MONITORING OF ORGANOCHLORINE PESTICIDE RESIDUES IN LAMB, BUFFALO MEAT AND HUMAN POPULATION

Thesis

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in

VETERINARY PUBLIC HEALTH
(Minor Subject: Veterinary Pharmacology and Toxicology)

By

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CERTIFICATE- I

This is to certify that the thesis entitled, "**MONITORING OF ORGANOCHLORINE PESTICIDE RESIDUES IN LAMB, BUFFALO MEAT AND HUMAN POPULATION"** submitted for the degree of M. V. Sc. in the subject of **Veterinary Public Health** (Minor Subject: **Veterinary Pharmacology and Toxicology**) of Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, is a bonafide research work carried out by **Sital Kaji Shrestha** (L-2007-V-37-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.

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CERTIFICATE- II

This is to certify that the thesis entitled, "**MONITORING OF ORGANOCHLORINE PESTICIDE RESIDUES IN LAMB, BUFFALO MEAT AND HUMAN POPULATION"** submitted by **Sital Kaji Shrestha** (L-2007-V-37-M) to 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 after an oral examination on the same, in collaboration with an external examiner.

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ABSTRACT

The present study was undertaken to ascertain the levels of organochlorine pesticide residues in lamb, buffalo meat and human population. Lamb meat and products were collected from local butcher shops at Ludhiana, while buffalo meat was collected from slaughter house located at village Derabassi of Punjab. Contamination level for lamb, buffalo meat and their products were 88.6, 90.9 and 100 per cent, respectively. Mean total DDT, HCH, endosulphan, dieldrin in lamb were 0.696, 0.151, 0.607 and 0.053 mg kg^{-1}, respectively while respective values for DDT, HCH, endosulphan, dieldrin in buffalo meat were 0.308, 0.095, 0.043 and 0.013 mg kg^{-1}. In addition heptachlor was detected in lamb meat with mean level of 0.060 mg kg^{-1}. Seven different kinds of buffalo and mutton products were monitored for pesticide residues. DDT and endosulphan residues were found in all kinds of products where as HCH were only encountered in samples of sheep meat balls. Highest level of DDT was found in mutton kababs (0.186 mg kg^{-1}) followed by buffalo patties (0.179 mg kg^{-1}). Similarly, highest amount of endosulphan was found in sheep patties (1.022 mg kg^{-1}). None of them exceeded national MRLs except, two buffalo meat samples with higher levels of β- HCH than established by FAO/WHO, one buffalo meat exceeded MRLs for lindane and six sheep meat samples exceeded MRLs for endosulphan sulphate set by Codex Alimentarius Commission.

Analysis of 50 human serum samples each from Ludhiana and Nepal revealed that pesticide residues occurred with a frequency of 96 and 94 per cent, respectively. The pesticide load in human population of Ludhiana had DDT (68%), endosulphan (56%) and chlorpyrifos (16%) with mean levels as 0.019, 0.035 and 0.026 mg l^{-1}, respectively. While the Nepal population had DDT (46 %), endosulphan (38%) and chlorpyrifos (12 %) with mean levels as 0.079, 0.098 and 0.245 mg l^{-1}, respectively. The levels were higher in Nepali population probably due to the fact that they were residing near a dumping warehouse of obsolete pesticides for the last more than 30 years.

Key words: Organochlorine pesticides, residues, buffalo meat, lamb, human serum, Ludhiana, Nepal

Signature of Major Advisor   Signature of the Student

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ABBREVIATIONS USED

\$	Dollar
@	At the rate of
$\mu g\ g^{-1}$	Microgram per gram
$\mu g\ kg^{-1}$	Microgram per kilogram
μl	Microliter
BDL	Below Detectable Level
bn	Billion
Bt	*Bacillus thuringiensis*
CAC	Codex Alimentarius Commission
CDC	Center for Disease Control and Prevention
DCM	Dichloromethane
DDD	Dichloro Diphenyl Dichloroethane
DDE	Dichloro Diphenyl Dichloroethylene
DDT	Dichloro Diphenyl Trichloroethane
ECD	Electron Capture Detector
etc	et cetera
FAO	Food and Agriculture Organization
Fig.	Figure
g	Gram
GADVASU	Guru Angad Dev Veterinary and Animal Sciences University
GLC	Gas Liquid Chromatography
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
HPLC	High Performance Liquid Chromatography
i.d.	Internal diameter
ICAR	Indian Council of Agricultural Research
IPM	Integrated Pest Management
IRPTC	International Registry of Potentially Toxic Chemicals
Kg	Kilogram
$Kg\ ha^{-1}$	Kilogram per hectare
m	meter
$mg\ kg^{-1}$	Milligram per kilogram
$mg\ l^{-1}$	Milligram per liter
ml	Millilitre (s)
mm	Millimeter
MRL	Maximum Residue Limit
MT	Metric Tones
ND/nd	Not Detected
ng	Nanogram
Ni	Nickel
NPD	Nitrogen Phosphorus Detector
OC	Organochlorines
OP	Organophosphates

op’	Ortho Para
PAU	Punjab Agricultural University
PC	Personal Computer
PCB	Polychlorinated Biphenyls
PCT	Polychlorinated terphenyls
POP	Persistent Organic Pollutant
pp’	Para Para
ppb	Parts per billion
rpm	Revolutions per minute
SPE	Solid Phase Extraction
SPS	Sanitary and Phytosanitary
UN	United Nations
US	United States
v/v	Volume by volume
viz	Videlicet
WHO	World Health Organization
WHP	Washed high purity
WTO	World Trade Organization
WWF	World Wildlife Fund

Chapter- I

INTRODUCTION

Food and Agriculture Organization (FAO) of The United Nations (UN) has defined pesticides as "any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm or otherwise interfering with the production, processing, storage, transport, or marketing of food, agricultural commodities, wood, wood products or animal feedstuffs, or which may be administered to animals for the control of insects, mites/spider mites or other pests in or on their bodies." The use of pesticides started mainly after Second World War. During their initial application no one realized the harmful effects of the pesticides to human habitat, eco system and in general to the health of people. In 1960's a famous book *Silent Spring* written by Rachel Carson described adverse effects of pesticide residues in human, marines, wildlife and ecosystem which raised a global concern. In response to the publication of *Silent Spring* and the uproar that ensued, U.S. President John F. Kennedy directed his Science Advisory Committee to investigate Carson's claims. Their investigation vindicated Carson's work and led to an immediate strengthening of the regulation of chemical pesticides.

Due to their wide spread use in the production systems (both agriculture and the allied sectors), persistence in the environment and their varying toxicity make these pesticides a major components of public health consideration. In India, 51 per cent of food commodities were contaminated with pesticide residues and out of these, 20 per cent have pesticide residues above the maximum residue limits (MRLs) (Gupta 2004). Food of animal origin are the most contaminated one by pesticides followed by leafy vegetables and garden fruits (Rathore *et al* 1996). The most commonly noted residues of pesticide encountered in food are organochlorines followed by organophosphates and carbamate pesticides (Kulkarni and Mitra 1990). These synthetic pesticides are fat soluble, which are absorbed and rapidly stored in tissues but slowly excreted (Hansen and Lambert 1987). Due to dipping of cattle and spray of the chemicals for vector control or during feeding of feedstuffs contaminated with these chemicals meat may contain high residues of pesticides in the tissue (Darko and Acquaah 2007).

Owing to the adverse effect of the pesticides, international community had signed Stockholm Convention which entered into force on 17th May 2004. Till date, there are 94 different countries including India who have ratified the Stockholm Convention according to which the parties are to take measures to eliminate or reduce the release of Persistent Organic Pollutants (POPs) into the environment. The Stockholm Convention currently covers nine pesticides, one industrial chemical and two unintended by-products. International Registry of Potentially Toxic Chemicals (IRPTC) - an organ of the United Nation Environmental Protection Agency, has clearly stipulated that the meat and meat products should not have more than the maximum permissible limits because of human safety and from an environmental angle.

India ranks 10th in the world in pesticide consumption, its pesticide market being 12th largest in the world with a value of US$ 0.6 bn per annum, which is 1.6 per cent of the global market pie (Hundal *et al* 2006). Despite the fact that the consumption of pesticides in India is still very low, about 0.5 kg ha^{-1} of pesticides against 6.60 and 12.0 kg ha^{-1} in Korea and Japan, respectively, there has been a widespread contamination of food commodities with pesticide residues, basically due to non-judicious use of pesticides. Pesticide residues have been reported to cause cancer, epilepsy, liver and kidney dysfunctions, somatic growth depression, neuritis and testicular cancer (Ekbom *et al* 1996, Sandhu 1992 and Straube *et al* 1999). OCP's like DDT and PCB have been responsible for breast cancer and decreased fertility in human beings (Au *et al* 1999). pp' DDE, a metabolite of DDT, was found in brain samples of persons suffering from neurological disorders (Fleming *et al* 1994). In Leukemia or lymphoma patients, concentrations of OCP's in bone marrow were also found to be higher in comparison to normal cases (Scheele *et al* 1996).

According to statistics compiled by the Ministry of Food Processing, India (http://www.indiainbusiness.nic.in) the current production levels of buffalo meat in India were estimated at 1.9 million MT, of which about 21 per cent is exported (worth Rs 2600 crore / annum) while the export of sheep/ goat meat further generates Rs 80 crore / annum. India's export of meat and meat products reached Rs. 3,224 crores during 2006-07. Frozen bovine meat dominated the exports with a contribution of over 97 per cent. The demand for bovine meat in international market has sparked a sudden increase in the meat exports from India. The main markets for Indian bovine meat are Malaysia, Philippines, Mauritius and Gulf countries. India has embarked on policy of liberalization on domestic and international fronts. To compete in the international markets, it is necessary that Indian products meant for exports are of international standards. Animal husbandry is one of the most important areas of earning foreign exchange. Therefore, it is necessary to strengthen quality competitive aspects of products. In existing scenario, export of animal products is only limited to some developing countries and is negligible to developed countries due to their strict food hygiene standards and residue levels. With signing of India on World Trade Organization (WTO), in near future situation will become more grave. Though Indian Government has imposed a ban or restricted the use of various persistant pesticides, a rigorous monitoring system on meat and animal food products has to be introduced in India, so that meat industry could be effectively involved to produce residue free meat for domestic consumers and exports.

Very little work has been done regarding the presence of pesticide residues in buffalo and sheep meat and their products in India. In the absence of authentic reports, it is difficult to know the spectrum of residue level in those commodities. Thus, in the wake of liberalization and globalization of the markets, pesticide residues in food assume importance with special reference to codex and sanitary and phytosanitary (SPS) measures. Keeping in view of public health significance of pesticide residues, safety of consumers and legal restrictions on export, the present study was proposed to monitor the organochlorine pesticides levels in meat of lamb and buffalo and in human blood samples.

Objectives:

- Monitoring of organochlorine pesticide residues in lamb and buffalo lean meat.
- To study the levels of organochlorine pesticide residues in ready to cook lamb and buffalo meat products available in the market.
- Evaluation of organochlorine pesticide residues in serum of human population of Ludhiana.

REVIEW OF LITERATURE

Use of pesticides in India began in 1948 when DDT was imported for malaria control, while India started pesticide production in the year 1952. Currently, there are approximately 145 pesticides registered for use. Pesticide use in India has jumped hundreds fold from 154 metric tones (MT) in 1954 to 75, 033 MT in 200l (http://www.indiatogether.org/2005/jun/agr-bloodcide.htm). But with subsequent innovations of new technologies like integrated pest management (IPM) and also use of some other technical grades of pesticides, the amount again reduced to 40, 672 MT in 2005 (http://www.punenvis.nic.in/database_agri19.htm). This decline may also be attributed to the ban on some organochlorine pesticides such as technical HCH, DDT, aldrin etc. for agricultural use and introduction of Bt cotton in some parts of India (Agnihotri 2000). Among Indian states, Punjab is one of the largest user of pesticides with a consumption of 6,972 MT a year (http://www.punenvis.nic.in). Pesticides are widely used in conventional agriculture and residues remain on (and in) the food we eat. In many cases, small amounts of pesticides used to treat crops and animals remain in produce or in meat when they are marketed. In other cases, for instance, most food products from animals like meat and meat products contain measurable amounts of pesticides which comes about because of widespread contamination of the environment with pesticides.

2.1 Pesticide residues in meat

Saschenbrecker (1976) analyzed samples of bovine and ovine renal fat, porcine dorsal fat and pooled fowl fat for chlorinated hydrocarbon residues. γ- HCH (Lindane), HCH, heptachlor and its epoxide, chlordane, aldrin, dieldrin, DDT and its metabolites, methoxychlor, toxaphene and PCB were detected. DDT and metabolites were present in 53.34 per cent samples while 40.56 per cent of all tested samples failed to reveal the presence of any pesticide residue of the organochlorine and organophosphorus group. Beef exhibited an average DDT level of 2.6 times lower than pork.

Kasai *et al* (1979) surveyed domestic beef, imported beef, pork and chicken in Japan and reported the levels of 0.093, BDL, 0.008 and 0.010 mg kg^{-1} for total HCH 0.022, 0.030, 0.046 and 0.045 mg kg^{-1} for dieldrin, respectively. The levels of residues were higher from domestic beef of Japan than imported beef.

Kalra and Chawla (1980) surveyed market meat of Ludhiana city for pesticide residue levels. They reported maximum levels of DDT and HCH residues in pork and the lowest in sheep.

Battu *et al* (1984) carried out studies on determination of organochlorine insecticide residues in market samples of meat from Ludhiana, Punjab. Residues of both DDT and HCH were present in almost all the samples. DDT was found at the mean levels of 1.3, 0.3, 0.05, 0.23 and 3.02 mg kg^{-1} in pork, chicken, sheep, goat and fish, respectively and was below the prescribed tolerance level. Level of HCH was lower than that of DDT in meat of all animals.

Al-Omar *et al* (1985) analyzed levels of organochlorine insecticides in lamb and beef from Baghdad. Two lots of lamb and beef were collected randomly, each lot consisting of 20 samples. Average values of lindane, sum of aldrin and dieldrin, heptachlor, chlordane and total DDT in lamb were 0.225, 0.067, 0.067, 0.126 and 1.048 mg kg^{-1}, respectively. Similarly, in beef average levels were lindane (0.116 mg kg^{-1}), dieldrin (0.101 mg kg^{-1}), heptachlor (0.124 mg kg^{-1}), chlordane (0.068 mg kg^{-1}) and total DDT (0.875 mg kg^{-1}).

