CSE/PML/PR-21/2005

Analysis Of Pesticide Residues In Blood Samples From Villages Of Punjab

INVESTIGATORS

Prof. H. B. Mathur
Prof. H. C. Agarwal
Dr. Sapna Johnson
Dr. Nirmali Saikia

DATE
March, 2005

CENTRE FOR SCIENCE AND ENVIRONMENT
41, Tughlakabad Institutional Area, New Delhi 110062
Website: www.downtoearth.org.in; Email: cse@cseindia.org

POLLUTION MONITORING LABORATORY
India Habitat Centre, Core 6A, Fourth Floor
Lodhi Road, New Delhi - 110003

CONTENTS

1. ABOUT CSE LABORATORY

The Centre for Science and Environment, a non-governmental organisation based in New Delhi, has set up the Pollution Monitoring Laboratory (PML) to monitor environmental pollution. Its main aim is to undertake scientific studies to create public awareness about food, water, air and other environmental contamination. It is equipped with state of art equipments for the monitoring and analysis of air, water, food and other environmental contamination, including High Performance Liquid Chromatograph (HPLC), Gas Chromatograph (GC) with Electron capture detector (ECD), Nitrogen Phosphorus Detector (NPD), Flame ionization detector (FID) and other detectors, UV-VIS Spectrophotometer, Mercury Analyzer, Respirable Dust Sampler etc. It provides scientific services at nominal cost to communities that cannot obtain scientific evidence against polluters in their area. Given the state of scientific research in India --most of it being restricted to national defense and food security -- this is an effort to use science to achieve ecological security.

2. INTRODUCTION AND ORIGIN OF THE STUDY

Pesticide is a general term for substances, which are used to poison pests (weeds, insects, molds, rodents etc.). The pesticides most acutely dangerous to humans are insecticides and rodenticides. Synthetic pesticides have been popular with farmers, because of their widespread availability, simplicity in application, efficacy and economic returns. But they also have huge environmental costs. After India's Green Revolution started, the consumption of pesticides in India has increased several hundred folds, from 154 MT in 1954 to 88,000 MT in 2000-2001. According to industry estimates, the pesticide use has high growth potential in India, as the use of agricultural pesticides is markedly low at 0.54 kg /ha as against 3.7 kg/ha in USA and 2.7 kg/ha in Europe. Notwithstanding the fact that overall consumption of pesticides in India as a whole is low than that used in the developed countries of the world, there is still a widespread contamination of water, soil and air with pesticide residues. In India, among different states maximum consumption of pesticides-1999-2000 was in Uttar Pradesh (7459 MT) followed by Punjab (6972 MT), Haryana (5025 MT), Andhra Pradesh (4054 MT), Gujarat (3646 MT). Leading pesticides used in India include monocrotophos (10700 MT- highest consumed), acephate (6400MT), endosulfan (5600 MT) and chlorpyrifos (5000 MT - fourth highest consumed). (Source: Pesticide Information, Volume XXVIII, No. 3, October- December 2002).

About 54% of the total pesticides used in Indian agriculture are consumed on cotton alone, though it accounts for only 5% of the total cultivated area (Puri *et al*, 1999) and nearly 20-25 per cent are used for the control of sucking pests and bollworm. Pesticides have become integral part of villagers in Punjab. Bhatinda district in Punjab, an important cotton belt of the country irrigated by canal water grows largely cotton and rice crop –the two crops known for excessive use of pesticides. The Punjab Agricultural University at Ludhiana recommends only seven sprays on cotton in six months, but farmers in Bhatinda spray as many as 32 times. (www.indiatogether.org). Many people are using empty containers of pesticide for storing most of the food items. (Source: An epidemiological study of cancer cases reported from villages of Talwandi Sabo block district Bhatinda, Punjab, Final Report, Punjab Pollution Control Board, Patiala). Several studies have shown pesticide residues in breast milk (Kalra *et al*, 1994), bovine milk (Kalra *et al*, 1999), fruits and vegetables from Punjab and a few reports of high incidence of cancer have been coming from certain areas of Punjab since last few years. An epidemiological study was conducted in villages of Talwandi Sabo block, district Bhatinda by the Chandigarh based Post Graduate Institute of Medical Education and Research (PGIMER) on behalf of the Punjab Government to assess whether cancer cases are higher in these areas. A total population of 183243 consisting of 39732 families in 129 villages- a

population of 85315 in 36 villages of Talwandi Sabo block of Bhatinda district and a reference population of 97928 in 93 villages of Chamkaur Sahib block of Roop Nagar district - was surveyed. A total of 7441 deaths were recorded which occurred in last 10 years (1993-2003). Age adjusted cancer death rate per 1,00,000 population per year at Talwandi Sahib was 51.2 while in control area Chamkaur Sahib it was 30.3. Age adjusted prevalence of confirmed cancer cases was 103 per lakh at Talwandi Saboo and 71 per lakh in Chamkaur Sahib. Cancer of female reproductive system, i.e. breast, uterus/cervix and ovary were more common in Talwandi sabo whereas cancer of blood and lymphatic system, esophagus and bones were more common in Chamkaur Sahib block.(Source: An epidemiological study of cancer cases reported from villages of Talwandi Sabo block district Bhatinda, Punjab, Final Report, Punjab Pollution Control Board, Patiala)

Not many studies have been carried out to confirm that pesticides are responsible for various incidences of cancer and other diseases in Punjab but the research worldwide has shown that pesticides do produce these effects. Biological monitoring provides the basis for estimating an internal chemical doze by measuring pesticide and their metabolite compound concentrations in selected tissues, fluids, or bodily waste (feces and or urine) (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 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 can be monitored directly in blood products instead of their metabolites, which are usually measured in urine. Blood measurements provide an estimation of the dose available for the target site, allowing for prediction of dose-response relationships. Furthermore, because blood is a regulated fluid (the volume does not vary substantially with water intake or other factors), the blood concentrations of toxicants measured at a specific time interval after exposure will remain the same as long as the absorbed amounts are constant; therfore no corrections for dilution are necessary. (Wessels *et al* , 2003)

PML scientists visited Punjab and collected blood samples from 20 randomly selected people from 4 different villages of Punjab - Mahi Nangal, Jajjal and Balloh in Bhatinda district and Dher in Ropar district. Agricultural fields surrounded these villages and pesticide use was evident. The blood samples were analyzed with a widely and internationally used methodology based on United States Environment Protection Agency (USEPA) protocols for organochlorine pesticides with Electron Capture Detector and organophosphorus pesticide with Nitrogen Phosphorus detector using a capillary column. Results are presented in this report.

3. LITERATURE REVIEW

3.1 Blood

Blood is composed of a yellowish fluid, called plasma (55%), in which are suspended millions of cells that constitute 45% by volume of whole blood. In an average adult, the volume of the blood is about one –eleventh of the body weight and the volume of blood in an average adult is 4.7-5.0 litres. (http://hypertextbook.com/facts/1998/LanNaLee.shtml). The density of blood plasma is 1.025 g/ml and density of blood cells circulating in the blood is 1.125 g/ml. Blood plasma and its content is known as whole blood. The average density of whole blood for a human is 1.060 g/ml. Whole blood contains on an average about 0.5% lipid. (Covaci *et al*., 2002). Plasma is the straw-colored liquid in which the blood cells are suspended composed of water (90-92%), proteins (6-8%), salts (0.8%), lipids (0.6%) and glucose-blood sugar (0.1%) (http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/B/Blood.html). Serum lipids (0.6%) include High Density Lipoprotein cholesterol, Low Density Lipoprotein cholesterol and 20% of the triglyceride value. Plasma transports materials needed by cells and materials that must

be removed from cells, various ions (Na^+, Ca^{2+}, HCO_3 , etc.) glucose and traces of other sugars, amino acids, other organic acids, cholesterol and other lipids, hormones, urea and other wastes. Serum proteins make up 6–8% of the blood. They are about equally divided between serum albumin and a great variety of serum globulins. After blood is withdrawn from a vein and allowed to clot, the clot slowly shrinks. As it does so, a clear fluid called serum is squeezed out. Serum is blood plasma without fibrinogen and other clotting factors.

Suspended in the watery plasma are seven types of cells and cell fragments-red blood cells (RBCs) or erythrocytes, platelets or thrombocytes, five kinds of white blood cells (WBCs) or leukocytes, three kinds of granulocytes –neutrophils, eosinophils and basophils and two kinds of leukocytes without granules in their cytoplasm – lymphocytes and monocytes

3.2 Pesticide Residues in blood

Across the globe pesticides have been found in human blood, urine, breast milk, semen, adipose tissue, amniotic fluid, infant meconium and umbilical cord blood. Cumulative exposure to pesticides may come from food, water, air, dust, soil etc. Pesticides can be absorbed through skin contact, inhalation or accidental ingestion. Farm workers come into direct contact with pesticides at work as well and are occupationally exposed to them. When a person is exposed to pesticides, body's detoxification mechanisms are activated. Some pesticides are metabolized into different chemicals and excreted and some are stored in fatty tissues in the body. Body burden data from analysis of blood provides evidence of exposure to chemicals stored in our body. There are several studies - international and national on pesticide residues found in blood samples.

