphere* **74(7)**: 931-37

Bernier J, Brousseau P, Krystyniak K, Tryphonas H and Fournier M. 1995. Immunotoxicity of heavy metals in relation to Great Lakes. *Environmental Health Perpsectives* **103**(Suppl. 9): 23-34.

Bhanti M and Taneja A. 2007. Contamination of vegetables of difference seasons with organophosphorous pesticides and related health risk assessment in Northern India. *Chemosphere* **69**(1): 63-8.

Bhatnagar V K, Kashyap R, Zaidi S S A, Kulkarni P K and Saiyed H N. 2004. Levels of DDT, HCH and HCB residues in human blood in Ahmedabad, India. *Bulletin of Environmental Contamination and Toxicology* **72**: 261-65.

Battu R S, Singh B and Kang B K. 2004. Contamination of liquid milk and butter with pesticide residues in the Ludhiana district of Punjab state, India. *Ecotoxicology and Environmental Safety* **59(3)**: 324-31.

Birnbaum L S. 1994. Endocrine effects of prenatal exposure to PCBs, dioxins, and other xenobiotics: implications for policy and future research. *Environmental Health Perpsectives***102**: 676–79.

Bouwman H, Sereda B and Meinhardt H M. 2006. Simultaneous presence of DDT and pyrethroid residues in human breast milk from malaria endemic area in South Africa. *Environmental pollution* **144**: 902-17.

Burke E R, Holden A J and Shaw I C. 2003. A method to determine residue levels of persistent organochlorine pesticides in human milk from Indonesian women. *Chemosphere* **50**: 529-35.

Charlier C J and Plomteux G J. 2002. Determination of organochlorine pesticides reidues in blood of healthy individuals. *Clinical Chemistry and Laboratory Medicine* **40(4)**: 361-64.

Chitra G A, Muraleedharan V R, Swaminathan T, and Veeraraghavan D. 2006. Use of pesticides and its impact on health of farmers in South India. *International Journal of Occupational and Environment Health*. **12**: 228-33

Cohen M. 2007. Environmental toxins and health. The health impact of pesticides. *Australian family physician* 36(12): 102-104.

Colborn T, Dumanoski D, Myers J P. 1996. Our stolen future. A Dutton Book, published by Penguin Books, USA

Colborn T, Vom Saal F S and Soto A M. 1993. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environmental Health Perpsectives* **101(5)**: 378–84.

Corcellas C, Feo L M , Torres J P , Malm O, Ocampo-Duque W , Eljarrat E , Barcelo D. 2012. Pyrethroids in human breast milk: Occurrence and nursing daily intake estimation. *Environment International* **47**: 17–22.

Cruz S, Lino C And Siliveira M I. 2003.Evaluation of organochlorine pesticide residues in human serum from an urban and two rural populations in Portugal. *Science of the Total Environment* **317**: 23-35.

Czaja K, Ludwicki JK, Goralczyk and Strucinski P. 2001. Relationship between two consecutive lactations and fat level in persistent organochlorine compound concentrations human breast milk. *Chemosphere* **43**:889–93

Damalas Chrisros A and Eleftherohorinos Ilias G. 2011. Pesticide Exposure, Safety Issues, and Risk Assessment Indicators. *International Journal of Environmental Research and Public Health* **8**: 1402-19

Devanathan G, Subramanian A, Someya M, Sudaryanto A, Isobe T, Takahashi S, Paromita P, Tanabe S. 2009. Persistent organochlorines in human breast milk from major metropolitancities in India. *Environmental Pollution* **157**: 148–154

DeVoto E, Kohlmeier L and Heeschen W. 1988. Some dietary predictors of plasma organochlorine concentrations in an elderly German population. *Archives of Environmental Health* **53(2)**: 147-55.

Dirtu A C, Cernat R, Dragan D,Mocanu R, Grieken R V, Neels H and Covaci A.2006. Organohalogenated pollutants in human serum from Iassy Romania and their relation with age and gender. *Environment International* **32**: 797-803.