Kaphalia *et al* (1985) reported levels of DDT and HCH as 0.24 and 0.199 mg kg^{-1}, respectively for meat including poultry and fish. They further reported that the Indians take maximum levels of DDT (0.199 mg) and HCH (1.293 mg) per day.

Corrigan and Seneviratna (1990) reported result of Australian Quarantine and Inspection Service (AQIS) intensive test program for organochlorines. The samples were taken from cattle from approximately 137, 000 individual farms. A total of 813, 330 cattle slaughtered at export and domestic abattoirs were tested. Out of the total farms, 118, 000 of those farms had met the designated test result criteria and were declared clear under the program.

Frank *et al* (1990) analyzed 602 samples of abdominal fat from avian, bovine, caprine, lupine, ovine, porcine species together with hen eggs for organochlorine and organophosphorus pesticides and industrial organic pollutants. DDE, dieldrin, lindane, PCB, pentachlorophenol and tetrachlorvinphos were identified. Pentachlorophenol was the most frequently found contaminant and DDE was the second being present in 21 per cent of the samples. No residues of organophosphorus insecticides were detected in any animal tissue samples.

Tanabe *et al* (1991) analyzed raw foodstuffs collected from Bangkok for presence of organochlorine pesticides and polychlorinated biphenyls. Residues of PCBs and organochlorine pesticides were detected in all the samples analyzed. Meat samples recorded higher concentrations of total DDT and dieldrin than HCH. Concentrations of

PCBs in meat samples ranged from 2.3 to 12 ng g^{-1} of which pork fat exhibited the maximum. Higher DDTs were recorded in chicken fat and pork fat.

Lazaro *et al* (1991) screened 52 sausage samples from various animals in Spain for hexachlorobenzene (HCB), HCH, heptachlor, heptachlorepoxide, aldrin, dieldrin and endrin. HCB and HCH were detected in all samples (0.015 and 0.050 mg kg^{-1}, respectively). No chlorocyclodine residues were detected except dieldrin (0.002 mg kg^{-1}). In all the samples, residue levels were well below MRLs for meat and meat products.

Kannan *et al* (1992b) analyzed foodstuffs from different regions in India for the presence of HCH, HCB, DDT, aldrin, dieldrin, heptachlor and PCB's. Significantly high levels of food contamination with HCH, DDT, aldrin and dieldrin were evident throughout India. They reported that dairy products and livestock meat were the prime sources of human dietary exposure to these chemicals. On the whole, contamination of foods by organochlorine compounds followed the order HCH > DDT > aldrin/dieldrin > PCB's > HCB > heptachlor. HCH contamination in different groups of foodstuffs was rated as following, dairy products > meat > pulses > oils > spices > cereals > fish. A similar pattern was noticed for DDT and aldrin/dieldrin with minor variation. In general, oils and dairy products contained higher levels of HCB than other foods while in spices and oils heptachlor dominated. Contamination by PCB's in oil was greater followed by meat and fish.

Khalafalla *et al* (1993) measured levels of organochlorine residue in 350 carcasses of animals (175 cattle, 150 buffaloes and 25 sheep) in Giza, Egypt. Only beef muscle, fat and buffalo fat contained high levels of lindane.

Herrera *et al* (1994) investigated levels of organochlorine pesticides in 229 samples of Spanish meat products of different species (lamb, pork, beef and poultry). HCB and HCH were detected in all beef samples. The level of HCB averaged 49 ppb in lamb; varied between 8-18 ppb in pork and beef products and amounted to 26 ppb in fresh poultry sausages. HCH concentration varied between 20-40 ppb in beef products. No residue of aldrin, endrin, heptachlor epoxide, chlordane, methoxychlor, endosulphan or transnonachlor was detected.

Surendranath *et al* (1998) studied the occurrence pattern of organochlorine pesticide residues in adipose tissue, liver and kidney samples of goat, sheep and oxen collected from the local slaughterhouse in Bangalore, India. They found higher concentration of DDT in most of the tissues analyzed. They reported the mean concentration ($\mu g\ kg^{-1}$) levels of γ- HCH, $\sum$ HCH, pp' DDE and $\sum$ DDT residues as 21.7, 57.3, 56.9 and 174 in adipose tissue, 6.3, 13.1, 4.8 and 110.1 in liver and 1.6, 3.6, 1.4 and 3.8 in kidney tissue, respectively whereas endosulphan was not detected in any of the samples.

Glynn *et al* (2000) determined time trends of organochlorine concentrations in adipose tissues from swine and bovines slaughtered between 1991 and 1997 in Sweden. Multiple linear regression indicated that the concentrations of PCB, pp' DDE, HCB and α-HCH decreased by 4-17 per cent per year. The organochlorine concentrations were higher in bovines than in swine, and declined faster in swine than in bovines. Concentrations of CB 153 and pp' DDE were similar in bovines.

Kipcic *et al* (2001) assayed 466 fatty tissue samples of beef, pork, poultry and fish in Croatia between 1992 and 1996 for chlorinated hydrocarbons. Samples were divided into two groups, meat and fish imported to Croatia and meat from Croatian farms and fish from the Adriatic Sea. DDT level was 0.013 mg kg^{-1} and 0.059 mg kg^{-1} in domestic and imported beef, respectively. There was an average level of 0.001 mg kg^{-1} of HCB and 0.001 mg kg^{-1} of α- HCH in beef. However, imported beef had an average of 0.004 mg kg^{-1} of HCB and 0.002 mg kg^{-1} of α- HCH.

Manirakiza *et al* (2002) in Sene-Gambian region, reported that pp' DDE and HCB were detected in all the beef samples, while heptachlorepoxide, op' DDE, op' DDT, dieldrin, α- and β- endosulphan, methoxychlor and mirex were not detected in any sample. The sum of HCH ranged from 1.0 to 4.6 ng gm^{-1}, sum of DDT's from 27.5 to 63.2 ng gm^{-1} and total pesticides from 20.0 to 84.7 ng gm^{-1} with an average of 50.7 ng gm^{-1} and a standard deviation of 17.6 ng gm^{-1} on fat basis.

Bedi *et al* (2003) monitored residue levels of cyclodiene group of insecticide residues in different body tissues like fat, muscle, liver and kidney of goats. Mean dieldrin residues in fat, muscle and liver were recorded as 0.045, 0.024 and 0.014 mg kg^{-1}, respectively. Similarly mean residue levels of endosulphan was measured as 0.103, 0.093, 0.091 and 0.085 mg kg^{-1} in fat, muscle, liver and kidney, respectively. Mean residue levels of heptachlor were 0.020, 0.004, 0.002 and 0.003 mg kg^{-1} in fat, muscle, liver and kidney, respectively. Aldrin and chlordane residues were not detected in any of the tissue samples.

Bedi *et al* (2005) reported levels of DDT and HCH in various tissues of goat. DDT was found to be the most predominant pesticide with mean levels of 0.573, 0.156, 0.208 and 0.060 mg kg^{-1} in fat, muscle, liver and kidney, respectively while levels of HCH were 0.441, 0.137, 0.126 and 0.092 mg kg^{-1} in fat, muscle, liver and kidney, respectively. Out of HCH isomers, γ-HCH was the most predominant followed by β-, α- and δ- HCH isomers.

Matsumoto *et al* (2006) surveyed for PCBs and organochlorine pesticides in meats (beef, pork and poultry) and processed meat products in Osaka, Japan. It was found that for 35 years from 1970 to 2004, concentrations of PCB, HCH isomers, DDT analogues and dieldrin in all meats appeared to decline with each passing year. The concentrations of DDT analogues in all meats also showed sharp decline from the late 1970s to the early 1980s. The concentrations of each pesticide in processed meat products tended to be lower than those of the corresponding meat samples, respectively.

Darko and Acquaah (2007) found organochlorines in all the beef samples analyzed in Ghana. The average concentration of lindane in beef fat samples from Kumasi was 4.03 µg kg^{-1} and 1.79 µg kg^{-1} in beef fat from Buoho. The average levels of lindane were 2.07 µg kg^{-1} for lean meat samples from Kumasi abattoir and 0.60 µg kg^{-1} in lean meat samples from Buoho. Beef samples from Buho had DDE concentration of 31.89 µg kg^{-1} in the fat and 5.86 µg kg^{-1} in lean beef. Average concentration of DDT in beef fat from Buoho was 403.82 µg/kg but lean meat samples from the same sampling site recorded mean concentration of 10.82 µg kg^{-1} for DDT. Although, most of the organochlorine residues detected were below the maximum residue limits set by the FAO/WHO, bioaccumulation of these residues is likely to pose health problems in higher organisms like human beings.

2.2 Pesticide residues in blood of general human population

Pesticides have contaminated environment ubiquitously. They have been found in human blood, urine, breast milk, semen, adipose tissue etc. Pesticides after they come from various sources make a cumulative accumulation in human body. Though body detoxification mechanism come in to play after pesticides gain entry in human system and are metabolized into different chemicals and excreted, some still remain stored in fatty tissues of the body. Evaluation of pesticides in blood of people provides evidence of body burden due to those toxic chemicals.

Brown and Chow (1975) analyzed paired samples of adipose tissue and blood obtained from autopsies on accident victims residing in Norfolk County and 52 blood samples from persons engaged in the agricultural application of DDT in Ontario and 315 from residents of Holland Marsh were analyzed for total DDT. Mean value of total DDT for adipose tissue and blood were 5.83 and 0.032 mg kg^{-1}, respectively. There was a statistically significant correlation between total DDT in fat and blood. Mean value for total DDT in human blood samples was 0.032 mg kg^{-1} in Norfolk County and 0.016 mg kg^{-1} in Holland Marsh and in 26 persons exposed during formulation of DDT preparations it was 0.063 mg kg^{-1}.

Doguchi and Fukano (1975) analyzed 27 samples of human whole blood from 21 to 57 years old, in Tokyo Metropolitan Research Laboratory of Public Health. Mean values for polychlorinated terphenyls (PCTs), polychlorinated biphenyls (PCBs) and DDE were obtained as 3.2, 5.0 and 11.2 ppb, respectively.

Agarwal *et al* (1976) conducted a study in Delhi during which blood samples from 182 people were examined for DDT residues. It showed that all except 8 contained DDT and its metabolites. The average total DDT concentration in the whole blood ranged from 0.177 to 0.683 mg l^{-1} in males and from 0.166 to 0.329 mg l^{-1} in females. The DDT metabolites detected were pp' DDE, pp' DDD and op' DDT. DDE accounted for most of the total DDT.

Kaphalia and Seth (1983) detected DDT and HCH residues in all the 99 samples of blood and adipose tissue of normal and exposed persons from urban area of Lucknow, India. Total HCH concentration in normal population was 0.038 mg l^{-1} (children), 0.034 mg l^{-1} (females), 0.075 mg l^{-1} (males) and in exposed persons was 0.295 mg l^{-1}. Total DDT concentration in normal population was 0.023 mg l^{-1} (children), 0.023 mg l^{-1} (females), 0.028 mg l^{-1} (males) and in exposed person was 0.200 mg l^{-1}.

Ramachandran *et al* (1984) collected a total of 340 biopsies of body fat and blood samples from 162 males and 178 females from 3 government hospitals in Delhi for determining DDT and HCH residues. Mean DDT concentration of body fat was 22.25 ±1.66 mg kg^{-1} and for blood 0.71 ± 0.05 mg l^{-1}. The mean total HCH of body fat was 16.85 ± 0.94 mg kg^{-1} and for blood 0.49 ± 0.05 mg l^{-1}.

Saxena *et al* (1987) analyzed blood from 50 volunteers from residents of areas surrounding a DDT manufacturing factory in Delhi, India. It was found that mean total DDT in male (0.344 mg l^{-1}) was higher than that of female (0.229 mg l^{-1}). A very high level of DDT and its metabolites were found in whole blood of occupationally unexposed population of that area. Total DDT ranged from 0.053 to 0.663 mg l^{-1} with a mean value of 0.301 mg l^{-1}. Mean blood concentrations (mg l^{-1}) of pp' DDE, op' DDT, pp' DDD, pp' DDT and total DDT were 0.129 ± 0.061, 0.066 ± 0.044, 0.0005 ± 0.0007, 0.102 ± 0.079 and 0.301 ± 0.169, respectively.

Stehr- Green PA *et al* (1988) surveyed for measured levels and dietary sources of 11 organochlorine pesticide residues and metabolites in 85 human sera from a rural population. The mean serum concentrations in ppb were HCB (0.27 ±0.18), dieldrin (0.84 ± 0.72), γ- HCH (0.05 ± 0.01), β- HCH (0.87 ±1.43), heptachlor epoxide (0.50 ±0.89), oxychlordane (0.49 ± 1.08), trans- nonachlor (1.27 ± 3.00), pp' DDT (3.50 ±9.40), op' DDT (1.00 ± 0.90), pp' DDE (9.90 ± 13.54).

Krawinkel *et al* (1989) detected organochlorine pesticides in measurable concentrations in blood and fat concentrations in humans in Pakistan. All participants were inpatients, mostly who went laparotomies, of the Sandeman Provincial Hospital in Quetta, the capital of Baluchistan province in Pakistan. Blood samples were detected for α- HCH, β- HCH, T- HCH, 44' DDE and 44' DDT. A great range of inter individual differences were observed.

Waliszewski and Szymczynski (1991) elaborated an analytical procedure to find out the extent of contamination of the human body by persistent residues of organochlorine pesticides. Mean level (ng ml^{-1}) of pesticides in blood serum were HCB (93.8 ± 5.3), α- HCH (92.7 ±4.9), β- HCH (90.2 ±6.8), δ- HCH (91.8 ± 4.2), ε- HCH (88.4 ± 6.6), lindane (95.9 ±4.8), aldrin (89.9 ± 5.7), heptachlor (90.3 ± 6.0), heptachlor epoxide (92.3 ± 4.8), pp' DDE (89.7 ±6.4), op' DDT (92.0 ± 5.1), pp' DDT (93.1 ± 4.4), α- endosulphan (134.6 ± 8.7), β- endosulphan (72.6 ± 8.6), endosulphan sulphate (90.7 ± 5.9), kepone (84.7 ± 6.2) and mirex (94.1 ± 4.7).

Bhatnagar *et al* (1992) made a study in Ahemdabad (rural area) during which blood samples were collected from 31 healthy males during 1989-90 for analysis of DDT, HCH, heptachlor, heptachlor epoxide, aldrin, oxychlordane, HCB and dieldrin in serum. Mean serum levels of pp' DDE, op' DDT, pp' DDD, pp' DDT and total DDT were 37.25, 0.335, 1.33, 8.828 and 47.745 mg l^{-1}. pp' DDE was the major metabolite and it alone contributed about 78 per cent of total DDT. All serum samples were contaminated by HCH with an average of 147.335 mg l^{-1} with equivalent amounts of α-, β- and γ- HCH. Heptachlor, oxychlordane, aldrin and dieldrin were detected at an average concentration of 0.819 mg/l, 1.465 mg l^{-1}, 0.200 mg l^{-1}, 2.152 mg l^{-1}. Heptachlor epoxide and hexachlorbenzene were not detected in any sample.