3.2.1 International reports

A study from Ontario in which 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 the country 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 ppm respectively and 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 ppm in Norfolk County and 0.016 ppm in Holland Marsh and in 26 persons exposed during formulation of DDT preparations was 0.063 ppm (Brown and Chow, 1975).

Analysis of 27 samples of human whole blood of 19 males and 8 females from 21 to 57 year old, in Tokyo Metropolitan Research Laboratory of Public Health for polychlorinated terphenyls (PCTs), polychlorinated biphenyls (PCBs) and DDE showed a mean value of 3.2, 5.0 and 11.2 ppb respectively (Doguchi and Fukano, 1975).

A total of 41 samples of maternal blood, milk subcutaneous fat and umbilical cord blood were analysed 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 fat) of t-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. The mean levels of beta-hexachlorocyclohexane (β-HCH) in subcutaneous fat and milk fat were 0.034 and 0.26 mg/kg fat, respectively. (Kanja *et al* , 1992)

Blood samples of 135 residents living near the estuary of the Elb river (Schleswig-Holstein, Germany) were analysed for organochlorines (e. g, β-HCH- 0.5-22.9 ng/ml), benzene hexachloride (HCB-0.8-55.2 ng/ml), DDE-0.5-29.2 ng/ml and octachlorostyrene (n.d-9.2 ng/ml). (Lommel *et al*, 1992)

During 1986-87, 750 whole blood samples from residents of large and medium to small urban centers across Ontario showed a mean concentration of PCBs up to 9.2 μg/kg and DDE up to 3.7 μg/kg. 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 μg/kg and 0.05 - 0.77 μg/kg respectively. (Frank *et al*, 1993)

In a study from Veracruz, Mexico maternal adipose tissue, maternal blood serum from 64 volunteer mothers were analysed for organochlorine pesticide residues- HCB, α, β, γ, δ- HCH, aldrin, dieldrin, heptachlor, heptachlor epoxide, pp'-DDT, op'-DDT, pp'- DDD, α, β, endosulfan, endosulfan sulfate, chlordane, and methoxychlor. Concentration of t-HCH in maternal adipose tissue, maternal serum was 0.17 and 0.22 mg/kg on fat basis respectively and t-DDT was 5.851, 5.226 mg/Kg respectively and hexachloro benzene was 0.065 and 0.18 mg/kg respectively. (Waliszewski *et al*, 2000).

A total of 96 serum and 46 adipose tissue samples collected from infertile women attending centres for reproductive medicine in Belgium from 1996-98 were analyzed for seven organochlorine pesticides and seven polychlorinated biphenyls. There was a strong association between adipose tissue and serum residues. The adipose tissue levels in ng/g of CB-138, 153, 180 and pp'- DDE (68.3 vs. 78.6, 145.7 vs. 90.9, 93.5 vs. 69.1, 470.9 vs. 1274.5) were explained by serum residues. The accumulation pattern for CB-153 and CB –180 in serum and adipose tissue are mirror images of each other (Pauwels *et al*, 2000).

A survey of 577 whole blood samples from school children in Peninsular Malaysia, extracted and analysed for the residues of 11 organochlorine and 2 organophosphorus pesticides revealed the presence of pesticide residues in blood in nanogram per gram - aldrin, nd-47.6; dieldrin, nd; endrin, nd; alpha-endosulfan, nd-0.6; beta-endosulfan, nd; endosulfan sulfate, nd; heptachlor, nd-3.8; lindane, nd-5.7; p,p'-DDT, nd-3.4; o,p'-DDE, nd-1.4; p,p'-DDE, nd; chlorpyrifos, nd-10.3; diazinon, nd-103.0 (Mohammad *et al*, 2001)

In a study from Canada, 251 cord blood samples collected from 1994 through 2001 for plychlorinated biphenyls (PCBs), dichlorodiphenyl dichloroethylene (DDE), hexachlorobenzene (HCB), chlordanes, lead and mercury 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 (Dallaire *et al*, 2003).

In a study conducted in USA, plasma samples collected at birth between 1998 and 2001 from 230 mother and newborn pairs enrolled in the Columbia Centre for children's Environmental Health were analysed for 29 pesticides. Seven pesticides were detected in 48-83% of plasma samples (range, 1-270 pg/g) 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 ($p<0.001$) (Whyatt *et al* 2003).

Blood and abdominal tissue from 126 adult cadavers submitted for autopsy at the Institute of Forensic Medicine of the University of Veracruz, Mexico were analyzed 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 t- DDT – 3.258 vs. 1.706). Only pp' – DDT reveals 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. (Waliszewski *et al*, 2004)

According to 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 many U,S. residents carry toxic pesticides in their bodies above government assessed "acceptable" levels. Analyzing pesticide residue data collected by the US Centres 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 (Schafer *et al*, 2004).

According to a WWF report, 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, 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. 25 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 and in blood serum it was pp'- DDE (a DDT metabolite), at a concentration of 3300 pg/g and deca BDE, a neurotoxic chemical used as flame retardant was found at the highest concentration of 45 pg/g of all the flame retardants analysed (WWF Report, 2004).

3.2.2 India

Several studies conducted in India have shown organochlorine pesticide residues in human blood samples. According to a study conducted in Delhi, blood samples from 182 people were examined for DDT residues 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 in males and from 0.166 to 0.329 mg/l in females. The DDT metabolites detected were pp'- DDE, pp'-DDD and op'-DDT. DDE accounted for most of the total DDT (Agarwal *et al*, 1976).

DDT and BHC residues were detected in all the 99 samples of blood and adipose tissue of normal and exposed persons from urban area of Lucknow. Total HCH concentration in normal population was 0.038 ppm (children), 0.034 ppm (females), 0.075 ppm (males) and in exposed persons was 0.295 ppm. Total DDT concentration in normal population was 0.023 ppm (children), 0.023 ppm (females), 0.028 ppm (males) and in exposed person was 0.200 ppm (Kaphalia and Seth, 1983).

A total of 340 biopsies of body fat and blood samples from 162 males and 178 females collected from 3 government hospitals in Delhi showed a mean DDT concentration of body fat of 22.25 $\pm$ 1.66 mg/kg and for blood 0.71 $\pm$ 0.05 mg/l. The mean total HCH of body fat was 16.85 $\pm$ 0.94 mg/kg and for blood 0.49$\pm$ 0.05 mg/l (Ramachandran *et al*, 1984).

A survey of blood samples of general population of occupationally unexposed population from Delhi showed levels of DDT several times higher than that from other countries. Total DDT ranged from 0.053- 0.663 ppm with a mean value of 0.301 ppm. Mean total DDT in males (0.344 ppm) was higher than of females (0.229 ppm) (Saxena et *al*, 1987).

In a study from Ahemdabad (rural area) blood samples collected from 31 healthy males during 1989-90 were analysed for 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 t-DDT were 37.25, 0.335, 1.33, 8.828 and 47.745 μg/l. pp'-DDE was the major metabolite and it alone contributed about 78% of total DDT. All serum samples were contaminated by HCH with an average of 147.335 μg/l with equivalent amounts of α, β and γ- HCH). Heptachlor, oxychlordane, aldrin and dieldrin were detected at an average concentration of 0.819μg/l, 1.465 μg/l, 0.200 μg/l, 2.152 μg/l. Heptachlor epoxide and hexachlorbenzene were not detected in any sample (Bhatnagar *et al,* 1992).

Mean HCH and DDT contents in whole blood of general population of 37 males not involved in spraying from district Hardwar, UP were 21.50 μg/l and 20.79 μg/l respectively. 47 samples from the 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, analyzed for HCH and DDT contamination in whole blood was 68.0 μg/l and DDT was 58.43 μg/l i.e. 3.1 times and 2.8 times more as compared to general population (Dua *et al*, 1996).

In a study conducted in Delhi, 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 showed the presence of t-DDT at an average level of 1.27, 0.27 and 0.14 mg/l 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 t-HCH in maternal blood, breast milk and cord blood was 0.327, 0.050 and 0.033 mg/l. β- isomer was the predominant isomer accounting for more than 60 percent of the various isomers (Nair *et al* 1996).

Human blood samples from 18 male healthy volunteers of Ahmedabad (urban) area 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 μg/l in serum samples respectively. The concentration of α, β and γ- and t-HCH in serum samples was 4.49, 35.06, 1.69 μg/l and 41.23μg/l respectively. Hexachlorobenzene was present in 7 samples at an average concentration of 0.2 μg/l (Bhatnagar *et al,* 2004).

The available literature shows that a lot of investigations have been carried out on organochlorine pesticide residue levels in human blood in India, but we have not come across any reports of organophosphorus pesticide residues in blood in India. However widespread use of organophosphorus pesticides means that people are continuously re-exposed to these pesticides and they might be present in the body, therefore it is probably the first time that organophosphorus pesticides are being analyzed in blood samples in India.