Dorner G and Plagemann A. 2000. DDT in human milk and mental capacities in children at school age: an additional view on PISA. *Neuroendocrinology letters* **23**: 427-31.

Dua V K, Kumari R, Sharma V P and Subbarao S K. 2001. Organochlorine residues in human blood from Nainital (U.P.), India. *Bulletin of Environmental Contamination and Toxicol*ogy **67**: 42-45

Dua V K, Pant C S, Sharma V P and Pathak G K. 1996. Determination of HCH and DDT in finger- pricked whole blood dried on filter paper and its field application for monitoring concentrations in blood. *Bulletin of Environmental Contamination and Toxicol*ogy **56**: 50-57.

Dureja P and Gupta R L. 2009. Status of Pesticides in India. *Pesticide Research Journal*. **21 (2)**: 202- 10.

Ennaceur S, Gandoura N and Driss MR. 2008b. Distribution of polychlorinated biphenyls and organochlorine pesticides in human breast milk from various locations in Tunisia: levels of contamination, influencing factors, and infant risk assessment. *Environmental Research* **108**: 86-3.

Ennaceur S, Ridha D and Marcos R. 2008a. Genotoxicity of the organochlorine pesticides 1,1-dichloro-2,2-3 bis(p-chlorophenyl) ethylene (DDE) and hexachlorobenzene (HCB) in cultured human lymphocytes. *Chemosphere* **71**(7): 1335-39.

Eskenazi B, Harley K, Bradman A, Weltzien E, Jewell N P and Barr D B. 2004. Association of in utero organophosphate pesticide exposure and fetal growth and length of gestation in an agricultural population. *Environmental Health Perpsectives* **112(10)**:1116-24.

Falcon M, Olive J, Osuna E, Barba A and Luna A. 2004. HCH and DDT residues in human placentas in Murcia (Spain). *Toxicology* **195**: 203-08.

Fangstrom B, Strid A, Grandjean P, Weihe P, Bergman A. 2005. A retrospective study of PBDEs and PCBs in human milk from the Faroe Islands. *Environmental Health* **14**: 4-12.

FAO/WHO. 2008. Food standard programme, Codex alimentarius commission, vol. 11, pesticide residues in food.

Gill J P S. 2000. Studies on pesticide residues in milk and milk products and its public health significance. Ph.D Dissertation. Punjab Agricultural University, Ludhiana.

Giri S, Prasad S B, Giri A and Sharma G D. 2002. Genotoxic effects of malathion: an organophosphorus insecticide, using three mammalian bioassays in vivo. *Mutation Research* **514**: 223-31.

Gladen B C, Ragan N B and Rogan W J. 2000. Pubertal growth and development and prenatal and lactational exposure to polychlorinated biphenyls and dichlorophenyl dichloroethene. *Journal of Paediatrics* **136**: 490-96.

Gladen B C, Shkiryak-Nyzhnyk Z A, Chyslovska N, Zadorozhnaja T D and Little R E. 2003. Persistent organochlorine compounds and birth weight. *Annals of Epidemiology***13**: 151–57.

Glynn A W, Wolk A, Aune M, Atuma S, Zettermark S, Maechle-Schmid M, Darnerud P O, Becker W, Vessby B And Adami H. 2000. Serum concentrations of organochlorine in men: a search for markers of exposure. *Science of the Total Environment* **263**: 197-208.

Gupta P K. 2004. Pesticide exposure-Indian scene. *Toxicology*. **198**: 83–90.

Gupta M. 2010. Pesticide poisoning in India. *Asian labour update* (74) www.amrc.org.hk/node/1007 (accessed on 11 January, 2011).

Halder A. 2009. Premature greying of hairs, premature ageing and predisposition to cancer in Jajjal, Punjab: A preliminary observation. *Journal of Clinical and Diagnostic Research* **1**(6): 577-80.