Kanja *et al* (1992) analyzed a total of 41 samples of maternal blood, milk, subcutaneous fat and umbilical cord blood from mothers giving birth by caesarean operation at Kenyatta National Hospital in Nairobi in 1986. The main contaminants found in all the samples were pp' DDT (100 %), pp' DDE (100 %), op' 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 umbilical cord serum.

Mes J (1992) reported organochlorines in human blood and biopsy fat with their relationship. Paired blood and biopsy fat samples were obtained from 25 patients. The median concentrations of organochlorine residues in human blood in ng g^{-1} were as 1,2-Dichlorobenzene (<3.12), 1,2,3- Trichlorobenzene (<1.17), 1,3,5-Trichlorobenzene (<4.02), 1,2,3,5-Trichlorobenzene (0.06), Pentachloronebenzene (<0.02), HCB (0.11), α- HCH (<0.04), β- HCH (0.13), Octachlorostyrene (<0.06), Oxychlordane (0.07), α- Chlordane (0.02), γ- Chlordane (0.01), t-Nonachlor (<0.03), c-Nonachlor (<0.01), pp' DDE (0.87), op' DDT (<0.13), pp' DDT (0.13), Dieldrin (<0.01), Heptachlor epoxide (<2.35), Mirex (<0.04).

Frank e*t al* (1993) analyzed 750 whole blood samples during 1986-87, from residents of large and medium to small urban centers across Ontario, Canada. They showed a mean concentration of PCBs up to 9.2 mg kg^{-1} and DDE up to 3.7 mg kg^{-1}. Dietary levels of PCBs and DDE in foods consumed by Ontario residents during 1986-87 on whole food basis ranged from 0.1-3.0 mg kg^{-1} and 0.05 - 0.77 mg kg^{-1}, 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 Hardwar, India. Mean concentrations of HCH and DDT were 21.50 mg l^{-1} and 20.79 mg l^{-1}, respectively. Similarly 47 samples of occupationally exposed persons, involved in spraying operation of HCH and DDT during Ardh Kumbh Congregation at Hardwar in April, 1992 for the control of mosquitoes and flies, were screened for HCH and DDT contamination in whole blood. Mean concentrations of HCH was 68.0 mg l^{-1} and DDT 58.43 mg l^{-1} i.e. 3.1 times and 2.8 times more as compared to general population.

Nair *et al* (1996) conducted a study in Delhi, in which samples of maternal blood, breast milk and cord blood from 25 mothers (23.4 ± 1.085 years of age with a range of 18-40 years) and their new born from Irwin Hospital, Delhi were screened for concentration of DDT and HCH. DDT was present at an average level of 1.27, 0.27 and 0.14 mg 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. Average value of HCH in maternal blood, breast milk and cord blood was 0.327, 0.050 and 0.033 mg l^{-1}. β- isomer was the predominant isomer accounting for more than 60 per cent of the various isomers.

Paumagarteen *et al* (1998) measured serum levels of organochlorine pesticides in agricultural workers from Rio de Janeiro, Brazil. Blood were taken from 26 volunteers.

They detected pp’ DDE in 16 samples, but pp’ DDE concentration exceeded 1.4 µg l^{-1} in only 3 of these. Limits of residue quantification for the study were validated as follows: op’ DDT, pp’ DDT and pp’ DDT as 2.8 µg l^{-1}; pp’ DDE as 1.4 µg l^{-1}; aldrin, dieldrin, endrin, heptachlor and heptachlor epoxide as 1.4 µg l^{-1}; α- and γ- HCH as 0.7 µg l^{-1}, β- HCH as 1.4 µg l^{-1}; HCB as 0.7 µg l^{-1}. β- HCH was found in 6 samples. Dieldrin was detected in only one sample. The percentage of positive pp’ DDE increases from the youngest to the oldest age group. A similar trend was found for β- HCH.

Stellman *et al* (1998) measured levels of pesticides and PCBs in 173 women. pp’DDE was the most abundant comprising 71 per cent of total serum pesticides. The next most common pesticide was β- HCH. Mean concentration (ng ml^{-1}) of pesticides in serum were HCB (0.214 ng ml^{-1}), β- HCH (0.824 ng ml^{-1}), oxychlordane (0.249 ng ml^{-1}), *trans* nonachlor (0.236 ng ml^{-1}), pp’ DDE (4.72 ng ml^{-1}), op’ DDD (0.218 ng ml^{-1}), pp’ DDT (0.216 ng ml^{-1}), total OCP’s (6.677 ng ml^{-1}).

Laden *et al* (1999) evaluated predictors of plasma concentrations of DDE, and PCBs in a group of 240 women, controls from a breast cancer case- control study. They chose to evaluate levels of DDE as opposed to DDT because of its presence in higher concentration in blood and as it reflect better as a sign of long term exposures. Plasma concentrations of DDE ranged from 0.14 to 39.44 ppb with a mean value of 7.09 ± 6.06 in the control series and with a mean of 6.12 ± 4.58 in the case series. Total plasma PCBs ranged from 1.61 to 16.62 ppb (mean as 5.22 ± 2.35) and from 1.55 to 17.44 ppb (mean as 5.15 ± 2.77) in the control and case series, respectively.

Naqvi and Jahan (1999) took samples of serum from different laboratories of Karachi, Pakistan and were analysed for the presence of DDT, DDE, Dieldrin, Aldrin, Malathion and Deltamethrin. Majority of the samples had presence of more than one pesticide. With their result they concluded that organochlorine pesticides were dominant than other groups of pesticides (organophosphate and pyrethroid) in the exposed people of Pakistan

Waliszewski *et al* (1999) analyzed 65 blood samples during the period of October 1997 to June 1998 from volunteer mothers admitted to hospital for delivery. All of the samples were analyzed for HCB, α, β and γ- HCH, aldrin, heptachlor, heptachlor epoxide, pp' DDT, op' DDT, pp' DDE, pp' DDD, α, β- endosulphan and endosulphan sulphate. Mean levels (ng/ml) of pesticides were as HCB (1.1 ± 0.8), α- HCH (0.1 ± 0.2), β- HCH (1.4 ± 1.8), γ-HCH (0.2 ± 0.6), total HCH (1.6 ± 2.0), pp' DDE (14.5 ± 28.0), pp' DDD (0.3 ± 0.2), op' DDT (1.1 ± 4.0), pp' DDT (1.2 ± 3.8) and total DDT (16.4 ± 30.8). HCB was detected in 100 per cent of analyzed samples followed by pp' DDE in 98 per cent of samples. B- HCH revealed a frequency of 71 per cent.

Dua *et al* (2001) analysed 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. ε- HCH isomer was reported highest in comparison to other 3 isomers of HCH.

Manirakiza *et al* (2002) measured concentrations of organochlorine pesticides in human serum samples from the Sene-Gambian region using a validated analytical methodologies. In serum samples, α- HCH, pp' DDE, op' DDT and pp' DDT were detected in all 16 pooled serum samples. Endosulphan sulphate, methoxychlor, mirex, heptachlor epoxide and endrin were detected in 15 samples with most of the concentrations below 10 ng ml^{-1}.

Waliszewski *et al* (2002) determined persistent organochlorine pesticide levels in maternal blood serum, colostrum and mature milk from 100 randomly selected volunteer mothers among hospital patients admitted for delivery in the period of 1998- 1999. The mean levels (mg kg^{-1} on fat basis) of HCB in serum, colostrum and mature milk were 0.09, 0.04 and 0.03 respectively. Similarly mean values of β- HCH in serum, colostrum and mature milk were 0.18, 0.11 and 0.07, respectively. The mean (mg kg^{-1} on fat basis) value of pp' DDE reached 3.78 in maternal blood serum, 4.21 in colostrum and 3.24 in mature milk. The same tendency revealed by pp' DDT, reaching 0.82 mg kg^{-1} in maternal blood serum, 0.83 mg kg^{-1} in colostrum and 0.58 mg kg^{-1} in mature milk. The DDT total presented mean values as 4.50 mg/kg in maternal blood serum, 4.96 mg kg^{-1} in colostrum and 3.79 mg kg^{-1} in mature milk.

Dallaire *et al* (2003) in a study in Canada collected 251 cord blood samples from 1994 through 2001 for detection of polychlorinated biphenyls (PCBs), dichlorodiphenyl dichloroethylene (DDE), hexachlorobenzene (HCB), chlordanes, lead and mercury. It showed significantly decreasing trends for PCBs (7.9 % per year, $p<0.001$), DDE (9.1 % per year, $p<0.001$), DDT (8.2 % per year, $p<0.001$) and HCB (6.6 % per year, $p< 0.01$). No significant trend was detected for chlordanes.

Whyatt *et al* (2003) in a study conducted in USA, collected plasma samples at birth between 1998 and 2001 from 230 mother and newborn pairs enrolled in the Columbia Centre for Children's Environmental Health. Those samples were analysed for 29 pesticides. Seven pesticides were detected in 48-83 % of plasma samples (range, 1-270 pg g^{-1}) the organophosphates chlorpyrifos and diazinon, carbamates bendiocarb and 2- isopropoxyphenol (metabolite of propoxur) and fungicides- dicloran, phthalimide (metabolite of folpet and captan) and tetrahydrophthalimide (metabolite of captan and captafol). Maternal and cord plasma levels were similar, except for phthalimide and were highly correlated ($p<0.001$).

Chan *et al* (2004) made a survey of 577 whole blood samples from school children in Peninsular Malaysia for extraction and analysis of the residues of 11 organochlorine and 2 organophosphorus pesticides. It revealed the presence of pesticide residues in blood in ng g^{-1}, aldrin- ND-47.6; dieldrin- nd, endrin- nd, α-endosulphan- ND-0.6, β-endosulphan-ND, endosulphan sulphate-ND, heptachlor-ND- 3.8; Lindane-ND-5.7, pp' DDT- ND-3.4, op' DDE- ND-1.4, pp' DDE-ND, chlorpyrifos- ND-10.3, diazinon-ND-103.0.

According to a World Wildlife Fund report (WWF 2004) analysis of blood samples of 14 European ministers from 13 European countries, for 103 different man made chemicals from 7 different chemical families: organochlorine pesticides, polychlorinated biphenyls, synthetic musks, per fluorinated chemicals, brominated flame retardants, phthalates and anti bacterial were made. It revealed that 55 of the 103 chemicals analyzed were detected. A cocktail of hazardous chemicals contaminated every volunteer tested and six of the 7 chemical groups were detected. Twenty five of the same chemicals were detected in every individual – including pp'- DDE and HCB. The chemical found in highest concentration in whole blood was Diethyl hexyl phthalate (endocrine disrupter) at concentrations of 160 ng g^{-1} and in blood serum it was pp' DDE (a DDT metabolite), at a concentration of 3300 pg g^{-1} and deca BDE, a neurotoxic chemical used as flame retardant was found at the highest concentration of 45 pg g^{-1} of all the flame retardants analyzed.

In a study conducted at urban area of Ahmedabad by Bhatnagar *et al* (2004) human blood samples from 18 male healthy volunteers showed the presence of pp' DDE, op' DDT, pp' DDD, pp' DDT and t-DDT at an average value of 20.85, 1.15, 2.03 9.28 and 32.61 mg l^{-1} in serum samples, respectively. The concentration of α-, β- γ- and total HCH in serum samples was 4.49, 35.06, 1.69 mg l^{-1} and 41.23 mg l^{-1}, respectively. Hexachlorobenzene was present in 7 samples at an average concentration of 0.2 mg l^{-1}.

Schafer *et al* (2004) in "Chemical Trespass: Pesticides In Our Bodies And Corporate Accountability" a report by Pesticide Action Network North America (PANNA) and partner groups in more than 20 cities has stated that many U.S. residents carry toxic pesticides in their bodies above government assessed "acceptable" levels. Analyzing pesticide residue data collected by the US Centre for Disease Control and Prevention (CDC) on levels of chemicals in 9,282 people nationwide revealed that government and industry have failed to safeguard public health from pesticide exposures. CDC found that among the people who had their blood and urine tested, 100 per cent showed pesticide residues. The average person carried a toxic cocktail of 13 of 23 pesticides analyzed. Two insecticides chlorpyrifos and methyl parathion- were found at levels up to 4.5 times higher than what U.S government deems acceptable. Children, women and Mexican Americans shouldered the heaviest pesticide burden. Children, the population most vulnerable to pesticides are exposed to a higher level of nerve damaging organophosphorus pesticides.

Waliszewski *et al* (2004) analyzed blood and abdominal tissue from 126 adult cadavers submitted for autopsy at the Institute of Forensic Medicine of the University of Veracruz, Mexico for HCB, β- HCH, pp' DDE, op' DDT and pp' DDT. The comparison of mean and standard deviation values for all organochlorine pesticides between both sample groups indicated significantly higher values of serum lipids vs. adipose lipids expressed as mg/kg on lipid basis (HCB 0.178 vs. 0.055, β- HCH 0.504 vs. 0.216, pp' DDE 2.789 vs. 1.063, op'- DDT 0.130 vs. 0.062, pp' DDT 0.340 vs. 0.585 and total DDT 3.258 vs. 1.706). Only pp' DDT revealed inverse levels which could be due to higher accumulation in adipose fats. The higher levels in blood serum lipids express that these organochlorines are inclined to blood lipids as a body compartment and that the equilibrium pattern favors blood serum lipids.

Mathur *et al* (2005) analyzed blood samples from four Punjab Villages for 14 organochlorines and 14 organophosphorus pesticides. Blood samples contained 0.057 mg l^{-1} of HCH and 0.023 mg l^{-1} of lindane which ranged from 0.014 to 0.057 mg l^{-1}. Similarly the levels of DDT, Endosulphan were 0.065 mg l^{-1} and 0.045 mg l^{-1}, respectively. The levels of monocrotophos, chlorpyrifos, malathion and phosphamidon were detected at mean levels of 0.095, 0.066, 0.030and 0.037 mg l^{-1}, respectively. Hexachlorocyclohexane was detected in all the blood samples and DDT in 95 per cent of the samples.