4. MATERIALS AND METHODS

4.1. Sampling methodology

Venous blood (10ml) of 20 people from 4 different villages of Punjab - Mahi Nangal, Jajjal and Balloh in Bhatinda district and Dher in Ropar district - were collected in the month of October' 2005. Blood samples were collected in residue free heparinised 20 ml glass vials containing 200 USP units of heparin in 0.2 ml solution with the help of sterilized syringe. Blood samples were transported in dry ice to the laboratory and stored at - 20^0 C until analysed. Details of the samples collected are given in Annexure-I

4.2. Equipments

Gas Chromatographs used for pesticide residue analysis were Thermoquest-Trace GC with ^{63}Ni selective Electron-Capture Detector with advanced software (Chromcard-32 bit Ver 1.06 October 98) and Nucon –GC- 5765 series equipped with Nitrogen Phosphorus Detector. GC columns employed were capillary column, DB- 1701 14%-Cyanopropyl-phenyl-methylpolysiloxane (length 30m, ID 0.25 mm and film 0.25μm), DB- 17 50% phenyl methylpolysiloxane (length 30m, ID 0.25 mm and film 0.25μm), and DB-5, coated with 5% diphenyl and 95% dimethylpolysiloxane (length 30m, ID 0.25 mm and film 0.25μm) J & W make for cross verification. Rotatory evaporator (Buchi type) and a 10-μl syringe from Hamilton Co. were employed.

4.3. Solvents and Glassware

All the solvents acetone, di ethyl ether, hexane (HPLC grade) used for the analysis was purchased from E-Merck, India. Organic solvents were glass distilled and checked for any pesticide contamination. All glassware were washed with detergent, rinsed with water, dipped in chromic acid for 24 hr and finally rinsed with distilled water and then hexane.

4.4. Chemicals

Pesticide reference standards of organochlorine pesticides - α, β, γ and δ HCH, heptachlor, aldrin, dieldrin, α,β endosulfan and endosulfan sulfate, chlordane, pp'-DDD, pp'-DDE, pp-'DDT and organophosphorus pesticides - dichlorvos, acephate, phorate, diazinon, monocrotophos, dimethoate, phosphamidon, chlorpyrifos, malathion, fenthion, quinalfos, phenthoate, profenofos and ethion were obtained from Sigma chemicals. Other chemicals were purchased from s. d. Fine Chem Ltd.

4.5.Sample extraction and clean up

The samples were analysed for organochlorines by using USEPA method 8081A for organochlorines by Gas chromatography and USEPA Method 8141A for organophosphorus compounds by gas chromatography -capillary column technique.

Extraction: Extraction was based on the method followed by Agarwal *et al*, 1976 with some modifications. Blood (5 ml) was diluted with 25 ml distilled water and 2 ml of saturated brine solution added and transferred to a 125 ml capacity separatory funnel and extracted with hexane: acetone (1:1) (20ml) (thrice) by shaking the separatory funnel vigorously for 2-3 min, releasing the pressure intermittently. The layers were allowed to separate. The three combined extracts were passed through anhydrous sodium sulfate and concentrated to about 1-2 ml using rotary vacuum evaporator.

Clean up: Clean up was done by USEPA Method 3620B- Florisil clean up by column chromatography. Florisil was activated at 130^0 C overnight and cooled in a dessicator before use. Weight of florisil taken was predetermined by calibration using lauric acid. 1g florisil was packed in the 20 cm length and 12 mm ID glass chromatographic column, anhydrous sodium sulfate was added to the top of the florisil column (0.5 cm) and the column was pre-eluted with hexane and discarded. Transferred the extract to the column and eluted with hexane (10 ml), 6% diethyl ether in hexane (10 ml), 15% diethyl ether in hexane (10 ml), 50% diethyl ether in hexane (10 ml) and finally with diethyl ether (10 ml). Eluent was collected and evaporated to dryness. Final samples were prepared in 2ml hexane (HPLC grade) and analyzed by GC-ECD for organochlorines and GC equipped with NPD for organophosphorus pesticides.

4.6. Sample Analysis

Calibration of GC system: GC system was calibrated using *external standard technique*.

Stock standard solution (1000mg/l): Individual stock solutions were prepared by weighing appropriate amounts of active ingredients in a brown bottle with a Teflon-lined screw cap and dissolving the weighed standard in HPLC grade hexane. The resulting concentration was corrected for the stated purity if purity was less than 96%. Stock standard solution was used to prepare primary dilution standards.

Composite stock standard solution: Appropriate volume of each individual stock solution was taken in a volumetric flask and mixed the solutions to obtain composite stock standard solution.

Calibration standard: Calibration standard was prepared at different concentrations by dilution of the composite stock standard solution with hexane, corresponding to the expected range of concentrations found in samples and it was used to calibrate (retention time, area count) the instrument response with respect to analyte concentration.

For Organochlorine pesticides

Organochlorine were analysed by Gas Chromatograph (Thermoquest-Trace GC) with the ^{63} Ni selective electron-capture detector. The column used was DB- 1701. The carrier gas and the makeup gas was nitrogen with a 1.0 ml/min and 40 ml/min-flow rate respectively employing the split less mode. 2.0µl of the final extract (2 ml) was injected at a temperature of 270^0 C. The oven temperature was kept at 120^0C with a hold time of 1 minute, then from 120^0C to 205 ^{0}C at a rate of 25^0 C/minute with a hold time of 1 minute then finally from 205 to 290^0C at a rate of 2^0 C / minute with a hold time of 12 min. The total run length was 59.9 minutes. The detector was maintained at 290^0C. Peak identification was performed by the GC software (Chromcard-32 bit Ver 1.06 October 98) calibration table set up with a relative retention time window of 0.65%.

For Organophosphorus pesticides

Organophosphorus pesticides were analysed by Gas Chromatograph (Nucon –5765 series equipped with Nitrogen Phosphorus detector). The capillary column used was another GLC capillary column – DB- 17. The carrier gas and the makeup gas was nitrogen with a 2.0 ml/min and 30 ml/min-flow rate respectively, hydrogen at 5 ml/min and air at 80ml/min respectively employing the split less mode. 2.0µl of the final extract was injected at a temperature of 270^0 C. The oven temperature was kept at 120^0C with a hold time of 1 minute, then from 120^0C to 205 ^{0} C at a rate of 25^0 C/minute with a hold time of 1 minute then finally from 205 to 270^0C at a rate of 2^0 C / minute with a hold time of 1 min. The total run length was 38.90 minutes. The detector was maintained at 290 ^{0}C.

Linearity checks

Gas chromatograph equipped with ECD and NPD were checked for linearity. Instrumental limit of detection for GC- ECD was 0.01 ng/ml for organochlorines and 0.1 ng/ml for organophosphorus pesticides.

Laboratory Reagent Blank

An aliquot of reagent grade water was treated exactly as a sample including exposure to all glassware, equipments, solvents, and reagents used with the sample matrix. No analyte peak was detected in laboratory reagent blank.

Laboratory fortified blank

An aliquot of reagent grade water to which known amount of pesticides were added in the laboratory in ppb range was analysed exactly like the sample. The recovery of the pesticides over the background values obtained from unfortified samples was more than 80 per cent for all the pesticides.

Laboratory fortified sample matrix

An aliquot of sample matrix (blood) was prepared to which known quantities of the pesticides were added in the laboratory in ppb range. This laboratory fortified matrix was analyzed exactly like the sample.15-25% of the samples (minimum) were fortified with a known concentration of pesticides and percent recovery was calculated. Extraction and clean up was done as mentioned and the recovery of the pesticides over the background values obtained from unfortified samples were more than 80 per cent. Standard deviation and coefficient of variation were less than 10 indicating repeatability of the method. All calculations were done as described in US EPA method and the amount of residues in samples was obtained in mg/l (ppm) of whole blood**.**

4.7 Confirmation and Quantification

Spiking

Identifications of the analytes were confirmed by spiking with known standards.

Dual column

The identifications were crosschecked with another column - DB-5 of different polarity. Elution pattern was different from the elution pattern in DB-1701.

5. RESULTS AND DISCUSSION

Twenty blood samples collected from 4 different villages of Punjab - Mahi Nangal, Jajjal and Balloh in Bhatinda district and Dher in Ropar district were analysed for 14 organochlorines and 14 organophosphorus pesticides following methodology based on USEPA protocols and the results are given in Annexure II, III

- **Organochlorine pesticides -** Among the 14 organochlorines and their isomers analyzed in whole blood samples from Punjab the mean levels of α, β, γ and δ isomers of HCH were 0.0281, 0.0063, 0.0227 mg/l and n. d and ranged from n.d – 0.1054, n.d – 0.0382, 0.0136-0.0569 mg/l (ppm) and n.d respectively. Total content of HCH (sum of α, β, γ and δ HCH) in whole blood samples from Punjab was 0.057 mg/l and ranged from 0.024 –0.128 mg/l. Hexachlorocyclohexane (HCH) is used against sucking and biting pests and as smoke for control of pests in grain stores. It is used as dust to control various soil pests such as flea beetles and mushroom flies. It is in the list of banned pesticides in India (with effect from April 1, 1997). Hexachlorcyclohexane, previously called BHC (benzene hexachloride), is a mixture of eight isomers of which five are found in the crude product ($\alpha, \beta, \gamma, \delta, \varepsilon$). Only the γ isomer or lindane has powerful insecticidal properties.

γ-HCH (Lindane) detected in 100% of the whole blood samples analysed which might be because γ-HCH is more resistant to biological and chemical degradation under aerobic conditions (El Beit *et al*, 1981) and is most commonly used. It appears in the list of pesticides for restricted use (the use of lindane formulations generating smoke for indoor use is prohibited; it can be used for the control of insect pests of field crops).