Hanaoka T, Takahashi Y, Kobayashi M, Sasaki S, Usuda M And Okubo S. 2002. Residuals of beta- hexachorocyclohexane and relations with consumptions of dietary components in rural residents in Japan. *Science of the Total Environment* **286**: 119-27

Harris C A, Hagan S O and Merson G H J. 1999. Organochlorine pesticide residues in human milk in the United Kingdom. *Humans and Experimental Toxicology***18**: 602-06

Hayat K, Ashfaq M, Ashfaq U and Saleem M A. 2010. Determination of pesticide residues in blood samples of villagers involved in pesticide application at District Vehari (Punjab), Pakistan. *African Journal of Environmental Science and Technology*. **4(10)**: 666-84.

Herrero-Mercado M, Waliszewski S M, Caba M, Martinez-Valenzuela C, Gomez Arroyo S, Villalobos Pietrini R, Cantu Martinez P C , Hernandez-Chalate F. 2011. Organochlorine Pesticide Gradient Levels Among Maternal Adipose Tissue, Maternal Blood Serum and Umbilical Blood Serum. *Bulletin of Environmental Contamination and Toxicology* **86**: 289–93

Herrero-Mercedo M, Waliszewski S M, Caba M, Martinez-Valenzuela C and Hernandez-Chalate F. 2010. Organochlorine pesticide levels in umbilical cord blood of newborn in Veracruz, Mexico. *Bulletin of Environmental Contamination and Toxicology*. **85**: 367-71

Hodgson A. 2003. The high cost of pesticide poisoning in northern Ghana. *Pesticide News* **62**(3): 4- 8.

Hosie S, Loff S, Witt K, Niessen K and Waag K L. 2000. Is there a correlation between organochlorine compounds and undescended testes? *European Journal of Paediatric Surgery***10**: 304–09.

International Programme on Chemical Safety (IPCS). 2001. Neurotoxicity risk assessment for human health: principles and approaches. Environmental health criteria.Geneva: World Health Organization (WHO); ISBN 94 4157223 X. 223

Iyer R. 1993. The chemical industry in India: occupational hazards and pollution. Centre for Development and the Environment, University of Oslo, Oslo.

Jaga K and Dharmani C. 2003. Sources of exposure to and public health implications of organophosphate pesticides. *Pan American Journal of Public Health* **14:** 171-85.

Janssen P A H, Dalessi D L W M, Lambert J G D, Vethaak A D, Van Wezel A P and Goos H J T H. 1997. Oestrogenic effects in the flounder, Platichthys flesus, after exposure to polluted harbour sediment. (Abstract)Seventh Annual Meeting of SETAC-Europe, Amsterdam, The Netherlands.

Jaraczewska A, Lulek J, Covaci A, Voorspoels S, Kaluba-Skotarczak A, Drew K And Shepens P. 2006. Distribution of polychlorinated biphenyls, organochlorine pesticides and polybrominted diphenyl ethers in human umbilical cord serum, maternal serum and milk from Wielkopolska region, Poland. *Science of the Total Environment* **372**: 20-31.

Jayashree R and Vasudevan N. 2007. Effect of tween 80 added to the soil on the degradation of endosulfan by *Pseudomonas aeruginosa*. *International Journal of Environmental Science and Technology* **4(2)**: 203-10.

John P J, Bakore, N and Bhatnagar P. 2001. Assessment of organochlorine pesticide residues levels in dairy milk and buffalo milk from Jaipur city, Rajasthan, India. *Environment International.* **20**: 231-36

Kalra R L, Singh B and Battu R S. 1994. Organochlorine pesticide residues in human milk in Punjab, India. *Environmental Pollution* **85**: 147–51.

Kanja L W, Skaare J U, Ojwang S P O and Maitai C K. 1992. A comparison of organochlorine pesticide residues in maternal adipose tissue, maternal blood, cord blood and human milk from mother/ infant pairs. *Archives of Environment Contamination and Toxicol*ogy **22**: 21-24.