Waliszewski *et al* (2005) made a study of organochlorine pesticides level in blood serum lipids in women bearing babies with undescended testis. Blood samples were taken post partum from thirty mothers, after diagnosis of undescended testicles. All samples were analysed for HCB, β- HCH, pp' DDE, op' DDT and pp' DDT. Serum organochlorine pesticide levels (mg kg^{-1} on fat basis) were HCB (0.210 ± 0.142), β- HCH (0.212 ± 0.121), pp' DDE (2.562 ± 0.804), op' DDT (0.108 ± 0.044), pp' DDT (0.427±0.045) and DDT (3.042 ± 0.929).

Lino and Silveira (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, pp' DDE, op' DDT and pp' 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 the op' DDT and pp' DDT highest levels were 24.8 and 21.9 µg l^{-1}, respectively.

Subramaniam and Solomon (2006) made a study of blood samples taken from two groups of people, one that has direct exposure to pesticides, the second group, which has indirect exposure to pesticides through food chain. Twenty two people of various occupations with minimum health complaints and skin diseases were selected from Aandipatti area of Madurai, South India for the study. The pesticide residue concentration ranged from 0.006 to 0.130 mg l^{-1} for HCH and 0.002 to 0.033 mg l^{-1} for DDE. High concentrations of HCH and DDE were noted both in the serum of agricultural and non agricultural people.

Glynn *et al* (2007) determined serum concentrations of organochlorine compounds in Swedish pregnant women during a cross sectional study. Concentrations of *trans*- nonachlor and pp' DDE increased with increased age and were highest in women sampled early during the 4 year study period. pp' DDE increased significantly with number of months women had been breast- fed during infancy. Mean serum concentrations of chlorinated pesticides/ metabolites were HCB (23 ng g^{-1} lipid), γ- HCH (1 ng g^{-1}), α- HCH (1 ng g^{-1}), γ- HCH (9 ng g^{-1}), oxychlordane (3 ng g^{-1}), trans Nonachlor (5 ng g^{-1}), op' DDT (2 ng g^{-1}), op' DDE (2 ng g^{-1}), pp' DDT (5 ng g^{-1}), pp' DDD (2 ng g^{-1}), pp' DDE (88 ng g^{-1} lipid).

Li *et al* (2007) studied the relationship between organochlorine pesticides and level of residues in serum and daily foodstuff. One hundred and seven men and 142 women, all healthy and living in the communities, were investigated. The relationship between organochlorine pesticides contents in foods and residues in serum were analyzed by ridge regression. Salted meat was negatively correlated to men's serum level of β-HCH and pp' DDT. It was concluded that pickled meat and vegetable foodstuff might contain low-level of organochlorine pesticide residues.

Ntow *et al* (2008) analyzed 115 serum samples from vegetables farmers from Ghana, during 2005 for DDT and its metabolites, HCH isomers, HCB and dieldrin. DDD was not detected in any of the serum samples analyzed. Among DDT isomers DDE represented the highest levels 7.1 ng/g in serum. The mean level of DDT was 901 ng g^{-1} in blood serum. Mean total DDT levels were higher than the mean concentration of sum HCHs. HCHs were found in serum samples with a mean level of 7.3 ng g^{-1} and were detected in over 50 per cent of sample. Dieldrin and HCB appeared in at least 60 per cent of serum samples. The mean level of HCB was 5.3 ng g^{-1}.

Pathak *et al* (2008) reported the pesticides level of 68 maternal and cord blood samples from mothers undergoing normal full term delivery at Guru Teg Bahadur Hospital, Delhi during the period of January 2006 to September 2007. HCH contributed maximum towards total OCP residues present in maternal and cord blood. This was followed by endosulphan, pp' DDE and pp' DDT contributing the least. The mean levels α, β, γ and total HCH in maternal blood vs. cord blood (ng ml^{-1}) were 7.26 ± 4.08 vs. 4.71 ± 3.12, 10.05 ± 7.01 vs. 7.23 ± 4.24, 5.23 ± 3.11 vs. 2.77 ± 2.41 and 22.54 ± 7.32 vs. 14.71 ± 5.18 respectively. Similarly mean levels of α, β and total Endosulphan were 2.21

± 2.04 vs. 1.39 ± 1.42, 1.49 ± 2.10 vs. 0.88 ± 1.27 and 3.70 ± 4.20 2.27 ± 2.24 respectively. Levels of pp' DDE and pp' DDT were 4.26 ± 3.66 vs. 3.08 ± 2.72 and 1.46 ± 2.99 vs. 1.03 ± 2.63, respectively.

Monitoring pesticide residues in buffalo meat, mutton and human serum:

3.1 Sampling

3.1.1 Buffalo, sheep meat and their products:

Samples of sheep meat and products were procured from local butcher shops at Ludhiana, while buffalo meat was collected from slaughter houses located at village Derabassi of Punjab. Sheep products were procured from local market. Buffalo meat products were prepared in the Department of Livestock Production and Technology, GADVASU, Ludhiana.

Hundred gram sample was collected and brought to laboratory under chilled conditions and a representative sample of 10 g was processed. All samples were processed in duplicate.

3.1.2 Human serum

Blood samples were collected from human population of Ludhiana, Punjab and Amlekhgunj, Bara of Nepal. Venous blood (10 ml) from 50 people in Ludhiana, Punjab and 50 samples from Amlekhgunj, Nepal was collected. Samples from Ludhiana consisted of male population visiting Department of Veterinary Public Health, GADVASU for various purposes. Most of them comprised of animal handlers and veterinary pharmacists. While the participants from Nepal mainly comprised of school children aged between 15 to 19 years old and were of grade ninth and tenth and few of them were teaching staff aged between 36-57 years old. Except 7 students, all of them were non vegetarian. Among those 50 candidates from Nepal, 29 of them were female and 19 were male. With few exceptions almost all of them were the residents of the local village and were being continuously being exposed to the obsolete pesticides which had been housed in the ware house located at the premises of their school.

Blood samples were collected in serum separator vacutainer blood tubes. Blood tubes were allowed to clot, centrifuged and the serum was transferred into 15 ml vials. Field samples were transported in an ice pack to the laboratory and stored at -20^0 C until analyzed.

3.2 Chemicals and glassware

All the chemicals were of Analytical Reagent (AR) quality and obtained from M/s E Merck (India) Ltd. The solvents were glass distilled before use. The suitability of the reagents/solvents was checked by running reagent blank analysis. All the glass ware used in the present study was obtained from Borosil (Mumbai). The glassware was thoroughly washed with Extran (E Merck India Ltd.) and after proper rinsing with distilled water was dried in hot air oven. Before use it was again rinsed with solvents to make them free from residual contamination, if any.

3.3 Pesticide standards

Most of the pesticide standards were imported from M/s Dr. Ehrenstorfer GmbH, Augsburg, Germany, while pesticide standards of pp' DDE, pp' DDD, chlorpyriphos were provided by Department of Entomology, College of Agriculture, PAU, Ludhiana.

3.4 Extraction of pesticide residues

3.4.1 Extraction of residues in sheep and buffalo meat

Procedure given by Mills *et al* (1972) was followed with slight modifications. Sub sample weighing 10 g of sheep/buffalo tissue (muscle) was taken in a mortar and was ground with double the amount of anhydrous sodium sulphate. The free flowing granular material thus formed was extracted by Soxhlet for 8 hours in 200 ml hexane-acetone (1:1, v/v) mixture. The extract was transferred to 250 ml beaker. The flask and the condenser were rinsed with additional 10 ml of hexane and the washing was combined with the original extract. The extract was made to dry completely and then 20 ml of hexane was added to the extract and was dissolved, transferred into 125 ml separatory funnel. Beaker was again rinsed with 10 ml of hexane.

Thirty ml acetonitrile saturated with hexane was added to the separatory funnel and shaken vigorously for 1 min. After layers were separated acetonitrile was drained into 1 litre separatory funnel containing 400 ml water, 30 ml saturated sodium chloride solution, and 50 ml of 15 per cent dichloromethane in hexane. Partitioning was repeated twice with acetonitrile saturated with hexane. All acetonitrile were combined in 1 litre separatory funnel while hexane was discarded. Now separatory funnel containing 400 ml water, 30 ml saturated sodium chlorine solution, 50 ml 15 per cent dichloromethane was partitioned twice. Then extract was evaporated till it became 2 ml and was stored for further clean up process.

3.4.2 Clean up of pesticide residues

Clean up of the samples were performed by florisil column clean up chromatography. Sintered chromatographic column (22 mm id) was packed with 4 g florisil sandwiched between 2 gm sodium sulphate. The column was washed with 8 ml hexane. Sample extract solution was transferred to the column. Rinsing was done twice with 2 ml portions of hexane. Column was eluted @ 5 ml/ min with 40 ml of 20 %

dichloromethane and hexane (v/v). Eluate was concentrated to 2 ml for determination of residues by GLC.

Flow diagram for extraction of pesticide residues in meat

Muscle (10 g)

↓

Add 10 g of anhydrous sodium sulphate and grind in mortar

↓

Make thimble of free flowing granular material

↓

Extract by Soxhlet apparatus for 8 hours in 200 ml hexane acetone (1:1, v/v) mixture

↓

Transfer extract to 250 ml beaker

↓

Rinse flask and condenser with 10 ml of hexane and combine washing with original extract

↓

Completely evaporate extract

↓

Dissolve extract with 20 ml hexane and transfer to 125 ml separatory funnel, rinse with 10 ml hexane and transfer to the same separatory funnel

↓

Partition thrice with 30 ml acetonitrile saturated with hexane

↓

Transfer acetonitrile to another 1 liter separatory funnel containing 400 ml water, 30 ml saturated sodium chloride solution and 50 ml 15% dichloromethane in hexane

↓

Partition twice with 50 ml of 15% dichloromethane in hexane

↓

Completely evaporate extract for clean up

Flow diagram for clean up of pesticide residues in meat

Pack 4 g of activated florisil in a glass column in between 2 layers of anhydrous sodium sulphate

↓

Wash column with 8 ml of hexane

↓

Add concentrated sample extract to the column.

↓

Elute by 40 ml of dichloromethane and hexane (1:4, v/v)

↓

Concentrate the resultant elute to 2-5 ml

↓

Stored in labeled glass stoppered vials for estimation in GLC

3.4.3 Extraction of pesticide residues in meat products

The methods mentioned in 3.4.1 and 3.4.2 was followed for the extraction and clean up of the samples.

3.4.4 Extraction of pesticide residues in serum

The method developed by Gill *et al* (1996) was followed with some modifications for extraction of pesticide residues in serum. The serum samples were thawed and vortexed. An aliquot (1 ml) was placed into a centrifuge tube (15 ml). After equilibration at room temperature for 15 min, acetic acid (1.0 ml) was added and the tube vortexed for 1 min. The analytes were extracted with hexane/ DCM (9:1 v/v, 3.0 ml), vortexed for 1 min and centrifugation was done at 1800 rpm for 2 min. The top organic layer was drawn into a clean centrifuge tube (15 ml). The extraction was repeated twice with hexane/DCM with 3 ml amount each time. One ml concentrated sulphuric acid was added to the combined organic extract tube, and was vortexed for 1 min in order to remove lipids and other interfering compounds. The contents of the tube were centrifuged at 1800 rpm for 2 min to ensure good phase separation. The organic layer was pipetted out. The sulphuric acid layer was further extracted with hexane/DCM (1.5 ml). The combined organic phase was concentrated to 0.5 ml for clean up.

3.5.5 Clean up of pesticide residues

Clean up of the samples was performed by column chromatography using florisil. Florisil (1 g) was packed into a glass column in between two layers of anhydrous sodium sulphate (0.5 g). The column was pre-washed with 8 ml of hexane and then the concentrated sample extract was added to the column. The extract tube was rinsed twice with 0.5 ml of hexane. For recovering organochlorine residues, elution was carried out with 8 ml hexane, while for organophosphorus residues elution was done with 8 ml hexane and dichloromethane (1:9, v/v). The resultant elute was concentrated to 2-5 ml and stored in labeled glass stoppered vials for analysis in GLC.

Flow diagram for extraction of pesticide residues in serum

1 ml aliquot of serum

↓

Place into centrifuge tube (15 ml)

↓

Add acetic Acid (1.0 ml) and vortex tube for 1 min

↓

Extract with 3.0 ml of hexane: DCM (9:1, V/V), vortex, centrifuge at 1800 rpm for 2 min

↓

Draw organic layer into a clean centrifuge tube

↓

Repeat extraction twice

↓

Add 1 ml sulphuric acid to combined organic extract (9 ml)

↓

Vortex and centrifuge at 1800 rpm for 2 mins

↓

Extract organic layer

↓

Extract sulphuric acid layer further with hexane: DCM (1.5 ml)

↓

Concentrate combined sulphuric acid layer to 0.5 ml for fractionation on column

Flow diagram for clean up of pesticide residues in serum

Pack 1 g of activated florisil in a glass column in between 2 layers of anhydrous sodium sulphate

↓

Wash column with 8 ml of hexane

↓

Add concentrated sample extract to the column.

↓

Extract tube washing with 0.5 ml of hexane

↓

Carry out elution with 8 ml hexane for organochlorine and with 8 ml hexane: DCM (1:9, v/v) for organophosphorus

↓

Concentrate resultant elute to 2-5 ml

↓

Store in labeled glass stoppered vials for analysis in GLC

3.5 Estimation of residues

3.5.1 Gas Liquid Chromatography

The residues in cleaned up extracts were estimated using Gas Liquid Chromatography (GLC). The Electron Capture Detector (ECD) was used for organochlorine compounds while Nitrogen Phosphorus Detector (NPD) was used for organophosphate detection in human serum samples only. Instrumental parameters and operating conditions were as follows.

3.5.2 Organochlorine compounds

Gas chromatograph	:	Nucon 5700
Detector	:	ECD, Ni^{63}
Columns	:	Glass column of 2m length x 3 mm i.d. packed with 1.5% OV-17+1.95%OV-210 Support-Chromosorb W.H.P.-80-100 mesh
Temperature (^{0}C)	:	Injection port : 230 Oven : 210 Detector : 240
Gas flow	:	Highly purified nitrogen gas (Lobar-1) at flow rate: 1.6 kg cm^{-2}

The cleaned up extract measuring 2-5 µl was injected in GLC. Winacid 6.2 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 area of the sample chromatographs with those of standards run under the same operating conditions. The standards were run before and after each analysis.

The formula used for the quantification of the residues was:

$$\text{mg kg}^{-1}\text{ (residues)} = \frac{\text{Pesticide standard injected (ng)}}{\text{Area of the standard}} \times \frac{\text{Area of the sample}}{\text{Sample extract injected (µl)}} \times \frac{\text{Final volume of the sample extract (ml)}}{\text{Weight of the sample (g)}}$$

3.6 Confirmation of results

The confirmation of residues of organochlorine pesticides were done on an alternate glass columns packed with 3% DEGS (2 m x 3 mm i.d.) on chromosorb WHP, 80-100 mesh.