Dichlorodiphenyltrichloroethane (DDT) is a potent nonsystemic insecticide. Use of DDT is banned in agriculture. However its restricted use is allowed in public health sector (10,000 MT per annum). pp'- DDT was detected in 50% of the samples at mean levels of 0.0100 mg/l. A major metabolite of DDT is 2,2-bis (*p*-chlorophenyl)-1,1-dichloroethylene(pp' DDE) was detected at mean levels of 0. 0450 mg/l in the range of n.d–0.2554 mg/l in 95% of the whole blood samples analysed from villages in Punjab. DDE is more persistent than DDT.

2,2-bis(p-chlorophenyl)-1,1-dichloroethane(DDD) another metabolite of DDT was detected at mean levels 0.0102 in 55% of the samples. t-DDT(sum of pp'DDE+pp'DDD+pp'DDT) was detected at mean levels of 0.0652 mg/l in 95% of blood samples collected from villages of Punjab. DDT was detected in blood samples perhaps due to its persistent nature. Since DDT is known to undergo metabolic conversion and dehydrochlorination, presence of metabolites of DDT i.e. DDD and DDE encountered in this study might be due to such metabolic processes. The observed trend for t-DDT and t-HCH are comparatively lower than the earlier reports from India (Dua, *et al* 1996) but higher than a recent report from Ahemdabad. (Bhatnagar *et al*, 2004).

Chlordane, a banned organochlorine compound once used on agricultural crops and lawns and in buildings to kill termites was detected at mean levels 0.0090 mg/l in 70% of the samples analysed in the range of n.d–0.0539 mg/l .

Heptachlor (banned with effect from September 20, 1996) was detected in only one sample out of 20 blood samples analysed from Punjab at mean levels of 0.0006 mg/l. Aldrin (banned with effect from September 20, 1996), was detected in 80% of the blood samples analysed from Punjab at mean level 0.0062 mg/l and ranged from n.d –0. 0159 mg/l. Dieldrin (banned with effect from May, 1990) was not detected in any of the samples analysed.

Endosulfan, an organochlorine insecticide of the cyclodiene subgroup acts as a poison to a wide variety of insects and mites on contact and as a stomach acaricide. Technical grade endosulfan contains 94% α and β endosulfan. The α and β isomers are present in the ratio of 7:3 respectively. α isomer has been shown to be 3 times more toxic than the β isomer. α and β endosulfan were detected in 25% and 5% of the whole blood samples analyzed from villages of Punjab at mean levels of 0.0044 and 0.0002 mg/l respectively. In rabbits, the beta-isomer was cleared from blood plasma more quickly than the alpha-isomer, with reported blood half-lives of approximately 6 hours and 10 days respectively, which may account in part for the observed differences in toxicity. (Smith, 1991) Endosulfan sulfate a reaction product found in technical grade endosulfan as a result of oxidation, is considered to be equally toxic and more persistent than the parent compound. However it was not detected in any of the samples.

Residues of organochlorines were detected in most of the samples, as they are persistent in nature due to their slow decomposition rate, long half-life and high stability in the environment.

Organophosphorus pesticides

Among fourteen oragnophosphorus pesticides analysed in whole blood samples four were commonly detected - monocrotophos, chlorpyrifos, malathion and phosphamidon. Monocrotophos, a non-specific, systemic insecticide and acaricide is shown to cause delayed neuropathy. It is included in the PIC (Prior Informed Consent) procedure, an international convention that recognizes certain

acutely hazardous pesticides as a human health risk. Monocrotophos was detected in 75% of the whole blood samples analysed from Punjab villages at mean levels of 0.0948 mg/l in the range of- n.d–0.4915 mg/l. Higher levels of monocrotophos in blood samples in Punjab could be due to higher repeated exposure of test samples to this pesticide during application in fields in Punjab.

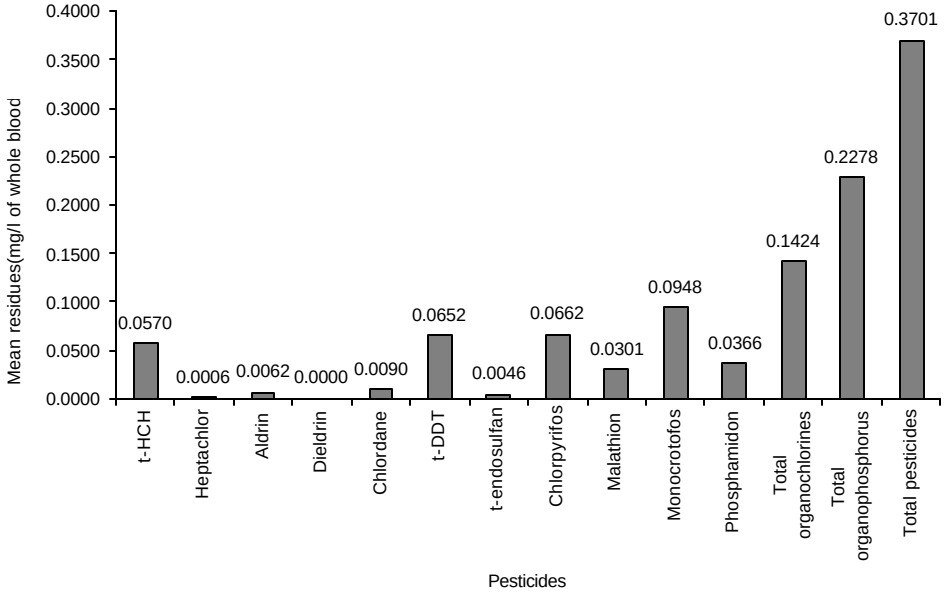
Phosphamidon was detected in 70% of the samples at mean levels 0.0366 mg/l and ranged from n.d-0.1282 mg/l in whole blood samples collected from villages of Punjab. Phosphamidon 85% SL is banned for import, manufacture and use.

Chlorpyrifos, fourth highest consumed pesticide in India was detected in 85% of the samples at mean level of 0.0662 mg/l and ranged from n.d–0.4965 mg/l in 20 whole blood samples analysed. It is a moderately persistent insecticide effective against mosquito and fly larvae, cabbage root fly and aphids. It has become one of the most widely applied insecticides in homes and restaurants against cockroaches and termites.

Malathion was detected in 70% of the whole blood samples collected from Punjab at mean levels of 0.0301 mg/l and ranged from n.d -0.0753 mg/l. Malathion is a widely used contact insecticide and acaricide for the control of aphids, red spider mites, leaf hoppers and thrips on a wide range of vegetable and other crops. It is also used to control insect vectors like mosquitoes.

Dichlorvos, acephate, phorate, diazinon, dimethoate, fenthion, quinalfos, phenthoate, profenofos and ethion were not detected in any of the samples.

Graph-I Organochlorine and organophosphorus pesticide residues in human blood samples from villages of Punjab



- **Total organochlorines, total organophosphorus and total pesticides**

  Total organochlorines were detected at mean levels of 0.1424 mg/l, total organophosphorus was 0.2278 mg/l and total pesticides was 0.3701 mg/l in whole blood samples analyzed from Punjab (Graph I)

- **All the samples from Punjab had pesticides in blood**

  In 20 blood samples collected from 4 villages in Punjab analysed for14 organochlorines and 14 organophosphorus pesticides – 11 of the 14 organochlorine pesticides were detected - α, β, γ isomers of HCH were detected in 95%, 35% and 100% respectively, aldrin (80%), heptachlor (5%), chlordane

(70%), DDD (55%), DDE (95%), DDT (50%), α endosulfan (24%) and β-endosulfan (5%) of the samples analysed. Among organophosphorus pesticides only 4 of 14 pesticides were detected – Monocrotophos (75%), Phosphamidon (70%), Chlorpyrifos (85%) and Malathion (70%) of the samples analysed indicating regular and widespread exposure to these pesticides. (Annexure III)

Total number of pesticides detected in blood samples from Punjab (15 of 28 analysed) which indicates that each person is exposed to and carries a body burden of multiple pesticides which might be due to a combination of direct and indirect exposure to these pesticides. Major contribution to total pesticide concentration in blood samples from Punjab is of organophosphorus pesticides. Presence of organophosphorus pesticides in blood means that they do persist n the body for good amount of time. It also indicates the presence in the body of the pesticide in its form as a primary compound.