Kannan K, Tanabe S and Tatsukawa R. 1995. Geographical distribution and accumulation features of organochlorine residues in fish in tropical Asia and Oceania. *Environmental Sciences and Technology* **29**: 2673–83.

Kannan K, Tanabe S, Giesy J P and Tatsukawa R. 1997. Organochlorine pesticides and polychlorinated biphenyls in foodstuffs from Asian and oceanic countries. *Reviews of Environmental Contamination and Toxicology* **152**: 1–55.

Karmaus W, Davis S, Chen Q, Kuehr J and Kruse H. 2003. Atopic manifestations, breast-feeding protection and the adverse effect of DDE. *Paediatric and Perinatal Epidemiology* **17**: 212-20.

Kaur R and Sinha A K. 2011. Globalization and health: a case study of Punjab human geographies. *Journal of Studies and Research in Human Geography* **5**(1): 35-42.

Kelce W R, Lambright C R, Gray L E and Roberts K P. 1997. Vinclozolin and p, p-DDE alter androgen dependent gene expression: in vivo confirmation of an androgen receptor mediated mechanism. *Toxicology and Applied Pharmacology*.**142**: 192–200.

Kelce W R, Monosson E, Gamcsik M P, Laws S C and Gray L.E. 1994. Environmental hormone disrupters: evidence that vinclozolin developmental toxicity is mediated by antiandrogenic metabolites. *Toxicology and Applied Pharmacology*. **126(2)**: 276–85.

Kumar A, Baroth A, Soni I and Bhatnagar R and John P J. 2006b. Organochlorine pesticide residues in milk and blood of women from anupgarh, Rajasthan, India. *Environmental monitoring and assessment*. **116**: 1–7

Kumar A, Dayal P, Shukla G, Singh G and Joseph P E. 2006a. DDT and HCH residue load in mother's breast milk: A survey of lactating mother's from remote villages in Agra region. *Environment International*. **32**: 248-51

Kunisue T, Muraoka M, Ohtake M, Sudaryanto A, Minh N H and Ueno D. 2006. Contamination status of persistent organochlorines in human breast milk from Japan: recent levels and temporal trend. *Chemosphere* **64**: 1601-08

Kunisue T, Someya M, Kayama F, Jin Y and Tanabe S. 2004. Persistent organochlorines in human breast milk collected from primiparae in Dalian and Shenyang, China. *Environmental pollution* **131**: 381-92

Leng J H, Karyama F, Wang P Y, Nakamura M, Nakata T And Wang Y. 2009. Levels of persistent organic pollutants in human milk in two Chinese coastal cities, Tianjin and Yantai Influence of fish consumption. *Chemosphere* **75(5)**: 634-39

Li Y F, Scholtz M T and Van Heyst B J. 2003. Global gridded emission inventories of b-hexachlorocyclohexane. *Environmental Sciences and Technology* **37**:3493-98.

Liem A K D and Theelen R M C. 1997. Dioxins: chemical analysis, exposure and risk assessment. Thesis Utrecht Universiteit, Netherlands. ISBN 90-393-2012-8.

Lino C M and Silveira M I N. 2006. Evaluation of organochlorine pesticides in serum from students in Coimbra, Portugal: 1997-2001. *Environmental research* **102**: 339-51.

Longnecker M P, Klebanoff M A, Zhou H and Brock J W. 2001. Association between maternal serum concentration of the DDT metabolite DDE and preterm and small-for-gestationalage-babies at birth. *Lancet* **358**: 110-14.

Luzardo O P, Goethals M, Zumbado M, Alvarez-Leon E E, Cabera F, Serra- Majem L I And Boada L D. 2006. Increasing serum levels of non-DDT-derivative organochlorine pesticides in the younger population of Canary Islands (Spain). *Science of the Total Environment* **367**: 129-38.