Chapter IV

RESULTS AND DISCUSSION

Indiscriminate and injudicious use of pesticides by the end user further adds their residues to the environment which many times get accumulated in food of animal origin. The pesticide industry which was instrumental in the green revolution in the country has now come under heavy scanner due to widespread damage to environment, making agro-ecosystem vulnerable to a new host of pests and causing pesticide-residue effects in a variety of foodstuffs. Pesticides even in minute quantities are commonly cited as significant contaminants because of their general environmental ubiquity, ease of assimilation in food and serious health hazards.

Owing to their lipophilic and persistent nature in the environment, bioaccumulative and biomagnification properties and ending up with the residue problems in humans (Aulakh *et al* 2003, Rodas-Ortız *et al* 2008, Singh *et al* 2007 and Wadhwa *et al* 2004) most of the organochlorine pesticides have been banned from their normal use. However, long term, low exposure of these chemicals has been linked to human health effects and their bioaccumulation can’t be neglected due to the recycling of those chemicals in the nature and due to their persistent character.

The present study was under taken to ascertain the level of organochlorine pesticides in buffalo and sheep meat and in human population.

Recovery test

Recovery tests were performed to check the efficiency of methods for the estimation of pesticide residue in the substrate analyzed during the study. Two different concentrations, i.e. 0.5 and 1 mg kg^{-1} of purified standards of pesticides were spiked in 10 g of buffalo and sheep muscle samples and processed for extraction and clean up as per the method described in section 3.4. The recovery was calculated by subtracting the value obtained for the unspiked sample and was expressed in percentage.

Average recoveries were between 83-94 per cent for DDT metabolites, endosulphan and lindane. The results did not include recovery corrections. The limit of detectability was 0.001 mg kg^{-1} for all the pesticides analyzed. The retention time of various compounds are given in table 1.

Table 1: Retention time of various organochlorine pesticide standards

S.No.	**Pesticide Standards**	**Retention Time (min)**
1.	α- HCH	1:20
2.	γ- HCH	1:52
3.	β- HCH	2:12
4.	δ- HCH	2:36
5.	Aldrin	2:48
6.	α- Endosulphan	5:08
7.	op’ DDE	5:09
8.	pp’ DDE	6:45
9.	op’ DDD	7:28
10.	pp’ DDD	9:54
11.	op’ DDT	10:24
12.	Dieldrin	10:32
13.	β- Endosulphan	10:45
14.	pp’ DDT	14:40
15.	Endosulphan sulphate	20:25

Pesticide residues in buffalo meat

In the present study, 44 samples of buffalo lean meat were subjected to analysis of different organochlorine pesticides. Forty samples were above detectable levels and out of which ∑ HCH, ∑ DDT, ∑ endosulphan and ∑ dieldrin were 0.095, 0.308, 0.043 and 0.013 mg kg^{-1} with frequency occurrence of 88.6, 90.9, 18.1 and 40.9 per cent, respectively. Of the total contamination in buffalo meat highest was by endosulphan (43%), followed by DDT (42%) and then by HCH (13%) and dieldrin (2%) (Fig.1). The percentage of occurrence of α-HCH, γ- HCH, β- HCH and δ- HCH were 77.27, 84.0, 52.2 and 59.0 per cent, respectively. The levels of various HCH isomers along with their range and mean are given in Table 2.

Table 2: Levels of HCH isomers in buffalo meat

S.No.	Pesticides	Positive Samples			Range		Mean (mg kg^{-1})
		No.	Frequency	Percentage	Minimum	Maximum	
1.	α- HCH	34	34/44	77.27 %	0.002	0.088	**0.016**
2.	γ- HCH	37	37/44	84.0 %	0.002	0.174	**0.042**
3.	β- HCH	23	23/44	52.2 %	ND	0.15	**0.019**
4.	δ- HCH	26	26/44	59.0 %	0.003	0.080	**0.017**
5.	∑ HCH						**0.095**

The level of total HCH was little higher than reported by Kasai *et al* (1979).The mean value of α- HCH was 0.016 with a range of 0.002 to 0.088 mg kg^{-1}. Similarly, the levels of γ-HCH ranged from 0.002 to 0.174 with mean value of 0.042 mg kg^{-1}. One of the buffalo meat samples exceeded MRLs for γ- HCH set by Codex Alimentarius Commission (CAC 1993). Likewise β- HCH ranged up to 0.15 mg kg^{-1} with mean value of 0.019 and δ- HCH had a range of 0.003 to 0.080 mg kg^{-1} with mean value of 0.017. Two of the buffalo meat samples exceeded MRLs for β- HCH established by WHO/FAO. The level of lindane (0.019 mg kg^{-1}) was far below than reported by Al-Omar *et al* (1985) and several other workers including Waliszewski *et al* (2004) and Surendranath *et al* (1998). However, the reported

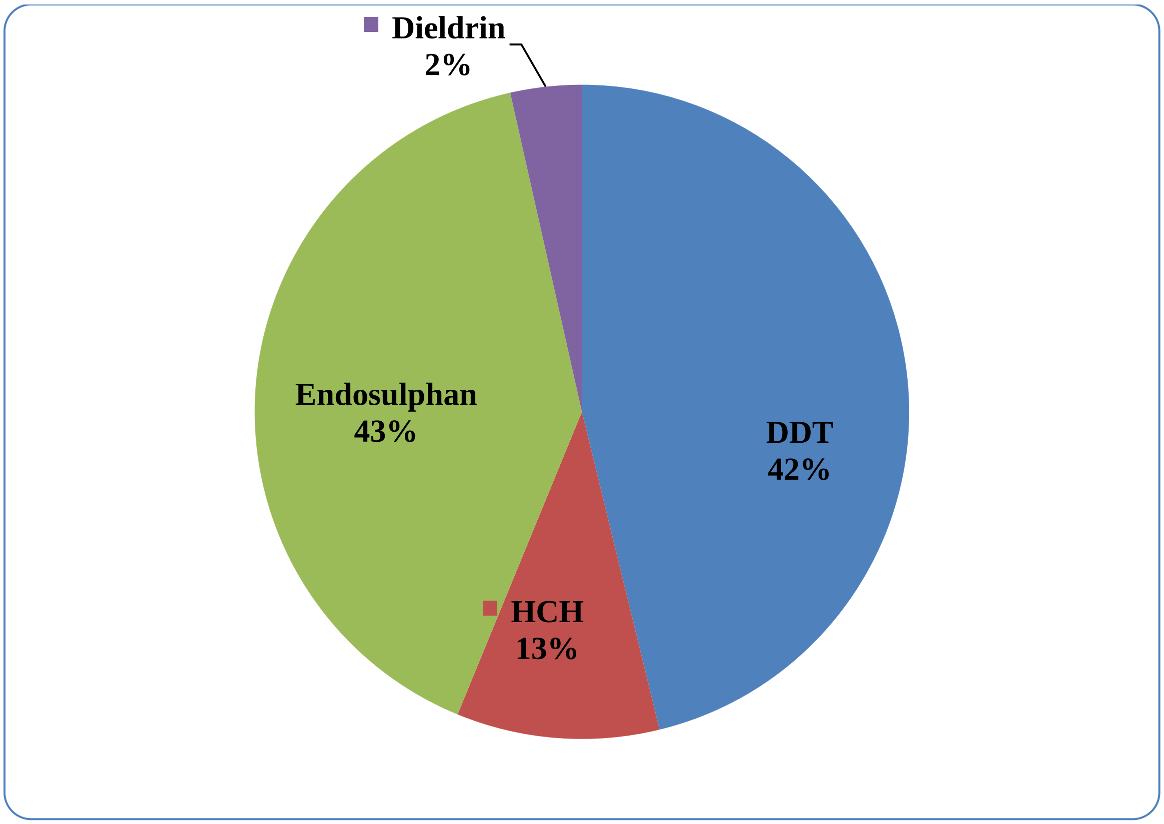

Fig. 1: Proportional contribution of pesticides towards total load in buffalo meat

levels of lindane were further low in the works carried by Lazaro *et al* (1996) and Kannan *et al* (1992b). Darko and Acquaah (2007) reported mean levels of lindane as 4.04, 1.79, 2.07, and 0.60 µg kg^{-1} from beef fat from Kumasi, Buoho, beef from Kumasi and Buoho abattoir, respectively. In their findings, although all the organochlorine residues detected in lean meat, were below the WHO/FAO recommended MRLs but were present at concentration close to the WHO/FAO MRLs in the meat samples.

The occurrence of pp' DDE, pp' DDT, pp' DDD, op' DDE, op' DDT and op' DDD in buffalo meat were 77.27, 70.4, 40.9, 18.1, 11.3 and 6.8 per cent, respectively (Table 3). Amount of total DDT, in buffalo meat, in the present study was much less than those reported by Al- Omar *et al* (1985) which was 0.875 mg kg^{-1}. Among DDT metabolites, pp' DDE had a major share with mean value of 0.101 mg kg^{-1} and frequency occurrence of 77.27 per cent and range from 0.009 to 0.563 mg kg^{-1}. While op' DDD had a least share with mean value of 0.021 mg kg^{-1} and range from 0.004 to 0.096 mg kg^{-1}. Mean residue levels of pp' DDT, pp' DDD, op' DDE and op' DDT were 0.066, 0.046, 0.043 and 0.031 mg kg^{-1}, respectively.

Table 3: Levels of DDT metabolites in buffalo meat

S.No.	Pesticides	Positive Samples			Range		Mean (mg kg^{-1})
		No.	Frequency	Percentage	Minimum	Maximum	
1.	pp' DDE	34	34/44	77.27 %	0.009	0.563	**0.101**
2.	pp' DDT	31	31/44	70.4 %	0.004	0.149	**0.066**
3.	pp' DDD	18	18/44	40.9 %	0.008	0.074	**0.046**
4.	op' DDE	8	8/44	18.1 %	0.006	0.066	**0.043**
5.	op' DDT	5	5/44	11.3 %	0.020	0.043	**0.031**
6.	op' DDD	3	3/44	6.8 %	0.004	0.096	**0.021**
	∑ DDT						**0.308**

pp' DDE constituted major share because of the metabolic conversion of pp' DDT to pp' DDE in animals. Matsumoto *et al* (2006) reported declining levels of DDT's in beef samples in Japan for 35 years from 1970 to 2004, maximum DDT were observed during 1970 to 1974 with mean DDT as 290 ppb and least were observed during 1995-1999 with mean residue level of 2.9 ppb. Residue levels of DDT during 2000 to 2004 were reported as 3.3 ppb which were below than reported in this study. Among DDT metabolites pp' DDE were highest throughout 1970 to 2004 as reported by Matsumoto *et al* (2006) resembling the trend made in the present study, though our was higher than in that Japanese study. Manirakiza (2002) reported mean levels of DDT as 39.5 ng g^{-1} in cattle fat. Though the figures were far below than our report but pp' DDE was found to occur in highest amount (34.8 ng g^{-1}) as reported in our study.

Mean levels of dieldrin, α- endosulphan and β- endosulphan and endosulphan sulphate were 0.013, 0.016, 0.012 and 0.015 mg kg^{-1}, respectively with frequency occurrence of 40.9 per cent, 15.9 per cent, 4.5 per cent, and 9.0 per cent, respectively (Table 4). Singh and Kumar (2003) mentioned that 6.5 per cent of buffalo tissue and 25 per cent of cattle tissues showed the presence of endosulphan residues in a study conducted in Pantnagar.

Table 4: Levels of cyclodienes in buffalo meat

S.No.	Pesticides	Positive Samples			Range		Mean (mg kg^{-1})
		No.	Frequency	Percentage	Minimum	Maximum	
1.	α- Endosulphan	7	7/44	15.9 %	0.003	0.037	**0.016**
2.	β- Endosulphan	2	2/44	4.5 %	0.007	0.017	**0.012**
3.	Endosulphan sulphate	4	4/44	9.0 %	0.002	0.021	**0.015**
4.	∑ Endosulphan						**0.043**
5.	Dieldrin	18	18/44	40.9 %	0.002	0.076	**0.013**

Tyagi (2007) reported levels of α- endosulphan, β- endosulphan and endosulphan sulphate in buffalo muscle as 0.083, BDL and 0.071 mg kg^{-1}, respectively. His figures were higher than the result reported in the present study. However, none of the samples in his study was found to exceed the MRLs established by FAO/WHO.

Pesticide residues in Sheep meat

Organochlorine pesticide residues were present in 88.6 per cent of the mutton samples analyzed. DDT was reported in higher amount than HCH. Total DDT reported was 0.696 mg kg^{-1} and total HCH was 0.151 mg kg^{-1}. Among DDT metabolites, pp' DDT was present in the highest amount. pp' DDE, pp' DDT, pp' DDD, op' DDE, op' DDT residues were detected in 45.4, 56.8, 25.0, 22.7, 4.5 per cent of samples, respectively with respective mean levels of 0.115, 0.256, 0.203, 0.09, 0.032 mg kg^{-1} (Table 5). In sheep meat samples, highest contamination was by DDT (44%), followed by endosulphan (39%), HCH (10%), heptachlor (4%) and dieldrin (3%) (Fig.2).

Mean level of total HCH was 0.151 mg kg^{-1} though was detected in only 3 out of 44 samples. α-, γ-, β- and δ- HCH were detected in 6.8, 4.5, 6.8 and 2.2 per cent of samples, respectively with respective mean levels of 0.031, 0.039, 0.041 and 0.040 mg kg^{-1}(Table 6).