6. HEALTH IMPACTS

6.1 Toxicity of pesticides

Pesticides must be toxic, or poisonous to be effective against the pests they are intended to control. Because pesticides are toxic, they are also potentially hazardous to humans and animals. Toxicity is a measure of the capacity of a pesticide to cause injury; it is a property of the chemical itself. The toxicity of a particular pesticide is determined by subjecting test animals (usually rats, mice, rabbits, and dogs) to different dosages of the active ingredient and to each of its formulated products. Toxicity can be acute or chronic (www. pubs.cas.psu.edu/FreePubs/pdfs/uo198.pdf)

6.1.1 Acute Toxicity and Acute Effects

The acute toxicity of a pesticide refers to the ability of the chemical to cause injury to a person or animal from a single exposure, generally of short duration. Acute toxicity is determined by at least three methods: (1) dermal toxicity is determined by exposing the skin to the chemical; (2) inhalation toxicity is determined by permitting test animals to breathe vapors of the chemical; and (3) oral toxicity is determined by feeding the chemical to test animals. The harmful effects that occur from a single exposure by any route of entry (dermal, inhalation, oral) are termed acute effects. In addition, the effect of the chemical as an irritant to the eyes and skin is examined under laboratory conditions. Acute toxicity is usually expressed as LD_{50} (lethal dose 50) or LC_{50} (lethal concentration 50), which is the amount or concentration of a toxicant required to kill 50 percent of a test population of animals under a standard set of conditions. LD_{50} values of pesticides are recorded in milligrams of pesticide per kilogram of body weight of the test animal (mg/kg bw). LC_{50} values of pesticides are recorded in milligrams of pesticide per volume of air or water (ppm). The lower the LD_{50} value of a pesticide, the less it takes to kill 50 percent of the test population, and therefore the greater the toxicity of the chemical. Parathion, for example, is considered to be highly toxic because the oral lethal dose is less than 4 milligram per kilogram (mg/kg) of body weight, compared with 1200 mg/kg for Malathion, or 5,000 mg/kg for methoxychlor

6.1.2 Chronic Toxicity and Chronic Effects:

Chronic toxicity is determined by subjecting test animals to long-term exposure to a pesticide. The harmful effects that occur from small doses repeated over a period of time, usually years, are termed chronic effects. Some of the chronic effects found in test animals exposed to certain pesticides include birth defects (teratogenesis); toxicity to a fetus (fetotoxic effects); production of tumors (oncogenesis), either benign (noncancerous) or malignant (cancerous/carcinogenesis); genetic changes (mutagenesis); blood disorders (hemotoxic effects); nerve disorders (neurotoxic

effects); endocrine disruption; and reproductive effects. The chronic toxicity of a pesticide is more difficult to determine through laboratory analysis than the acute toxicity.

6.2 Chronic Pesticide Exposure and Diseases

Studies indicate a strong linkage between pesticide exposure and chronic diseases, as very low –level exposures can result in effects long after the initial exposure occurs.

Cancer: Pesticides are a risk factor for several types of cancer- evidence on the links between pesticide exposure and breast cancer is mixed, with many studies showing no co-relation and others showing strong linkages. In a recent study it was shown that mixture of 4 organochlorines (op' DDT, pp' DDE, β-BHC and pp' DDT) acted together to produce proliferative effects in MCF-7 human breast cancer cells and the combined effect was additive (Gertrudis *et al*, 2001). Comparison of blood levels of HCB and total DDT in 159 women with breast cancer and 250 presumably healthy controls showed that mean levels of total DDT and HCB were significantly higher for breast cancer patients than for controls. The results showed significant differences between the two groups of women those with breast cancer were over five times as likely to have detectable levels of DDT above 0.5 parts per billion as the healthy women, and more than nine times as likely to have detectable levels of HCB in their blood. The highest levels detected were 20 parts per billion. (British Medical Association, 2003 at http://www.innovations-report.de/home.php).

Malathion has been shown to induce changes in the epithelium of rat mammary glands, influencing the process of carcinogenesis; such alterations occur at the level of nervous system by increasing the cholinergic stimulation (Vladimir *et al*, 2002). Multiple studies have shown that farmers are more likely to develop leukemia, brain, prostrate and skin cancer and non hodgkin's lymphoma than the general population (Dich and Zahm, 1997)

Lymphoma: In utero and early childhood exposure to pesticides is associated with a significantly increased risk for developing non-Hodgkin's lymphoma. Non-Hodgkin's lymphoma is a cancer that begins in cells of the lymph system (Zahm, 1992). Studies indicate an association between pesticide exposure and NHL in children exposed to lindane, DDT, organophosphorus pesticides. The lymph system includes the spleen, thymus, tonsils, bone marrow, lymph nodes and circulating white blood cells, called lymphocytes. Lymphocytes and the lymph system are part of the immune system, which protects the body from disease and infection. Non-Hodgkin's lymphoma is characterized by the excessive accumulation of atypical lymphocytes. These lymphocytes crowd the lymph system and suppress the formation and function of blood and immune cells. This leads to a diminished ability of the body to fight infection.

Mutagenesis (genetic changes): Pesticides have been shown to cause genetic changes; DDT and its metabolites induced DNA damage in peripheral blood mononuclear cells shown by comet assay. A significant correlation between blood levels of DDT, DDD, DDE and DNA damage was found in women with different amount of environment exposure to DDT and its metabolites (Yanez *et al*, 2004). Monocrotophos was shown to induce single/double strand DNA breaks in mice *in vivo* using comet assay when oral dose of 0.046, 0.186, 0.373 and 0.746 mg/kg body weight and assay was performed on whole blood (Mahboob *et al*, 2002). Malathion was found to cause DNA abnormalities at all doses (0.02, 0.2, 2 and 20 ug/l) when added to human blood cells drawn from three healthy non-smoking men, aged 23, 24 and 25 years. It causes a dose-dependent increase in chromosomal aberrations as well as sister chromatid exchanges in human leukocyte cultures. A dose dependent

decrease in mitotic index was also observed which suggests that malathion is a mild mutagen and at higher concentrations it might cause genotoxicity in humans (Source: Mutation Research, 301:13-17, 1993).

Neurotoxic (toxicity of the brain or nervous system): Chlorpyrifos, one of the most widely used organophosphorus pesticide has been reported as a developmental neurotoxicant specifically targeting the immature brain. Developmental neurotoxicity of chlorpyrifos is thought to involve both neurons and glia, increasing the vulnerability of the developing brain. The vulnerability increases from the gestational exposure through later periods of development which glial neuronal interactions influence brain architectural, circuitary and function. Exposures occurring during childhood are as important as those occurring prenatally (Barone *et al* 2000; Pope 1999). Fetal and childhood exposure to chlorpyrifos has raised concerns about developmental neurotoxicity. Exposure to chlorpyrifos resulted in adverse effects on brain cell development and cholinergic biomarkers. Neonatal rats were more sensitive to chlorpyrifos than the fetal rats and animals exposed prenatally developed behavioral deficits in adolescence and adulthood. (Qio et *al*, 2003)

Parkinsons Disease: Epidemiological studies have suggested an etiologic relationship between pesticide exposure and Parkinson's disease. Organochlorine pesticides were assayed in postmortem brain samples from 20 Parkinson's disease, 7 Alzheimer's disease, and 14 non-neurological control cases. Dieldrin, a lipid-soluble, long-lasting mitochondrial poison, was investigated as a potential etiological agent of Parkinsonism (Fleming *et al*, 1994).

Fetotoxic (toxicity to fetus): A strong relationship has been found between prematurely delivered and low birth weight babies and mothers' level of DDE, metabolic break down product of DDT. Studies carried out to evaluate potential toxicological effects of chlorpyrifos in rats showed that repeated exposure to sub threshold doses of chlorpyrifos may lead to growth retardation, behavioral abnormalities and muscle weakness. (Terry and Stone, 2003). Chlorpyrifos was evaluated for potential developmental toxicity in rats and was found to show fetotoxic and terratogenic effects at maternal dose of 25 mg/kg per day, a dose that also produced maternal toxicity. Fetal weight and viability were decreased and fetal death and early resorption increased at this dose (Farag *et al*, 2003).

Terratogenic (birth defects): Children born to women who live in a high pesticide use area while pregnant have an increased risk of birth defects- cleft lip, limb reduction defects and neural tube defects (Garry *et al*, 1996).

Recent findings suggest that chlorpyrifos, is a suspected neuroteratogen has a shifting cellular target, initially impacting the development of neurons and subsequently affecting the glia, which develop much later (Garcia *et al* 2001, 2002). Malathion has been shown to cause birth defects in a variety of wildlife and at levels lower than other pesticides. When administered to adult animals, malathion and related thiophosphonates stimulate, and subsequently inhibit the nicotinic sites in skeletal muscle, resulting in muscle weakness and paralysis. Neonates (newborn babies) are far more sensitive to these agents than adults, mainly because of a slower rate of detoxification of the metabolite (the metabolite in this case would be the liver breakdown product of malathion – malaoxon), which has been shown to be far more toxic than malathion itself. (*Source:* Teratology, 36:7-9, 1987)

Immunological changes: Associations of DDT, dichlorodiphenyldichloroethylene (DDE), and dichlorodiphenyldichloroethane (DDD) in levels of blood with several immune parameters in patients occupationally exposed to insecticides have been reported. The majority of 49 patients who worked as farmers or farm hands in the former German Democratic Republic were contaminated with more than 1 chemical-- most commonly DDE, PCBs, and HCB. Occupational exposure to insecticides for at least 6 months, 80% of them had been exposed for more than 20 yr had resulted in frequent infections and immunological abnormalities (Daniel, 2002). Chronic exposure to chlorpyrifos has been shown to cause immunological change. Comparison of chronic health complaints of twenty-nine individuals exposed to chlorpyrifos with respect to peripheral lymphocyte phenotypes; auto antibodies (nucleic acids and nucleoproteins, parietal cell, brush border, mitochondria, smooth muscle, thyroid gland, and central nervous system/peripheral nervous system myelin); mitogenesis to phytohemagglutinin and concanavillin and compared with 3 control groups (i.e., 1 positive 2 negative) showed an increase in CD 26 expression, a decrease in percentage of CD5 phenotype, decreased mitogenesis in response to phytohemagglutinin and concanavillin, and an increased frequency of auto antibodies. The alterations in these peripheral blood markers were unaffected by medication, age, sex, or season. (Thrasher *et al*, 2002)

Other effects: Some studies link pesticide exposure with decreased sperm quality, a higher sperm density with lower pesticide exposure (Swan and Kruse, 2003). Hormone disruption is considered a possible contributor to low sperm count and dozen of pesticides are known or suspected hormone disrupters. Lindane was found to be estrogenic to female rats and mice, and also caused the testes of male rats to become atrophied. Seminiferous tubules and Leydig cells (important for production of sperms) were completely degenerated at doses of 8 mg/kg/day over a 10-day period (Gallo and Lawryk, 1991).