Mathur H B, Agarwal H C, Johnson S and Saikia N. 2005. Analysis of pesticide residues in blood samples from villages of Punjab. *Centre for Science and Environment, New Delhi, India. Report* 1-21.

Minelli E V and Ribeiro M L. 1996. DDT and HCH residues in the blood serum of malaria control sprayers. *Bulletin of Environmental Contamination and Toxicology* **57**: 691-96.

Minh N H, Minh T B, Watanabe M, Kunisue T, Monirith I, Tanabe S, Sakai S, Subramanian A, Sasikumar K, Viet P H, Tuyen B C, Tana T S and Prudente M S. 2003. Open dumping sites in Asian countries: a potential source of polychlorinated dibenzo-pdioxins and polychlorinated dibenzofurans. *Environmental Sciences and Technology* **37**: 1493–502.

Minh N H, Someya M, Minh T B, Kunisue T, Iwata H, Watanabe M, Tanabe S, Viet PH and Tuyen BC. 2004. Persistent organochlorine residues in human breast milk from Hanoi and Hochiminh City, Vietnam: contamination, accumulation kinetics and risk assessment for infants. *Environmental Pollution* **129**(3):431-41.

Mishra K and Sharma R C. 2011. Assessment of organochlorine pesticides in human milk and risk exposure to infants from North-East India. *Science of the Total Environment*. **409**: 4939–949

Mishra K, Sharma R C and Kumar S. 2011. Organochlorine pollutants in human blood and their relation with age, gender and habitat from North-east India. *Chemosphere* **85**: 454-64

Mueller J F, Harden F, Toms L M, Symons R, Furst P . 2008. Persistent organochlorine pesticides in human milk samples from Australia. *Chemosphere* **70**: 712–720.

Mukherjee I, Gopal M and Mathur D S. 2007. Behaviour of b-cyfluthrin after foliar application on chickpea (*cicer aretinium* L) and pigeon pea (cajanus cajan L). *Bulletin of Environmental Contamination and Toxicology* **78**: 76-80.

Nagayama J, Kohno H and Kunisue T. 2007. Concentrations of organochlorine pollutants in mothers who gave birth to neonates with congenital hypothyroidism. *Chemosphere* **68**(5): 972-76.

Nair A and Pillai M K K. 1992. Trends in ambient levels of DDT and HCH residues in humans and environment of Delhi, India. *Science of the Total Environment* **121**: 145-57.

Nair A, Mandapati R, Dureja P and Pillai M K K. 1996. DDT and HCH load in mothers and their infants in Delhi, *India. Bulletin of Environmental Contamination and Toxicol*ogy **56**: 58-64.

Newsome W H and Ryan J J. 1999. Toxaphene and other chlorinated compounds in human milk from northern and southern Canada: a comparison. *Chemosphere* **39**: 519-26

Ntow W J, Gijzen H J and Drechsel P. 2006. Farmer perceptions and pesticide use practices in vegetable production in Ghana. *Pesticide Manage Sciences* **62**(4): 356-65.

Ntow W J, Tagoe L M, Drechsel P, Kelderman P, Gijzen H J and Nyarko E. 2008. Accumulation of persistent organochlorine contaminants in milk and serum of farmers from Ghana. *Environmental Research* **106**(1):17–26.

Ooostdam J V, Gilman A, Dewailly E and Kuhnelein H. 1999. Human health implications of environmental contaminants in Arctic Canada: a review. *Science of the Total Environment* 230: 1-82.

Pathak R, Ahmed R S, Tripathi A K, Guleria K, Sharma C S, Makhijani S D and Banerjee B D. 2009. Maternal and cord blood levels of organochlorine pesticides: association with preterm labor. *Clinical Biochemistry* **42**: 746-49.