Table 5: Levels of DDT metabolites in sheep meat

S.No.	Pesticides	Positive Samples			Range		Mean (mg kg^{-1})
		No.	Frequency	Percentage	Minimum	Maximum	
1.	pp' DDE	20	20/44	45.45 %	0.004	1.1	**0.115**
2.	pp' DDT	25	25/44	56.81 %	0.010	1.276	**0.256**
3.	pp' DDD	11	11/44	25.00 %	0.053	0.519	**0.203**
4.	op' DDE	10	10/44	22.72 %	0.002	0.022	**0.09**
5.	op' DDT	2	2/44	4.54 %	0.015	0.049	**0.032**
	∑ DDT						**0.696**

Table 6: Levels of HCH isomers in sheep meat

S.No.	Pesticides	Positive Samples			Range		Mean (mg kg^{-1})
		No.	Frequency	Percentage	Minimum	Maximum	
1.	α- HCH	3	3/44	6.81 %	0.01	0.072	**0.031**
2.	γ- HCH	2	2/44	4.54 %	0.034	0.045	**0.039**
3.	β- HCH	3	3/44	6.81 %	0.032	0.058	**0.041**
4.	δ- HCH	1	1/44	2.27 %	0.040	0.040	**0.040**
	∑ HCH						**0.151**

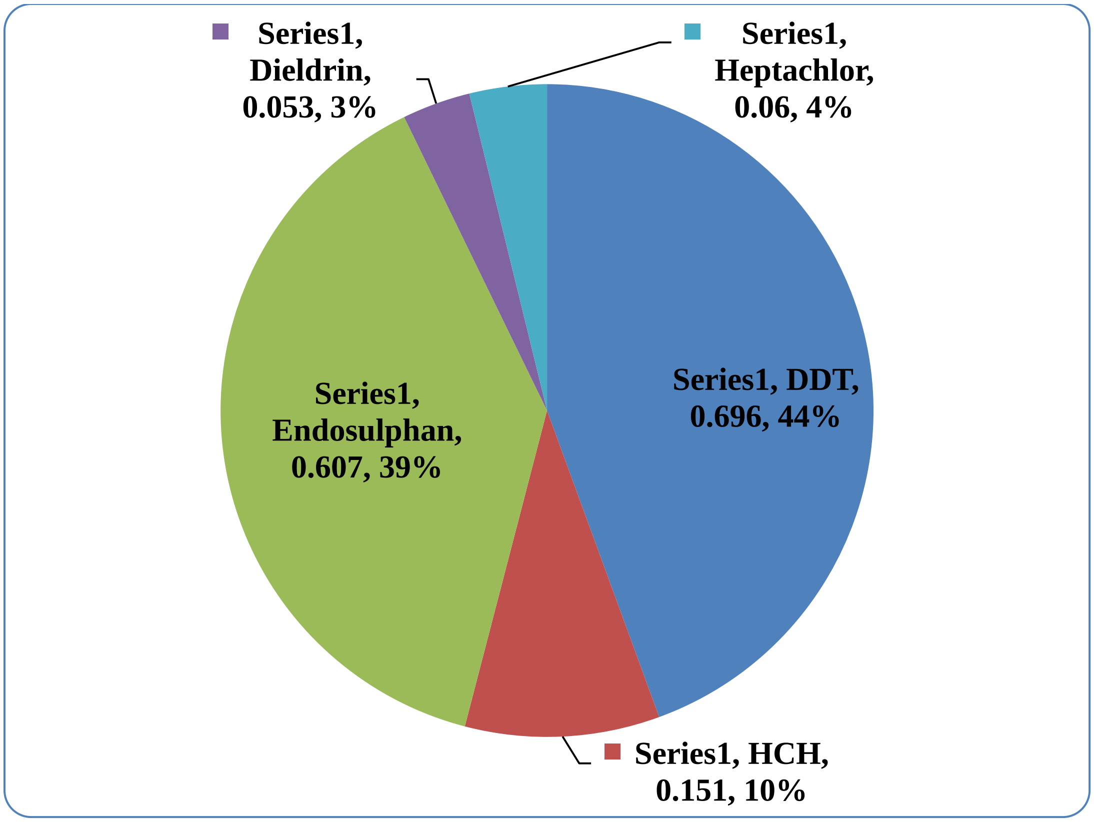

Fig. 2: Proportional contribution of pesticides towards total load in sheep meat

In the present study, mean residue levels of HCH and DDT in meat samples were 0.151 and 0.696 mg kg^{-1}, respectively. Previous studies show that this level was quite high than the levels reported in goat. Tripathi (1996) reported that 96 per cent of samples of goat meat from Pantnagar (Uttrakhand) were contaminated with DDT residues with a mean level of 0.538 mg kg^{-1}. But Seth and Kaphalia (1983) reported 0.21 mg kg^{-1} of HCH in sheep which was quite high than our report.

Kannan *et al* (1992b) reported mean residue levels of HCH, DDT, aldrin, dieldrin and heptachlor in meat samples to be 2.2, 1.0, 0.13, 0.24, 0.004 mg kg^{-1}, respectively. Similarly Bedi *et al* (2003) reported mean residue levels of DDT and HCH in goat muscle samples as 0.159 and 0.137 mg kg^{-1}, respectively. Herrera *et al* (1994) reported values of α, β, γ and ∑ HCH as 0.024, 0.012, 0.076 and 0.112 mg kg^{-1}, respectively in lamb meat. Bedi *et* *al* (2004) reported mean residue levels of DDT and HCH in goat meat samples as 0.462 and 0.359 mg kg^{-1}, respectively. Al-Omar (1985) reported 1.048 mg kg^{-1} of DDT in lamb samples from Baghdad markets. However, the result reported was on fat basis and this might be the probable reason for its high concentration as DDT highly concentrates in fat portion. The main contaminant metabolite of DDT in lamb was pp' DDT followed by pp' DDD, pp' DDE, op' DDE and op' DDT. Metabolic transformation of DDT under oxidative conditions, lead to DDE in animal bodies and thereby account for higher levels (Kannan *et al* 1992a). However, pp' DDT was the predominant analogue present in animals body when animals were dermally treated (Kalra *et al* 1986).

Frank *et al* (1990) reported mean residue levels of DDE and dieldrin as 0.2 and 0.008 mg kg^{-1}, respectively and frequency of occurrence as 43 per cent and 2 per cent, respectively in ovine fat samples. In a recent study made by Shinde and Karim (2009) organochlorine pesticides were not detected at even < 5 ppb level in sheep meat maintained in institute as well as in field flocks of Rajasthan, India. A study conducted in Pantnagar indicated the presence of endosulphan and chlorpyrifos in tissues of sheep and goat (Singh and Kumar 2003).

Animal exposure to HCH can occur through the use of lindane as dipping chemical, to control scab, mostly in sheep (Sumner 1984). However, in India technical HCH has been banned since 1997 and this may be the reason for its low frequency of occurrence in low figures. The residue of pesticides in sheep was reported within the limit largely due to fact that sheep are raised on pastures, which are free from pesticides application during agronomical practices.

Among cyclodiene group of pesticides, heptachlor was found in 34.09 per cent of samples with mean level of 0.060 mg kg^{-1}. Dieldrin was present in only five of the samples with 11.3 per cent occurrence and mean value of 0.053 mg kg^{-1}. Similarly contamination level of α- endosulphan, β- endosulphan and endosulphan sulphate were 0.017, 0.028 and 0.562 mg kg^{-1}, respectively and their frequency of occurrence was 11.3, 9 and 13.6 per cent, respectively (Table 7). In the present study, endosulphan residues were detected in 25 per cent of lamb samples. It may be due to the fact that the restriction imposed on the use of aldrin might have led to shift towards the use of endosulphan. Six samples of sheep meat exceeded MRLs established by Codex Alimentarius Commission for endosulphan sulphate (CAC 1993).

Table 7: Levels of cyclodiene group of pesticides in sheep meat

S.No.	Pesticides	Positive Samples			Range		Mean (mg kg^{-1})
		No.	Frequency	Percentage	Minimum	Maximum	
1.	α- Endosulphan	5	5/44	11.36 %	0.008	0.035	**0.017**
2.	β- Endosulphan	4	4/44	9.09 %	0.005	0.062	**0.028**
3.	Endosulphan Sulphate	6	6/44	13.63 %	0.313	0.812	**0.562**
4.	∑ Endosulphan						**0.607**
4.	Dieldrin	5	5/44	11.36 %	0.0110	0.087	**0.053**
5.	Heptachlor	15	15/44	34.09 %	0.0054	0.600	**0.060**

The presence of restricted cyclodienes viz., dieldrin and heptachlor in sheep meat samples might be due to the fact that fodder and pasture may have been contaminated from dust sprayed on the other major crops or due to translocation from soils and water with the pesticides. Endosulphan sulphate has been reported in high amount and was not detected to this extent in previous works carried out by various workers. In the present study, endosulphan sulphate was there in 6 out of 44 samples accounting for 13.6 per cent frequency of occurrence. This was markedly different to Surendranath *et al* (1998) where he encountered no endosulphan sulphate in any of the samples of goat meat. Tyagi (2007) also reported none of the sheep/goat tissue samples (muscle, liver and kidney) positive for α-endosulphan, β- endosulphan and endosulphan sulphate. Singh and Kumar (2003) mentioned that twelve out of a total of 108 tissue samples of goat carcasses collected from various parts of Uttrakhand showed the presence of endosulphan residues which ranged from 0.010 to 0.528 mg kg^{-1}. In the present study, the maximum level detected for endosulphan sulphate was 0.812 mg kg^{-1} which was in accordance to the level reported by Uttrakhand study.

Pesticide residues in ready to eat meat products

Seven different types of meat products were analyzed for pesticide residues. A total of 30 samples including 3 samples each of sheep patties, buffalo meat ball, buffalo nuggets, buffalo patties, sheep meat balls and sheep nuggets and 12 samples of mutton kababs were screened.

Buffalo Meat Balls: Four DDT metabolites were observed with highest value of pp' DDT as 0.023 mg kg^{-1}. Only β- endosulphan and endosulphan sulphate were detected among cyclodienes with 0.01 mg kg^{-1} of β- endosulphan and 0.216 mg kg^{-1} of endosulphan sulphate. Average levels of DDT and endosulphan in buffalo meat balls were 0.053 and 0.226 mg kg^{-1}, respectively.

Buffalo Nuggets: Among DDT metabolites, op' DDT and pp' DDT were found with equal level of contamination of 0.015 mg kg^{-1}. α- endosulphan which was detected in all the products except in buffalo meat balls was not detected in buffalo nuggets as well. Average levels of DDT and Endosulphan were 0.03 and 0.386 mg kg^{-1}, respectively.

Buffalo Patties: They exhibited contamination by op' DDT, pp' DDE and pp' DDD among DDT metabolites with mean level of total DDT as 0.179 mg kg^{-1} while α-endosulphan had mean levels of 0.16 mg kg^{-1}. Herrera *et al* (1994) detected no DDT residues in fresh sausage of beef, pork and poultry. But samples were contaminated with HCH the levels being 0.041 mg kg^{-1} in fresh beef sausage. Matsumoto *et al* (2006) in their review have mentioned the concentrations of organochlorine pesticides in processed beef meat products collected in Osaka, Japan for 15 years (1990-2004). They have reported the average levels of HCH as 0.00071 mg kg^{-1}, DDT as 0.0022 mg kg^{-1}. Levels of DDT were below than our study. It's worth mentioning that the levels of HCH were far below than the DDT levels. Here too in our study, no HCH was detected in buffalo products.

Sheep Patties: Average levels of DDT and endosulphan in sheep patties were 0.016 and 1.022 mg kg^{-1}, respectively. Level of endosulphan in sheep patties was highest among the products with levels of α- endosulphan, β- endosulphan and endosulphan sulphate as 0.653, 0.039 and 0.33 mg kg^{-1}, respectively. op' DDD and pp' DDD were the only metabolites of DDT present in sheep patties with equal average level of 0.008 mg kg^{-1}.

Sheep Meat Balls: pp' was the only DDT metabolite found in sheep meat balls with average level of 0.015 mg kg^{-1}. Average level of total endosulphan was 0.041 mg kg^{-1} with an average levels of α- and β- endosulphan as 0.02 and 0.021 mg kg^{-1}, respectively.

Sheep Nuggets: They contained no detectable levels of DDT metabolites. Mean level of endosulphan was 0.223 mg kg^{-1} with respective means of α-, β- and endosulphan sulphate as 0.022, 0.011 and 0.19 mg kg^{-1}, respectively.

Mutton Kababs: They revealed highest contamination level of DDT with average being 0.186 mg kg^{-1}. Levels of op' DDT, op' DDE, pp' DDT, pp' DDE and pp DDD were 0.018, 0.004, 0.062, 0.036 and 0.066 mg kg^{-1}, respectively (Table 8). However, levels of op' DDD were not detected in mutton kababs. Levels of α-, β- and endosulphan sulphate were 0.01, 0.016 and 0.347 mg kg^{-1}, respectively with average level of endosulphan as 0.373 mg kg^{-1}. Mutton kababs were the only products with significant level of HCH residues. Levels of α- and β- HCH were 0.029 and 0.06 mg kg^{-1}, respectively with total HCH as 0.089 mg kg^{-1}.

Table 8: Levels of pesticide residues in mutton kababs

S.No.	Pesticides	Positive Samples			Range		Mean (mg kg^{-1})
		No.	Frequency	Percentage	Minimum	Maximum	
1.	op' DDT	4	4/12	33.3 %	0.015	0.022	**0.018**
2.	op' DDE	2	2/12	16.6 %	0.003	0.006	**0.004**
3.	pp' DDE	7	7/12	58.3 %	0.007	0.102	**0.036**
4.	pp' DDD	2	2/12	16.6 %	0.016	0.117	**0.066**
5.	pp' DDT	9	9/12	75.0 %	0.012	0.206	**0.062**
6.	**∑ DDT**						**0.186**
7.	α- HCH	3	3/12	25 %	0.020	0.039	**0.029**
8.	γ- HCH	3	3/12	25%	0.008	0.155	**0.060**
9.	**∑ HCH**						**0.089**
10.	α- Endosulphan	1	1/12	8.3%	0.01	0.01	**0.01**
11.	β- Endosulphan	4	4/12	33.3%	0.008	0.028	**0.016**
12.	Endosulphan Sulphate	2	2/12	16.6%	0.22	0.47	**0.3470**
13.	**∑ Endosulphan**						**0.373**

Pesticide residues in serum of human population

Biological monitoring provides the basis for estimating an internal chemical dose by measuring pesticide and their metabolite compound concentrations in selected tissues, fluids, or bodily wastes, (Woollen 1993). Analysis of blood provides evidence of exposure of the body to pesticides and gives an indication of the body burden of the pesticide residues. Monitoring of OC concentration in blood is most appropriate because these pesticides are lipophilic in nature. Similarly, monitoring OP concentrations in blood or blood products (serum, plasma) offers several advantages. The parent compounds could be monitored directly in blood.