Treatment with 1-40 mg of lindane/kg of body weight disrupts testicular morphology, decreases spermatogenesis, inhibits testicular steroidogenesis, reduces plasma androgen concentrations and may adversely affect reproductive performance in males. In females lindane disrupts the estrous cycle, reduces serum estrogen and progesterone levels decreases sexual receptivity (Pages *et al*, 2002).

7. CONCLUSIONS

Twenty blood samples randomly selected from 4 different villages of Punjab - Mahi Nangal, Jajjal and Balloh in Bhatinda district and Dher in Ropar district - were analyzed for 14 organochlorines and 14 organophosphorus pesticides.

- Total content of HCH (sum of α, β, γ and δ HCH) in whole blood samples from villages of Punjab was 0.057 mg/l**.** γ isomer of HCH (lindane) was detected at a level of 0.0227 mg/l in blood samples and ranged from 0.0136 to 0.0569 mg/l.
- Total content of DDT (sum of DDD, DDE and DDT) in blood samples from villages of Punjab was 0.0652 mg/l. Mean levels of pp'- DDE in whole blood samples from Punjab was 0. 0450 mg/l.
- Total-endosulfan (α,β and endosulfan sulfate) in whole blood samples from Punjab was 0.0046 mg/l however isomers of endosulfan .
- Heptachlor, aldrin and chlordane were detected in 1, 16 and 14 out of 20 blood samples analyzed from Punjab.
- Monocrotophos, chlorpyrifos, malathion and phosphamidon were detected at mean levels of 0.0948, 0.0662, 0.0301and 0.0366 mg/l respectively in Punjab blood samples.

- Mean levels of total organochlorines in whole blood samples from Punjab were 0.1424, total organophosphorus was 0.2278 and total pesticides were 0.3701mg/l respectively.
- Total number of pesticides detected in blood samples from Punjab was 15 out of 28 pesticides analysed which indicates that each person is exposed to and carries a body burden of multiple pesticides which might be due to a combination of direct and indirect exposure to these pesticides.
- Major contribution to total pesticide concentration in blood samples from Punjab is of organophosphorus pesticides. Presence of organophosphorus pesticides in blood means that they do persist n the body for good amount of time. It also indicates the presence in the body of the pesticide in its form as a primary compound.
- It can be concluded from this study that human pesticide residue is a biological index of pesticide exposure and studies on blood can be used for assessing the total body burden data of pesticides in the occupationally exposed and unexposed population.

8. REFERENCES

1. Agarwal HC, Pillai MK, Yadav DV, Menon KB and Gupta RK (1976), Residues of DDT and its metabolites in human blood samples in Delhi, India. *Bull. World Health Organ*. 54:349-351
2. Aspelin AL (1994) Pesticides industry sales and usage, 1992 and 1993 market estimates: U.S. EPA, Office of Pesticides Programs, Biological and Economic Analysis Div., Economic Analysis Branch Report 733-K-94-001: 33.
3. Barone S, Das KP, Lassiter TL and White LD (2000). Vulnerable process of nervous system development. A review of markers and methods. *Neurotoxicology*. 21: 15-36
4. Bhatnagar VK, Kashyap R, Zaidi SSA, Kulkarni PK and Saiyed HN (2004). Levels of DDT, HCH and HCB residues in human blood in Ahemdabad, India. *Bull. Environ. Contam*. *Toxicol*. 72: 261-265
5. Bhatnagar VK, Patel S, Variya MR, Venkaih MP and Kashyap SK (1992). Levels of Organochlorines in human blood from Ahmedabad (Rural), India. *Bull. Environ. Contam. Toxicol* . 48: 30-307
6. Brown JR and Chow LY (1975). Comparative study of DDT and its derivatives in human blood samples in Norfolk county and Holland Marsh, Ontario. *Bull. Environ. Contam. Toxicol*. 13(4): 483-488
7. Covaci, A., Jorens, P., Jacquemyn, Y. and Schepens, R. (2002) Distribution of PCBs and organochlorine pesticides in umbilical cord and maternal serum *Sci. Total Environ.* 298, 45-53
8. 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. *Environ. Health Perspect*. 111(13): 1660-1664
9. Daniel V (2002). Associations of dichlorodiphenyltrichloroethane (DDT) 4.4 and dichlorodiphenyl dichloroethylene (DDE) 4.4 blood levels with plasma IL-4. *Arch. Environ. Health*. 57(6):541-547
10. Dich J and Zahm SH (1997). Pesticides and Cancer, *Can causes Cont*, 8 (3):420-43
11. Doguchi M and Fukano S (1975). Residue levels of polychlorinated terphenyls, polychlorinated biphenyls and DDT in human blood. *Bull. Environ. Contam. Toxicol.* 13(1):57-63.
12. Dua VK, Pant CS, Sharma VP and Pathak GK (1996). Determination of HCH and DDT in finger – prick whole blood dried on filter paper and its field application for monitoring concentrations in blood. *Bull. Environ. Contam. Toxicol* . 56:50-57
13. El Beit IOD, Wheelock JV, Cotton DE (1981) Factors affecting soil residues of dieldrin, endosulfan, gamma HCH, dimethoate. *Ecotoxicol and Environ Saf* 5:135-60.
14. Farag AT, El Okazy AM and El Aswed AF (2003). Developmental toxicity study of chlorpyrifos in rats. *Reprod. Toxicol.* 17(2): 203-208
15. Fleming L, Mann JB, Bean J, Briggle T and Sanchez-Ramos JR. (1994). Parkinson's disease and brain levels of organochlorine pesticides. *Annals of Neurology*. 36(1): 100-103.
16. Frank R, Braun HE and Thorpe P (1993). Comparison of DDE and PCB residues in the general diet and in human blood – Ontario 1986-87. *Bull. Environ. Contam. Toxicol* 51:146-152
17. Gallo M A and Lawryk NJ (1991). Organophosphorus Pesticides. *In* Handbook of Pesticide Toxicology. Hayes WJ Jr. and Laws ER Jr., Eds. Academic Press, New York, NY pp 3-5
18. Garcia SJ, Seidler FJ, Crumpton TL and Slotkin TA (2001). Does the developmental toxicity of chlorpyrifos involve glial targets? Macromolecule synthesis, adenylyl cyclase signaling, nuclear transcription factors, and formation of reactive oxygen in C6 glioma cells. *Brain Research*. 891: 54-68
19. Garcia SJ, Seidler FJ, Qiao D and Slotkin TA (2002). Chlorpyrifos targets developing glia: effects on glial fibrillary acidic protein. *Dev. Brain. Research*. 133: 151-161
20. Garry VF, Schreinemachers and Harkins ME (1996). Pesticide appliers, biocides and birth defects in Rural Minnesota. *Environ. Health. Perspect*, 104(4): 394-399