Pathak R, Suke S G, Ahmed R S, Tripathi A K, Guleria K, Sharma C S, Makhijani S D, Mishra M and Banerjee B D. 2008. Endosulfan and Other Organochlorine Pesticide Residues in Maternal and Cord Blood in North Indian Population. *Bulletin of Environmental Contamination and Toxicology* **81**: 216–19

Paumgartten F J R, Cruz C M, Chahoud I, Palavinskas R and Mathar W. 2000. PCDDs, PCDFs, PCB and other organochlorine compounds in human milk from Rio de Janeiro, Brazil. *Environmental Research* **83**: 293-97

Polder A, Skaare J U, Skjerve E, Loken KB and Eggesbo M. 2009. Levels of chlorinated pesticides and polychlorinated biphenyls in Norwegian breast milk (2002–2006),

and factors that may predict the level of contamination. *Science of the Total Environment* **407**: 4584–90

Porta M, De Basea M B, Benavides F G, Lopez T, Fernandez E, Marco E, Alguacil J, Grimalt J O and Puigdomenech E. 2008. Differences in serum concentrations of organochlorine compounds by occupational social class in pancreatic cancer. *Environmental Research* **108**: 370-79.

Punjab State Council for Science and Technology. 2007. *State of Environment Punjab*, Chandigarh.

Puri S N, Murthy K S and Sharma O P. 1999. 'IPM for sustainable crop production, Management' in Sundaram V et al, Handbook of cotton in India, ISCI, Bombay. International Programme on Chemical Safety (IPCS).

Quinsey P M, Donohue D C and Ahokas J T. 1995. Persistence of organochlorines in breast milk of woman in Victoria, Australia. *Food and Chemical Toxicology* **33**(1): 49-56

Rekha S N and Naik R P. 2006. Pesticide residue in organic and conventional food- risk analysis. *Chemical Health Safety* **13**: 12-19.

Ribas Fito N, Torrent M and Carrizo D. 2006. In utero exposure to background concentrations of DDT and cognitive functioning among preschoolers. *American Journal of Epidemiology* **164**(10): 955-62.

Rier S E, Martin D C, Bowman R E and Becker J L. 1995. Immunoresponsiveness in endometriosis: implications of estrogenic toxicants. *Environmental Health Perpsectives* **103**(Suppl. 7): 151–56.

Sagiv S K, Nugent J K, Brazelton T B, Choi A L, Tolbert P E, Altshul L M and Korrick S A. 2008. Prenatal organochlorine exposure and measures of behavior in infancy using the Neonatal Behavioral Assessment Scale (NBAS). *Environmental Health Perpsectives* **116**(5): 666-73.

Sala M, Ribas-Fito N, Cardo E, De Muga M E, Marco E And Mazon C. 2001. Levels of hexachlorobenzene and other organochlorine compounds in core blood: exposure across placenta. *Chemosphere* **43**: 895-901.

Sanghi R, Pillai M K, Jayalekshmi T R and Nair A. 2003. Organochlorine and organophosphorus pesticide residues in breast milk from Bhopal, Madhya Pradesh, India. *Human and Experimental Toxicology* **22**: 73–76.

Saxena S P, Khare C, Farooq A, Murugeasan K and Chandra J. 1987. DDT residues in blood of residents of area surrounding a DDT manufacturing factory in Delhi. *Bulletin of Environmental Contamination and Toxicol*ogy **38**: 392-95.

Schell L M, Gallo M V, Denham M, Ravenscroft J, Decaprio A P and Carpenter D O. 2008. Relationship of thyroid hormone levels to levels of polychlorinated biphenyls, lead, p, p′-DDE, and other toxicants in Akwesasne Mohawk Youth. *Environmental Health Perpsectives* **116(6)**: 806-13.

Skaare J U, Tuveng J M, Sande H A. 1988. Organochlorine pesticides and polychlorinated biphenyls (PCBs) in maternal tissue, blood, milk and cord blood from mothers and their infants living in Norway. *Archives of Environment Contamination and Toxicology* **17**: 55–63.