Pesticide residues in serum of human population of Ludhiana, Punjab

Results revealed that the samples contained DDT metabolites, endosulphan and chlorpyrifos with mean levels of 0.019, 0.035 and 0.026 mg l^{-1}, respectively (Table 9). The frequency of occurrence of pp’ DDE, pp’ DDT and pp’ DDD were 64, 10, 10 per cent, respectively, and their average level being 0.006, 0.011. 0.002 mg l^{-1}, respectively, highest being of pp’ DDT. Technical DDT consists primarily of p,p’ DDT and some op’ DDT. In human body, pp’ DDT is slowly dechlorinated to pp’ DDD and pp’ DDE, the latter being even more persistent than the parent compound (WHO 1989). Our results were in variance with several other studies conducted in India. In a study conducted by Mathur *et al* (2005) in Punjab, India they encountered levels of DDT higher than reported in the present study. Level of DDT reported by them was 0.0652 mg l^{-1}. However, their work was concentrated in Bathinda and Ropar districts of Punjab with less number of samples. These districts are surrounded by agricultural fields where pesticide use is greater. Our study was basically based in Ludhiana, an industrial hub of the state. Similarly, in a study conducted at Delhi, India, Saxena *et al* (1987) reported mean levels of DDT as 0.301 mg l^{-1}.

Table 9: Levels of pesticide residues in human population of Ludhiana

S.No.	Pesticides	Positive Samples			Range		Mean (mg l^{-1})
		No.	Frequency	Percentage	Minimum	Maximum	
1.	Chlorpyrifos	8	8/50	16 %	0.001	0.120	**0.026**
2.	pp' DDE	32	32/50	64 %	0.001	0.019	**0.006**
3.	pp' DDT	5	5/50	10 %	0.002	0.028	**0.011**
4.	pp' DDD	5	5/50	10 %	0.001	0.005	**0.002**
5.	**∑ DDT**						**0.019**
6.	α- Endosulphan	27	27/50	54 %	0.001	0.295	**0.026**
7.	Endosulphan Sulphate	3	3/50	6 %	0.005	0.013	**0.009**
8.	**∑ Endosulphan**						**0.035**

Noticeably Paumgartten *et al* (1998) did not find any derivatives of op' DDT, pp' DDT and pp' DDD in samples in Brazil. However, similar to our findings the frequency of occurrence of pp' DDE was 61.5 per cent in the same study. Subramaniam and Solomon (2006) reported range of DDT in serum in India from 0.002 to 0.003 mg l^{-1}. HCH was not encountered in any of the samples. Paumgartten *et al* (1998) also found only β- HCH in their study conducted in Brazil. They detected no α and γ- HCH. β- HCH though is a minor component of technical HCH but is the most persistent HCH isomer in human body (WHO 1992). It has been banned in India with effect from April 1, 1997. Among four isomers only the γ- isomer has powerful insecticidal properties.

Endosulphan was detected at mean levels of 0.035 mg l^{-1} with α endosulphan having frequency occurrence of 54 per cent and mean as 0.026 mg l^{-1}. Endosulphan sulphate, a reaction product found in technical grade endosulphan as a result of oxidation, which is considered to be equally toxic and more persistent than the parent compound was however detected only in 6 per cent of the samples. In study by Mathur *et al* (2005) endosulphan was not detected in any of the human serum samples.

Chlorpyrifos, fourth highest consumed pesticide in India was detected in 16 per cent of the samples collected from Ludhiana at mean levels of 0.026 mg l^{-1}. Mathur *et al* (2005) detected 0.066 mg l^{-1} of chlorpyrifos in Punjab blood samples. Presence of organophosphorus pesticides like chlorpyrifos in blood means that they do persist in the body for a good amount of time. It also indicates its presence in the body in its primary form. It is a moderately persistent pesticide effective against mosquito, fly larvae, cabbage root fly and aphids. It has become one of the most widely applied pesticides in homes and restaurants against cockroaches and termites.

Pesticide residues in serum of Human population of Nepal

As in samples from Ludhiana district, DDT, endosulphan and residues of chlorpyrifos were also detected in samples from Nepal and no residues of HCH were detected in any of the samples (Table 10). Among DDT metabolites pp' DDE and pp' DDT were detected. pp' DDD which was found in the samples from Ludhiana was not encountered at all in samples from Nepal. β- Endosulphan was detected in samples from Nepal but was not detected in any of the samples from Ludhiana. Levels of DDT, endosulphan and chlorpyrifos were 0.079, 0.098, 0.245 mg l^{-1}, respectively. Levels of pp' DDE and pp' DDT were 0.005 and 0.074 mg l^{-1}, respectively. Among endosulphan residues levels of α- endosulphan, β- endosulphan and endosulphan sulphate were 0.034, 0.023 and 0.041 mg l^{-1}, respectively. The most prevalent organochlorine pesticide residues found in human tissues are DDE, the major metabolite of DDT, and PCB's (Longnecker *et al* 1997).

Table 10: Levels of pesticide residues in human population of Nepal

S.No.	Pesticides	Positive Samples			Range		Mean (mg l^{-1})
		No.	Frequency	Percentage	Minimum	Maximum	
1.	Chlorpyrifos	6	6/50	12 %	0.014	0.440	**0.245**
2.	pp' DDE	22	22/50	44 %	0.001	0.018	**0.005**
3.	pp' DDT	4	4/50	8 %	0.002	0.149	**0.074**
4.	**∑ DDT**						**0.079**
5.	α- Endosulphan	18	18/50	36 %	0.007	0.079	**0.034**
6.	β- Endosulphan	4	4/50	8 %	0.02	0.03	**0.023**
7.	Endosulphan Sulphate	4	4/50	8 %	0.028	0.054	**0.041**
8.	**∑ Endosulphan**						**0.098**

Distribution of pesticides based on the basis of sex, age, food habits and rural/ urban habitation did not clearly indicate any pattern. However, 55.1 per cent of female population were carrying pesticide load and among male subjects 93.5 per cent of them were afflicted with various pesticides.

In comparison to Ludhiana, amount of pesticides in school children of Nepal were excessively high. The probable reason might be that school children of Nepal have been continuously exposed to the pesticides since their birth as the warehouse which is located at the premises of the school and the local village has been housing 50.9 MT of obsolete pesticides since 1975. Out of those 50.9 MT, amount of organophosphates and organochlorines were 8.10 and 35.40 MT, respectively while organomercurials contributed 7.40 MT (Mainali 2003). Water, air, dust and soil might be the major sources of these pesticides in those school children of Nepal as pesticides were being continuously leaked from the warehouse and making their way into the environment. Recurrent problems like nausea, vomition etc during wind blowing were reported by the students. There is a great need to analyze the water and soil samples from the area to know the pesticides load in them.

CHAPTER-V

SUMMARY

Foods of animal origin are one of the major sources of portal of entry of pesticides in human body. There are national and international regulatory bodies to monitor contamination in foods including meat and meat products. Very little work has been done regarding the pesticide residues in buffalo and sheep meat and their products in India. In the absence of authentic reports, it is difficult to know the spectrum of residue levels in those commodities. In the wake of liberalization and globalization of the markets, pesticide residues in food assume importance with special reference to codex and sanitary and phytosanitary (SPS) measures. This study was conducted to review the actual residue data in meats of buffalo and sheep, ready to eat meat products available in the market and in human sera samples so as to ascertain a realistic exposure.

In the present study, 44 samples each of buffalo and sheep meat were collected from the local market of Ludhiana and from a slaughter house located at Derabassi, respectively. Most of the samples were contaminated with pesticides. Contamination level was 90.9 per cent for buffalo meat and 88.6 per cent for sheep meat. However, 100 per cent contamination was observed for ready to cook buffalo and mutton products. None of them exceeded national MRLs except, 2 buffalo meat samples which exceeded levels of β- HCH established by FAO/WHO.

DDT levels were found to be highest in sheep meat followed by buffalo meat and were 0.696 and 0.308 mg kg^{-1}, respectively. Levels of DDT, HCH, endosulphan and dieldrin were all high in buffalo meat samples when compared to samples from sheep. Besides, sheep samples also contained residues of heptachlor. Levels of DDT, HCH, endosulphan and dieldrin in samples of buffalo were 0.308, 0.094, 0.308, and 0.013, mg kg^{-1}, respectively. Similarly the levels of DDT, HCH, endosulphan and dieldrin in sheep meat were 0.698, 0.151, 0.607 and 0.053 mg kg^{-1}, respectively. Level of Heptachlor in sheep meat was 0.06 mg kg^{-1}. Of the total contamination in buffalo meat highest was by endosulphan (43%), followed by DDT (42%) and then by HCH (13%) and dieldrin (2%). Whereas in sheep meat samples, highest contamination was by DDT (44 %), followed by endosulphan (39 %), HCH (10%), heptachlor (4%) and dieldrin (3%). Six samples of sheep meat exceeded MRLs established by CAC for endosulphan sulphate (CAC 1993).

Seven different kinds of products viz., buffalo meat balls, buffalo nuggets, buffalo patties, sheep patties, sheep meat balls, sheep nuggets and mutton kababs were monitored for pesticide residues. DDT and endosulphan residues were found in all kinds of products where as HCH were only encountered in samples of sheep meat balls. DDT was highest in mutton kababs followed by buffalo patties with an average of 0.186 and 0.179 mg kg^{-1}, respectively. Similarly highest amount of endosulphan was found in sheep patties with average as 1.022 mg kg^{-1}.

To assess the levels of pesticides in blood of human population, 50 samples each from Ludhiana and Nepal were analyzed. Residues of DDT, endosulphan and chlorpyrifos were found in samples from Ludhiana as well as in samples from Nepal. Among DDT metabolites only pp' DDE and pp' DDT were found in samples from Nepal while in Ludhiana samples besides these two metabolites pp' DDD was also found with an average level of 0.002 mg l^{-1}. Levels of all pesticides were high in samples from Nepal as compared to human population of Ludhiana. Levels of DDT in human population of Nepal and Ludhiana were 0.079 and 0.019 mg l^{-1}, respectively. Similarly average values of endosulphan and chlorpyrifos in Nepal were 0.098 and 0.245 mg l^{-1}, respectively whereas the values of endosulphan and chlorpyrifos in Ludhiana were 0.035 and 0.026 mg l^{-1}, respectively. Air, water and soil are thought to be a main source of portal of entry of pesticides into human body besides food as the levels of pesticides were found to be excessively high in children from Nepal as a warehouse was being accommodated at one the of the premises of the school with 50.9 MT of obsolete pesticides since 1975.

Conclusions

1. Most of the samples of meat and meat products were contaminated with pesticides (Buffalo meat: 90.9 per cent, Sheep meat: 88.6 per cent, meat products: 100 per cent).
2. Human population of Ludhiana and Nepal were carrying the burden of pesticides at frequency occurrence of 96 and 94 per cent, respectively. The presence of pesticides in human population indicated that humans were still carrying the load of pesticides which were banned long ago.
3. The levels of pesticides were very low in amount than the earlier reports. None of the samples exceeded national MRL, however, 2 buffalo meat samples exceeded levels of β HCH established by FAO/WHO, one buffalo meat samples exceeded MRLs for lindane and six sheep meat samples exceeded MRLs for endosulphan sulphate set by Codex Alimentarius Commission.

REFERENCES

Agarwal H C, Pillai M 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 Organi*zation **54**: 349-51.

Agnihotri N P. 2000. Pesticide consumption in agriculture in India-an update. *Pesticide Residue Journal* **12**: 150-55.

Al-Omar M, AI-Bassomy M, AI-Ogaily N and Shebi D A. 1985. Residue levels of organochlorine insecticides in lamb and beef from Baghdad. *Bulletin of Environmental Contamination and Toxicology* **34**: 509-12.

Au W W, Sierra Torres C H, Cajas Salazar N, Shipp B K and Legator M S. 1999. Cytogenetic effects from exposure to mixed pesticides and the influence from genetic susceptibility. *Environmental Health Perspectives* **107**: 501-05.

Aulakh R S, Joia B S, Gill J P S, Bedi J S, Pooni P A and Sharma J K. 2003. Occurecne of cyclodiene insecticide residues in human biopsy adipose tissues in Punjab, India. *Indian Journal of Environmental Toxicology* **13**: 75-79.

Battu R S, Gupta S C, Chawla R P and Kalra R L. 1984. Residues of DDT and BHC in market samples of meat of various animals. *Indian Journal of Ecology* **2**: 177-82.

Bedi J S, Gill J P S, Aulakh R S and Sharma J K. 2003. Presence of cyclodiene group of insecticides in goat tissues. *Journal of Veterinary Public Health* **1**: 31-35.

Bedi J S, Gill J P S, Aulakh R S, Joia B S and Sharma J K. 2004. Thermostability of organochlorine pesticides in chevon. *Toxicology International* **11**: 19-22.

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.

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 Ahemdabad, India. *Bulletin of Environmental Contamination and Toxicology* **72**: 261-65.

Bhatnagar V K, Patel S, Variya M R, Venkaih M P and Kashyap S K. 1992. Levels of Organochlorines in human blood from Ahmedabad (Rural), India. *Bulletin of Environmental Contamination and Toxicology* **48**: 303-07.

Brown J R and Chow L Y. 1975. Comparative study of DDT and its derivatives in human blood samples in Norfolk county and Holland Marsh, Ontario. *Bulletin of Environmental Contamination and Toxicology* **13**: 483-88.

CAC. 1993. Joint FAO/WHO food standards programme, Codex Alimentarius Commission, pesticide residues in food. Vol 2 pp. 475. Food and Agriculture Organization of the United Nation, World Health Organization, Rome, Italy.

Chan M P L, Mustafa A M, Hussein R, Hussain S H, Zulkifli S N and Abdullah A R. 2004. Pesticides residues in blood of school children from selected schools in Peninsular Malaysia. *Environmental Health Focus* **2**: 18-24.

Corrigan P J and Seneviratna. 1990. Occurrence of organochlorine residues in Australian meat. *Australian Veterinary Journal* **67**: 56-58.

Dallaire F, Dewailley E, Muckle G and Pierre A. 2003. Time trends of persistent organic pollutants and heavy metal in umbilical cord blood of intuit infants in Nunavik (Quebec, Canada) between 1999 and 2001. *Environmental Health Perspectives* **111**: 1660-64.

Darko G and Acquaah S O. 2007. Levels of organochlorine pesticides residues in meat. *International Journal of Environmental Science and Technology* **4**: 521-24.

Doguchi M and Fukano S. 1975. Residue levels of polychlorinated terphenyls, polychlorinated biphenyls and DDT in human blood. *Bulletin of Environmental Contamination and Toxicology* **13**: 57-63.

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

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

Ekbom A, Wicklund-Glynn A and Adami H O. 1996. DDT and testicular cancer. *Lancet* **347:** 553-54.

Fleming L, Mann J B, Briggle T and Sanchez-Ramas J R. 1994. Parkinson's disease and brain levels of organochlorine pesticides. *Annals of Neurology* **36**: 100-03.