21. Gertrudis C, Mario V, Arnaldo V, Viviana D, Isolde R, Nicolas H and Gloria C (2001). A rat mammary tumour model induced by the organophosphorus pesticides parathion and malathion, possibly through acetyl cholinesterase inhibition. *Environ. Health. Perspect.* 109(5): 211-214
22. Kalra RL, Kaur H, Sharma S, Kapoor SK, Chakraborty SS, Kshirasagar RB, Vaidya RC, Sagade RB, Shirolkar SB, Dikshith TSS, Raizada RB, Srivastava MK, Singh V, Nagarja K, Vappaiah KM, Srinivasa MA, Rani MU, Ramarao SN, Toteja GS, Dasgupta J, Ghosh PK and Saxena BN (1999). DDT and HCH residues in dairy milk samples collected from different geographical regions of India: a multicentre study. *Food Additives and Contaminants*. 16(10): 411-417.
23. Kalra RL, Singh B and Battu RS (1994). Organochlorine pesticide residues in human milk in Punjab, India. *Environmental pollution* 85: 147-151.
24. Kanja LW, Skaare JU, Ojwang SBO and Maitai CK (1992). A comparison of organochlorine pesticide residues in maternal adipose tissue, maternal blood, cord blood and human milk from mother/infant pairs. *Arch. Environ. Contam. Toxicol*. 22:21-24
25. Kaphalia BS and Seth TD (1983) Chlorinated pesticide residues in blood plasma and adipose tissue of normal and exposed human population. *Indian J. Med Res*. 77: 245-247.
26. Lommel A, Kruse H, Muller E and Wassermann O (1992). Organochlorine pesticides, Octachlorostyrene and Mercury in the blood of Elb river resident, Germany. *Arch. Environ. Contam. Toxicol*. 22:14-20
27. Mahboob M, Rahman MF, Danadevi K, Banu BS and Grover P (2002). Detection of DNA damage in mouse peripheral blood leucocytes by the comet assay after oral administration of monocrotophos. *Drug. Cem. Toxicol*. 25(1): 65-74.
28. Mohammed MA, Chan Pui Ling M, Abdullah AR and Zulkifli SN (2001). Pesticide residues in blood of school children from selected schools in Peninsular Malaysia. Available at http://landbase.hq.unu.edu/Symposia/2001Symposium/abstracts/17Ali.htm
29. Nair A, Mandapati R, Dureja P and Pillai MKK (1996). DDT and HCH load in mothers and their infants in Delhi, India. *Bull. Environ. Contam. Toxicol*. 56:58-64
30. Pages N, Sauvet MP, Bouvet S and Goudey-Perriere F. (2002) Reproductive toxicity of Lindane, *J. Soc. Biol*. 196(4): 325-338.
31. Pauwels A, Covaci A, Weyler L, Delbeke, Dhont M, Sutter PD, Hooghe TD and Schepens PJC (2000). Comparison of Organic pollutants residues in serum and adipose tissue in a female population in Belgium, 1996-1998. *Arch. Environ. Contam. Toxicol*. 39: 265-270
32. Pope CN (1999). Organophosphorus pesticides: Do they all have the same mechanism of toxicity? *J. Toxicol. Environ. Health*. 2:161-181
33. Puri SN, Murthy KS and Sharma OP (1999). 'IPM for sustainable crop production,' Management' in Sundaram V et al , Handbook of cotton in India, ISCI, Bombay.
34. Qio D, Seidler FJ, Tate CA, Cousins MM and Slotkin TA (2003). Fetal chlorpyrifos exposure: adverse effects on brain cell development and cholinergic biomarkers emerge postnatally and continue into adolescence and adulthood, *Environ. Health. Perspect*. 111(4): 536-544
35. Ramachandran M, Banerjee BD, Gulati, M, Grover A, Zaidi SSA and Hussain QZ (1984). DDT and HCH residues in the body fat and blood samples from Delhi hospitals. *Indian J. Med. Research*. 80:590-593
36. 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. *Bull. Environ. Contam. Toxicol*. 38:392-395.
37. Schafer KS, Reeves M, Spitzer S, Kegley S (2004) *In* Chemical trespass: Pesticides in our bodies and corporate accountability, Pesticide Action Network North America. Available at http://www.panna.org.

38. Smith, A. G. Chlorinated Hydrocarbon Insecticides. In Handbook of Pesticide Toxicology. Hayes, W. J., Jr.and Laws, E. R., Jr., Eds. Academic Press Inc., New York, NY, 1991.6-3
39. Swan SH and Kruse RL (2003). Semen quality in relation to biomarkers of pesticide exposure. *Environ. Health. Perspect.* 111(12): 1478-1484
40. Terry AV Jr. and Stone JD (2003). Repeated exposures to subthreshold doses of chlorpyrifos in rats: hippocampal damage, impaired axonal transport, and deficits in spatial learning. *J. Pharmacol. Exp. Theor.* 305(1): 375-84.
41. Thrasher JD, Heuser G and Broughton A (2002). Immunological abnormalities in humans chronically exposed to chlorpoyrifos. *Arch. Environ. Health*. 57(3): 181-187
42. Vladimir T, Valery R and Lorenzo T (2002). Dichlorodiphenyltrichloro ethane (DDT): Ubiquity, persistence and risks. *Environ. Health. Perspect.* 110(2): 125-128
43. Waliszewski SM, Aguirre AA, Infanzon RM, Carrillo LL and Sanchez LT (2000). Comparison of Organochlorine pesticide levels in adipose issue and blood serum from mothers living in Veracruz, Mexico. *Bull. Environ. Contam. Toxicol*. 64:8-15
44. Waliszewski SM, Carvajal O, Infanzon RM, Trujillo P and Hart MM (2004). Co partition ratios of persistent organochlorines pesticides between adipose tissue and blood serum lipids. *Bull. Environ. Contam. Toxicol* 73:732-738
45. Wessels D, Barr DB and Mendola P (2003). Use of Biomarkers to indicate exposure of children to organophosphate pesticides: Implications for a longitudinal study of children's environmental health, *Environmental Health Perspectives,*111(16):1939-1946
46. Whyatt RM, Barr DB, Camann DE, Kinney PL, Barr JR, Andrews HF, Hoepner LA, Garfinkel R, Hazi Y, Reyes A, RamirezJ, Cosme and Perera FP (2003). Contemporary- Use Pesticides in personal air samples during pregnancy and blood samples at delivery among urban minority mothers and new borns. *Environ. Health. Perspect.* 111 (5):749-756.
47. Woollen BH (1993) Biological Monitoring for pesticide absorption. *Am Occup. Hyg*. 37:525-540
48. WWF Report (2004). Bad Blood? A survey of chemicals in the blood of European ministers.
49. Yanez L, Borja AVH, Rojas E, de la Fuente H, Gonzalez-Amaro R, Gomez H, Jongitud AA and Diaz-Barriga F (2004). DDT induces DNA damage in blood cells: Studies in vitro and in women chronically exposed to this insecticide. *Environ. Research.* 94 (1) 18-24.
50. Zahm S (1992). Pesticides and non-hodgkins lymphoma. *Cancer Res* . 52:Suppl 19: 5485s-5488

Details of the blood samples collected from Villages of Punjab **Annexure I**

S.No	Sample code	Date of sampling	Name of person	Sex	Age (years)	District	Village/City	Any other information
1	Blood 1	October 5, 2004	Satya Devi	F	50	Bhatinda	Mahi Nangal	Earlier worked in cotton fields
2	Blood 2	October 5, 2004	Vimla Devi	F	37	Bhatinda	Mahi Nangal	Earlier worked in cotton fields
3	Blood 3	October 5, 2004	Jaspal Singh	M	28	Bhatinda	Mahi Nangal	Spraying pesticides for few years.
4	Blood 4	October 5, 2004	Gurjanta Singh	M	25	Bhatinda	Mahi Nangal	Spraying pesticides for past 5-6 years
5	Blood 5	October 5, 2004	Bhola Singh	M	35	Bhatinda	Jajjal	Spraying pesticides for past 15 years.
6	Blood 6	October 5, 2004	Baljeet	M	20	Bhatinda	Jajjal	Spraying pesticides for 2-3 years.
7	Blood 7	October 5, 2004	Jagtar Singh	M	20	Bhatinda	Jajjal	Sampled right after spraying pesticides.
8	Blood 8	October 5, 2004	Soma Rani	F	52	Bhatinda	Jajjal	Earlier worked in cotton fields
9	Blood 9	October 5, 2004	Angrez Kaur	F	55	Bhatinda	Jajjal	Earlier worked in cotton fields
10	Blood 10	October 5, 2004	Mandar Singh	M	40	Bhatinda	Balloh	Sprayed pesticides a day before sampling
11	Blood 11	October 5, 2004	Karm Singh	M	27	Bhatinda	Balloh	Spraying pesticides for 2 years
12	Blood 12	October 5, 2004	Satnam Singh	M	NA	Bhatinda	Balloh	Spraying pesticides for 2 years
13	Blood 13	October 5, 2004	Sarjeet	F	65	Bhatinda	Balloh	Earlier worked in cotton fields
14	Blood 14	October 5, 2004	Naseer Kaur	F	40	Bhatinda	Balloh	Earlier worked in cotton fields
15	Blood 15	October 6, 2004	Ram Pal	M	45	Ropar	Dher	Worked in agricultural fields
16	Blood 16	October 6, 2004	Sarwal Singh	M	40	Ropar	Dher	Worked in agricultural fields
17	Blood 17	October 6, 2004	Jasvinder Kaur	F	30	Ropar	Dher	Housewife, sometimes goes to fields.
18	Blood 18	October 6, 2004	Gurmeet Kaur	F	45	Ropar	Dher	Housewife
19	Blood 19	October 7, 2004	Sukhdev Raj	F	32	Ropar	Dher	Grows vegetables in kitchen garden
20	Blood 20	October 7, 2004	Surendra Singh	M	35	Ropar	Dher	Earlier worked in cotton fields

Note:

1.Female-F Male-M
2. NA- Not available

Organochlorine and organophosphorus pesticide residues in blood samples of 20 individuals from different villages in Punjab **Annexure II**