Subramanian A, Ohtake M, Kunisue T and Tanabe S. 2007. High levels of organochlorines in mothers' milk from Chennai (Madras) city, India . *Chemosphere* **68**: 928–39

Subramanian K and Solomon R D J. 2006. Organochlorine pesticides BHC and DDE in human blood in and around Madurai. *India Journal of Clinical Biochemistry* **21 (2)**: 169-72

Sudaryanto A, Kunisuet, Tanabe S, Niida M and Hashim H. 2005. Persistent organochlorine compound in humans breast from mothers living in Penang and Kedah, Malaysia. *Archives of Environment Contamination and Toxicology* **49**: 429-37.

Surendernath B, Unnikrishan V and Usha M A. 2001. Organochlorine pesticide residue contents of human milk and dairy milk. *The Indian Journal of Nutrition and Dietetics* **37**: 188-94

Tanabe S, Gondaira F, Subramanian A, Ramesh A, Mohan D, Kumaran P, Venugoplan V K and Tatsukawa R. 1990. Specific pattern of persistent organochlorine residues in human breast milk from south India. *Journal of Agricultural and Food Chemistry.* **38**: 899–03.

Thakur J S, Rao B T, Rajwanshi A, Parwana H K and Kumar R. 2008. Epidemiological study of high cancer among rural agricultural community of Punjab in Northern India', *International Journal of Environmental Research and Public Health* **5**: 399-07.

Thomas G O, Wilkinson M, Hodson S and Jones K C. 2006. Organohalogen chemicals in human blood fom the United Kingdom. *Environmental Pollution* **141**: 30-41.

Tiwana N S, Jerath N, Singh G and Singh R. 2009. Pesticide pollution in Punjab: A review. *Asian Journal of Water and Environmental Pollution* **6**(1): 89-96.

Tsang H L , Wu S , Leung C K M , Tao S , Wong M H. 2011. Body burden of POPs of Hong Kong residents, based on human milk, maternal and cord serum. *Environment International* **37**: 142–51.

Tsydenova V O, Sudaryanto A, Kajiwara N, Kunisue T, Batoev V B and Tanabe S. 2007. Organohalogen compound in human breast milk from Republic of Buryatia, Russia. *Environmental Pollution* **146**: 225-32.

Tutu A O, Yeboah P O, Golow A A, Denutsui D and Blankson S. 2011. Organochlorine Pesticides Residues in the Breast Milk of Some Primiparae Mothers in La Community, Accra, Ghana. *Research Journal of Environmental and Earth Sciences* **3(2)**: 153-59.

Ullah S, Ahmad M, Ahmad N, Khan M Z and Ahmad I. 2006. Toxic effect of cypermethrin in female rabbits. *Pakistan Veterinary Journal* **26**(4): 193-96.

Voldner E C and Li Y F 1995. Global usage of persistent organochlorines. *Science of the Total Environ*ment **160/161:** 201–10.

Waliszewski S M, Aguirre A A, Infanzon R M, Silva C S and Siliceo J. 2001. Orangochlorine pesticide levels in maternal adipose tissue, maternal blood serum, umblical blood serum and milk from inhabitants of Veracruz, Mexico. *Archives of Environment Contamination and Toxicology* **40**: 432-38.

Waliszewski S M, Aguirre A A, Infazon R M, Benitz J and Rivera J . 1999. Comparison of levels of organochlorine pesticides in adipose tissue and human milk of mothers living in Veracruz,mexico. *Bulletin of Environmental Contamination and Toxicology* **62**: 685-90.

Waliszewski S M, Melo S G, Villlalobos-Pietrni R, Gomez-Arroyo S, Amador-Munoz O, Herrero-Mercado M and Carvajo O. 2009. Breast Milk Excretion Kinetic of β-HCH, pp'DDE and pp'DDT. *Bulletin of Environmental Contamination and Toxicology* **83**: 869-73.