Frank R, Braun H E and Thorpe P. 1993. Comparison of DDE and PCB residues in the general diet and in human blood – Ontario 1986-87. *Bulletin of Environmental Contamination and Toxicology* **51**: 146-52.

Frank R, Braun H E, Stonefield K I, Rasper J and Luyken H. 1990. Organochlorine and organophosphorus residues in the fat of domestic farm animals, Ontario, Canada 1986- 1988. *Food Additives and Contaminants* **7**: 629-36.

Gill U S, Schwartz H M and Wheatley B. 1996. Development of a method for the analysis of PCB congeners and organochlorine pesticides in blood/ serum. *Chemosphere* **32**: 1055-61.

Glynn A W, Wernroth L, Atuma S, Linder C E, Aune M, Nilsson I and Darnerud P O. 2000. PCB and chlorinated pesticide concentrations in swine and bovine adipose tissue in Sweden 1991-1997: spatial and temporal trends. *Science of the Total Environment* **246**: 195-206.

Glynn A, Aune M, Darnerud PO, Cnattinggius S, Bjerselius R, Becker W and Lignell. 2007. Determinants of serum concentrations of organochlorine compounds in Swedish pregnant women: a cross sectional study. *Environmental Health* **6**: 2.

Gupta P K. 2004. Pesticide exposure: Indian scene. *Toxicology* **198**: 1-3.

Hansen G C and Lambert R J. 1987. Transfer of toxic trace substances by way of food animals: selected examples. *Journal of Environmental Quality* **16**: 200-05.

Herrera A, Arino A A, Conchello M P, Lazaro R, Bayarri S and Peres C. 1994. Organochlorine pesticide residues in Spanish meat products and meat of different species. *Journal of Food Protection* **57**: 441-44.

http://www.indiainbusiness.nic.in

http://www.indiatogether.org/2005/jun/agr-bloodcide.htm

http://www.punenvis.nic.in/database_agri19.htm

Hundal B S, Anand and Singh R. 2006. Pesticide marketing: the Indian scenario. *The Icfai University Journal of Managerial Economics* **4**: 32-37.

Kalra R L and Chawla R P. 1980. Studies of pesticide residues and monitoring of pesticide pollution. Final Technical Report PL 480 project. Department of Entomology, PAU, Ludhiana.

Kalra R L, Chawla R P, Joia B S and Tiwana M S. 1986. Excretion of DDT residues into milk of the Indian buffalo, *Bubalus bubalus* (L.) after oral and dermal exposure. *Pesticide Science* **17**: 128-34.

Kanja L W, Skaare J U, Ojwang S B 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 Environmental Contamination and Toxicology* **22**: 21-24.

Kannan K, Tanabe S, Quynh H T, Hue N D and Tatsukawa R. 1992a. Residue pattern and dietary intake of persistent organochlorine compounds in foodstuffs from Vietnam. *Bulletin of Environmental Contamination and Toxicology* **22**: 367-74.

Kannan K, Tanabe S, Ramesh A, Subramanian A and Tatsukawa R. 1992b. Persistent organochlorine residues in food stuffs from India and their implication on human dietary exposure. *Journal of Agricultural and Food Chemistry* **40**: 518-24.

Kaphalia B S and Seth T D. 1983. Chlorinated pesticide residues in blood plasma and adipose tissue of normal and exposed human population. *Indian Journal of Medical Research* **77**: 245-47.

Kaphalia B S, Siddiqui F S and Seth T D. 1985. Contamination levels in different food items and dietary intake of chlorinated pesticide residues in India. *Indian Journal of Medical Research* **81**: 71-78.

Kasai A, Asanama S, Sasaki K, Sazaki A, Kurosawa K and Abe E. 1979. Studies on pesticide residues in human body and meat. *Nippon Nosan Igakkaizesshi* **28**: 480-81. (Abstr.)

Khalafalla F A, Awad H A and Gergis A F. 1993. Organochlorine pesticide residues in meat and edible offals. *Veterinary Medical Journal Giza* **41**: 47-51.

Kipcic D, Vukusic J and Sebecic B. 2001. Monitoring of chlorinated hydrocarbon pollution of meat and fish in Croatia. *Food Technology and Biotechnology* **40**: 39-47.

Krawinkel M B, Plehn G, Kruse H and Kasi A M. 1989. Organochlorine residues in Baluchistan/Pakistan: Blood and fat concentrations on human. *Bulletin of Environmental Contamination and Toxicology* **43**: 821-26.

Kulkarni A P and Mitra A. 1990. Pesticide contamination of food in the United States. Food contamination from environmental sources. Vol 23 pp.257-93. Wiley Series on advance in environmental Science and Technology. New York, John Wiely and Sons,Inc.

Laden F, Neas L M, Spiegelman D, Hankinson S E, Willett W C, Ireland K, Wolff M S and Hunter D J. 1999. Predictors of plasma concentrations of DDE and PCBs in a group of US women. *Environmental Health Perspectives* **107**: 75-81.

Lazaro R, Herrera A, Arino A, Conchello M P and Bayarri S. 1996. Organochlorine pesticide residues in total diet samples from Aragon (Northeastern Spain). *Journal of Agricultural Food Chemistry* **44**: 2742-47.

Lazaro R, Herrera A, Conchello M P and Arino A. 1991. Contamination by organochlorine residues in fresh sausages. *Anales de Bromatologia* **43**: 373-82.

Li J Y, Zhou W D, Lei F M, Zeng H Y, Li S F and Wu D S. 2007. Study on the relationship between organochlorine pesticides and level of residues in serm and daily foodstuff. *Zhonghua Liu Xing Bing Xue Za Zhi.* **28**: 123-26.

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

Longnecker M P, Rogan W J and Lucier G. 1997. The human health effects of DDT (dichlorodiphenyl-trichloroethane) and PCBs (polychlorinated biphenyls) and an overview of organochlorines in public health. *Annual Review of Public Health* **18**: 211-44.

Mainali M. 2003. Greenpeace Briefing. Obsolete pesticides in the Himalayas, Greenpeace calls on Bayer and Shell to clean up Nepal. Obsolete Pesticides- A Global Poblem. Politics in Poison. (Eds) The Press Unlimited, Kathmandu, Nepal.

Manirakiza P, Akimbamijo O, Covaci A, Adrediran A, Cisse I, Fall S T and Schepens P. 2002. Persistent chlorinated pesticides in fish and cattle fat and their implications for human serum concentrations from the Sene-Gambian region. *Journal of Environmental Monitoring* **4**: 609-17.

Mathur H B, Agarwal H C, Johnson S and Saikia N. 2005. Analysis of Pesticide Residues in blood samples from villages of Punjab. Report. pp 1-21. Centre For Science and Environment, New Delhi, India.

Matsumoto H, Kuwabara K, Murakami Y and Murata H. 2006. Survey of PCB and organochlorine pesticide residues in meats and processed meat products collected in Osaka, Japan. *Shokuhin Eiseigaku Zasshi* **47**: 127-35.

Mes J. 1992. Organochlorine residues in human blood and biopsy fat and their relationship. *Bulletin of Environmental Contamination and Toxicology* **48**: 815-20.

Mills P A, Bong B A, Kamps La R and Burke J A. 1972. Elution solvent system for florisil column cleanup in organochlorine pesticide residue analyses. *Journal of Association of Official Analytical Chemists* **55:** 39-43.

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 Toxicology* **56**: 58-64.

Naqvi S N H and Jahan M. 1999. Pesticide Residues in serum and blood samples of the people of Karachi. *Journal of Environmental Biology* **20**: 241-44.

Ntow W J, Tagor 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**: 17-26.

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 S R, Delgado I F, Oliveira E S, Alleluia I B, Barretto H H C and Kussumi T A. 1998. Levels of organochlorine pesticides in the blood serum of agricultural workers from Rio de Janeiro state, Brazil. *Cadernos de Saude Publica* **14**: 33-39.

Ramachandran M, Banerjee B D, Gulati, M, Grover A, Zaidi S S A and Hussain Q Z. 1984. DDT and HCH residues in the body fat and blood samples from Delhi hospitals. *Indian Journal of Medical Research* **80**: 590-93.

Rathore H S, Saxena S K and Begum T. 1996. Handbook of Food Analysis. Vol 2 pp. 1382. Marcel Dekker, New York.

Rodas-Ortiz J P, Ceja-Moreno V, Gonzalez-Navarrete R L, Alvarado- Mejia J, Rodriguez-Hernandez M E and Gold-Bouchot G. 2008. Organochlorine pesticides and polychlorinated biphenyls levels in human milk from Chelem, Yucatan, Mexico. *Bulletin of Environmental Contamination and Toxicology* **80:** 255-59.

Sandhu T K. 1992. Pesticides in Food. *Proceedings of 3rd World Congress on Food Borne Infection and Intoxication*. pp. 710-40. Berlin, Germany.

Saschenbrecker P W. 1976. Levels of terminal pesticide residues in Canadian meat. *The Canadian Veterinary Journal* **17**: 158-63.

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

Schafer K S, Reeves M, Spitzer S and Kegley S. 2004. In Chemical trespass: Pesticides in our bodies and corporate accountability, Pesticide Action Network North America. Available at http://www.panna.org.

Scheele J, Teufel M and Niessen K H. 1996. Chlorintaed hydrocarbons in human bone marrow of healthy individuals and leukemia patients. *Archives of Environmental Health* **51**: 22-25.

Seth T D and Kaphalia B S. 1983. Residues levels of DDT in the body tissues of sheep. *Journal of Environmental Biology* **4**: 95-97.

Shinde A K and Karim S A. 2009. Screening of pesticide residues in meat and milk samples of sheep maintained in institute and field flocks. *Indian Journal of Small Ruminants.* **15**: 92-94.

Singh K, Malik A and Sinha S. 2007. Persistent organochlorine pesticide residues in soil and surface water of northern Indo-Gangetic Alluvial Plains. *Environmental Monitoring and Assessment* **125**: 147-55.

Singh S P and Kumar M. 2003. Status of pesticide residues in foods of animal origin in India. *Proceedings of IAVPHS 2nd Annual Conference and National Symposium on Challenges in Emerging WTO Scenario on Milk, Meat and Poultry Industry.* Sept. 20-22, Nagpur, India. pp. 51-54.

Stehr- Green P A, Farrar J A, Burse V W, Royce W G and Wohlleb J C. 1988. A survey of measured levels and dietary sources of selected organochlorine pesticide residues and metabolites in human sera from a rural population. *American Journal of Public Health* **78**: 828-30.

Stellman S D, Djordjevic M V, Muscat J E, Gong L, Bernstein D, Citron M L, White A, Kemeny M, Busch E and Nafziger A N. 1998. Relative abundance of organochlorine pesticides and polychlorinated biphenyls in adipose tissue and serum of women in Long Island, New York. *Cancer Epidemiology Biomarkers and Prevention* **7**: 489-96.

Straube E, Straube W, Kruger E, Bradatsch M, Jacob Meisel M, Rose H J, Leng G and Hadnagy W. 1999. Disruption of male sex hormones with regard to pesticides; pathophysiological and regulatory aspects. *Toxicology Letters* **107**: 225-31.

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

Sumner E R. 1984. Sheep meat exports to France. *The Veterinary Record* **9**: 254.

Surendranath B, Unnikrishnan V, Gayathri V, Chitra P S, Preeja C N and Murthy M K R. 1998. Organochlorine pesticide residues in animal tissues and their excretion through milk. *Journal of Food Science and Technology* **53**: 547-48.

Tanabe S, Kannan K, Tabucanon M S, Siriwong C, Ambe Y and Tatsukawa R. 1991. Organochlorine pesticide and polychlorinated biphenyl residues in foodstuffs from Bangkok, Thailand. *Environmental Pollution* **72**: 191-203.

Tripathi H C. 1996. 'Organochlorine insecticide residues in agriculture and animal products in Terai area.' M. Sc. Thesis, Uttar Pradesh Agriculture University, Pantnagar.

Tyagi A. 2007. 'Detection and quantification of chlorpyrifos and Endosulphan residues in dead animals using HPLC.' MVSc Thesis, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar.

Wadhwa B K, Sharma V and Sharma V. 2004. Pesticide residues in food products: consequences and future strategies. *Indian Dairyman* **56**: 33-37.

Waliszewski S M, Aguirre A A, Benitez A, Infanzon R M, Infanzon R and Rivera J. 1999. Organochlorine pesticide residues in human blood serum of inhabitants of Veracruz, Mexico. *Bulletin of Environmental Contamination and Toxicology* **62**: 397-402.

Waliszewski S M, Aguirre A A, Infanzon R M, and Siliceo J. 2002. Persistent organochlorine pesticide levels in maternal blood serum, colostrum and mature milk. *Bulletin of Environmental Contamination and Toxicology* **68**: 324-31.

Waliszewski S M, Carvajal O, Infanzon R M, Arroyo S G, Pietrini R V, Carvajal O, Trujillo P and Hayward- Jones P M. 2005. Persistent organochlorine pesticides levels in blood serum lipids in women bearing babies with undescended testis. *Bulletin of Environmental Contamination and Toxicology* **75**: 952-59.

Waliszewski S M, Carvajal O, Infanzon R M, Trujillo P and Hart M M. 2004. Co partition ratios of persistent organochlorines pesticides between adipose tissue and blood serum lipids. *Bulletin of Environmental Contamination and Toxicology* **73**: 732-38.

Waliszewski S M and Szymczynski G A. 1991. Persistent organochlorine pesticide in blood serum and whole blood. *Bulletin of Environmental Contamination and Toxicology* **46**: 803-09.

WHO. 1989. DDT and its derivatives: environmental aspects. Vol 83 Environmenral Health Criteria pp 98. UNEP, ILO and WHO.

WHO. 1992. Alpha- and beta- hexachlorocyclohexnes. Vol 123 Environmenral Health Criteria pp170. UNEP, ILO and WHO.

Whyatt R M, Barr D B, Camann D E, Kinney P L, Barr J R, Andrews H F, Hoepner L A, Garfinkel R, Hazi Y, Reyes A, RamirezJ, Cosme and Perera F P. 2003. Contemporary- Use Pesticides in personal air samples during pregnancy and blood samples at delivery among urban minority mothers and new borns. *Environmental Health Perspectives* **111**: 749-56.

Woollen B H. 1993. Biological monitoring for pesticide absorption. *The Annals of Occupational Hygiene* **37**: 525-40.

WWF. 2004. Bad Blood? A survey of chemicals in the blood of European ministers. (original not seen. Cited by Mathur H B, Agarwal H C, Johnson S and Saikia N. 2005. Analysis of Pesticide Residues in blood samples from villages of Punjab. Report. pp 1-21. Centre For Science and Environment, New Delhi, India.)

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