			Residues (mg/l of whole blood)																							
			ORGANOCHLORINES																	ORGANOPHOSPHORUS						
S.No	Sample	Location	a-HCH	b-HCH	g-HCH	d-HCH	t-HCH	Heptachlor	Aldrin	Chlordane	DDE	DDD	DDT	t-DDT	a-endosulfan	b-endosulfan	Endosulfan sulfate	t-endosulfan	Total Organochlorines	Monocrotofos	Phosphamidon	Chlorpyrifos	Malathion	Total Organophosphorus	Total Pesticides
1	Blood -1	Mahi Nangal	0.0336	0.0090	0.0155	0.0000	0.0581	0.0000	0.0054	0.0025	0.0529	0.0075	0.0077	0.0681	0.0000	0.0000	0.0000	0.0000	0.1341	0.1384	0.0796	0.0270	0.0658	0.3108	0.4449
2	Blood -2	Mahi Nangal	0.0131	0.0000	0.0137	0.0000	0.0268	0.0000	0.0122	0.0000	0.0104	0.0061	0.0000	0.0165	0.0000	0.0000	0.0000	0.0000	0.0555	0.0468	0.0312	0.0274	0.0523	0.1576	0.2131
3	Blood -3	Mahi Nangal	0.0401	0.0210	0.0166	0.0000	0.0777	0.0000	0.0052	0.0110	0.0293	0.0265	0.0485	0.1043	0.0058	0.0000	0.0000	0.0058	0.2040	0.1441	0.0488	0.0422	0.0400	0.2750	0.4790
4	Blood -4	Mahi Nangal	0.1054	0.0000	0.0225	0.0000	0.1279	0.0000	0.0071	0.0140	0.0065	0.0000	0.0000	0.0065	0.0000	0.0000	0.0000	0.0000	0.1555	0.0000	0.1282	0.0488	0.0000	0.1770	0.3325
5	Blood -5	Jajjal	0.0232	0.0000	0.0171	0.0000	0.0403	0.0000	0.0102	0.0012	0.0506	0.0068	0.0054	0.0628	0.0000	0.0000	0.0000	0.0000	0.1145	0.4915	0.0513	0.0275	0.0706	0.6409	0.7554
6	Blood -6	Jajjal	0.0140	0.0219	0.0186	0.0000	0.0545	0.0000	0.0000	0.0330	0.0579	0.0473	0.0248	0.1300	0.0000	0.0000	0.0000	0.0000	0.2175	0.0000	0.0000	0.0000	0.0000	0.0000	0.2175
7	Blood -7	Jajjal	0.0338	0.0000	0.0219	0.0000	0.0557	0.0000	0.0071	0.0000	0.0096	0.0079	0.0065	0.0240	0.0000	0.0000	0.0000	0.0000	0.0868	0.2330	0.0800	0.0532	0.0753	0.4415	0.5283
8	Blood -8	Jajjal	0.0176	0.0045	0.0167	0.0000	0.0388	0.0000	0.0000	0.0076	0.0245	0.0229	0.0286	0.0760	0.0000	0.0000	0.0000	0.0000	0.1224	0.0000	0.0000	0.0000	0.0000	0.0000	0.1224
9	Blood -9	Jajjal	0.0000	0.0000	0.0569	0.0000	0.0569	0.0000	0.0029	0.0000	0.0489	0.0000	0.0000	0.0489	0.0000	0.0000	0.0000	0.0000	0.1046	0.1659	0.0720	0.0616	0.0494	0.3488	0.4534
10	Blood -10	Balloh	0.0677	0.0382	0.0209	0.0000	0.1268	0.0000	0.0000	0.0000	0.0034	0.0000	0.0000	0.0034	0.0000	0.0048	0.0000	0.0048	0.1350	0.0299	0.0330	0.4965	0.0683	0.6277	0.7627
11	Blood -11	Balloh	0.0407	0.0000	0.0193	0.0000	0.0600	0.0000	0.0061	0.0000	0.0381	0.0000	0.0000	0.0381	0.0000	0.0000	0.0000	0.0000	0.1042	0.0000	0.0000	0.0104	0.0139	0.0243	0.1285
12	Blood -12	Balloh	0.0115	0.0142	0.0159	0.0000	0.0416	0.0000	0.0132	0.0011	0.2554	0.0139	0.0000	0.2693	0.0000	0.0000	0.0000	0.0000	0.3252	0.0482	0.0000	0.0121	0.0147	0.0750	0.4002
13	Blood -13	Balloh	0.0085	0.0000	0.0182	0.0000	0.0267	0.0000	0.0159	0.0085	0.0091	0.0000	0.0097	0.0188	0.0035	0.0000	0.0000	0.0035	0.0734	0.0237	0.0232	0.0295	0.0308	0.1072	0.1806
14	Blood -14	Balloh	0.0089	0.0000	0.0449	0.0000	0.0538	0.0000	0.0031	0.0031	0.0388	0.0000	0.0000	0.0388	0.0039	0.0000	0.0000	0.0039	0.1027	0.0184	0.0207	0.0550	0.0000	0.0941	0.1968
15	Blood -15	Dher	0.1054	0.0000	0.0169	0.0000	0.1223	0.0000	0.0056	0.0032	0.0926	0.0000	0.0000	0.0926	0.0199	0.0000	0.0000	0.0199	0.2436	0.1849	0.0000	0.1834	0.0180	0.3863	0.6299
16	Blood -16	Dher	0.0105	0.0000	0.0136	0.0000	0.0241	0.0000	0.0129	0.0539	0.0360	0.0289	0.0454	0.1103	0.0539	0.0000	0.0000	0.0539	0.2551	0.1497	0.0355	0.0456	0.0418	0.2726	0.5277
17	Blood -17	Dher	0.0053	0.0000	0.0241	0.0000	0.0294	0.0000	0.0016	0.0358	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0668	0.0552	0.0466	0.1188	0.0382	0.2588	0.3256
18	Blood -18	Dher	0.0109	0.0166	0.0388	0.0000	0.0663	0.0110	0.0091	0.0027	0.0736	0.0259	0.0141	0.1136	0.0000	0.0000	0.0000	0.0000	0.2027	0.0000	0.0000	0.0000	0.0000	0.0000	0.2027
19	Blood 19	Dher	0.0059	0.0000	0.0219	0.0000	0.0278	0.0000	0.0071	0.0029	0.0370	0.0110	0.0083	0.0563	0.0000	0.0000	0.0000	0.0000	0.0941	0.1030	0.0549	0.0262	0.0238	0.2079	0.3020
20	Blood 20	Dher	0.0050	0.0000	0.0197	0.0000	0.0247	0.0000	0.0000	0.0000	0.0250	0.0000	0.0000	0.0250	0.0000	0.0000	0.0000	0.0000	0.0497	0.0638	0.0280	0.0586	0.0000	0.1504	0.2001
		Average Punjab	**0.0281**	**0.0063**	**0.0227**	**0.0000**	**0.0570**	**0.0006**	**0.0062**	**0.0090**	**0.0450**	**0.0102**	**0.0100**	**0.0652**	**0.0044**	**0.0002**	**0.0000**	**0.0046**	**0.1424**	**0.0948**	**0.0366**	**0.0662**	**0.0301**	**0.2278**	**0.3701**

Note: 1.Each value is an average of duplicate

2.Female-F Male-M

3.t-HCH=α+β+γ+δ HCH , t-DDT-DDD+DDE+DDT, t- endosulfan- α+ β + endosulfan sulfate

4.Dieldrin, dichlorvos, acephate, phorate, diazinon, dimethoate, fenthion, quinalfos, phenthoate, profenofos and ethion were not detected in any of the samples.

Mean, Median and Range of pesticide residues (mg/l) in whole blood samples collected from 20 individuals from villages in Punjab **Annexue III**

RESIDUES(mg/l in whole blood)																								
												ORGANOCHLORINES									ORGANOPHOSPHORUS			
S.No		a-HCH	b-HCH	g-HCH	d-HCH	t-HCH	Heptachlor	Aldrin	Chlordane	DDE	DDD	DDT	t-DDT	a-endosulfan	b-endosulfan	Endosulfan sulfate	t-endosulfan	Total Organochlorines	Monocrotofos	Phosphamidon	Chlorpyrifos	Malathion	Total Organophosphorus	Total Pesticides
	PUNJAB																							
1	Total No of individuals	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
2	No of samples in which pesticide detected	19	7	20	0	20	1	16	14	19	11	10	19	5	1	0	6	20	15	14	17	14	17	20
3	Percentage tested positive	95	35	100	0	100	5	80	70	95	55	50	95	25	5	0	30	100	75	70	85	70	85	100
4	Mean (mg/l))	0.0281	0.0063	0.0227	0.0000	0.0570	0.0006	0.0062	0.0090	0.0450	0.0102	0.0100	0.0652	0.0044	0.0002	0.0000	0.0046	0.1424	0.0948	0.0366	0.0662	0.0301	0.2278	0.3701
5	Median(mg/l)	0.0136	0.0000	0.0190	0.0000	0.0542	0.0000	0.0059	0.0028	0.0365	0.0065	0.0027	0.0526	0.0000	0.0000	0.0000	0.0000	0.1185	0.0517	0.0321	0.0358	0.0273	0.1924	0.3290
6	Mininimum(mg/l)	0.0000	0.0000	0.0136	0.0000	0.0241	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0497	0.0000	0.0000	0.0000	0.0000	0.0000	0.1224
7	Maximum(mg/l)	0.1054	0.0382	0.0569	0.0000	0.1279	0.0110	0.0159	0.0539	0.2554	0.0473	0.0485	0.2693	0.0539	0.0048	0.0000	0.0539	0.3252	0.4915	0.1282	0.4965	0.0753	0.6409	0.7627

Note:

1.t-HCH=α+β+γ+δ HCH , t-DDT-DDD+DDE+DDT, t- endosulfan- α+ β + endosulfan sulfate

2.Dieldrin, Dichlorvos, acephate, phorate, diazinon, dimethoate, fenthion, quinalfos, phenthoate, profenofos and ethion were not detected in any of the samples.