Waliszewski S M, Valencia Quintana R, Corona C A, Herrero M, Sanchez K, Aguirre H, Aldave I A, Gomez Arroyo S and Villalobos Pietrini R. 2010. Comparison of organochlorine pesticide levels in human adipose tissue of inhabitants from Veracruz and Puebla, Mexico. *Archives of Environment Contamination and Toxicology* **58**: 230-36.

Weltman R H and Norback D H. 1983. Lack of hepatocarcinogenic activity after 2,3,6,2-,3-,6- hexachlorobiphenyl (HCB) exposure in Sprague- Dawley rats: a sequential ultra-structural study. *Toxicologist* **3**: 101.

Whyatt R M, Barr D B, Camann D E, Kinney P L, Barr J R and Andrews H F. 2003. Contemporaryuse pesticides in personal air samples during pregnancy and blood samples at delivery among urban minority mothers and newborns. *Environmental Health Perpsectives* **111**(5):749-56.

Whyatt R M, Rauh V, Barr D B, Camann D E, Andrews H F and Garfinkel R. 2004. Prenatal insecticide exposures and birth weight and length among an urban minority cohort. *Environmental Health Perpsectives* **112**(10):1125-32.

Wolff M S, Toniolo P G, Lee E W, Rivera M and Dubin N. 1993. Blood levels of organochlorine residues and risk of breast cancer. *Journal of National Cancer Institute*. **85 (8)**: 648–52.

Wolff M S, Zeleniuch-Jacquotte A, Dubin N and Toniolo P. 2000. Risk of breast cancer and organochlorine exposure. *Cancer Epidemiology, Biomarkers and Prevention* **9**: 271–77.

Wong C K C, Leung K M, Poon B H T, Lan C Y and Wong M H. 2002. Organochlorine hydrocarbons in human breast milk collected in Hong Kong and Guangzhou. *Archives of Environment Contamination and Toxicology* **43**: 364-72.

Wong F, Algeria H, Jantunen L M, Bidleman T F, Salvador Figuerosa M, Gold Bouchat G, Ceja Moreno V, Waliszewski S M and Infanzon R. 2008. Organochlorine pesticides in soil and air of southern Mexico: chemical profiles and potential for soil emissions. *Atmospheric Environment* **42**: 7737- 45.

Zhou P, Wu Y, Yin S, Li J, Zhao Y, Zhang L, Chen H, Liu Y, Yang X and Li X. 2011. National survey of the levels of persistent organochlorine pesticides in the breast milk of mothers in China. *Environmental Pollution* **159(2)**: 524-31.

Zietz B P, Hoopmann M, Funcke M, Huppmann R, Suchenwirth R, Gierden E. 2008. Long-term biomonitoring of polychlorinated biphenyls and organochlorine pesticides in human milk from mothers living in northern Germany. *International Journal of Hygiene and Environmental Health* **211**: 624–38.

Zumbado M, Goethals M, Alvarez-Leon E E, Luzardo O P, Cabrera F and Serra-Majem L I. 2005. Inadvertent exposure to organochlorine pesticides DDT and derivatives in people from the Canary Islands (Spain). *Science of the Total Environment* **339**: 49-62.

VITA

Name of the student	:	Anupama Sharma
Father's Name	:	Sh.Om Parkash Raina
Mother's Name	:	Smt. Kavita Kumari
Nationality	:	Indian
Date of Birth	:	16.07.1988
Permanent Home Address	:	Village- Bagnoti, Tehsil Nowshera District Rajouri, J&K 185152

EDUCATIONAL QUALIFICATION:

Bachelor's Degree	:	B.V.Sc. and A.H.
University	:	Sher- e- Kashmir University of Agricultural Sciences and Technology, Jammu (J&K).
Year of award	:	2010
OCPA	:	7.38/10
Master's Degree	:	M.V.Sc
OCPA	:	8.47/10
Awards/Distinction/ Fellowships/Scholarships	:	University Merit Fellowship, during M.V.Sc