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Research Article

DETERMINATION OF PESTICIDE RESIDUES IN BLOOD SERUM SAMPLES FROM INHABITANTS OF "DAL LAKE" HAMLETS 'IN J & K, INDIA (2008-2010)

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ABSTRACT

Dal Lake a Sub Himalyan urban Lake is one of the most beautiful lakes of India and second largest in state of Jammu & Kashmir. The surrounding area of Dal Lake and its floating gardens form the most fertile area where a variety of vegetables are grown and extensively consumed by the inhabitants of Dal Lake. During recent years an indiscriminate use of wide variety of pesticides have been witnessed to combat pest and increase the yield. The present study was planned to assess the burden of chronic exposure to pesticides by estimation of pesticide residual levels in human serum samples taken randomly from the inhabitants of Dal Lake hamlets. Exposure of humans to hazardous chemicals occurs directly in the fields and indirectly due to consumption of contaminated diet or by inhalation or by dermal contact. The study was conducted from 2008 – 2010 in which a total of 600 blood samples taken from three population groups designated as PG-1 ,PG-2 (Study groups) and PG-3 (Control group). Blood samples were analysed for seven commonly used pesticides viz. Butachlor, γ-HCH, chlorpyrifos, Hexaconazole, Endosulfan 1, Endosulfan 2 and Dichlorovos. Detection and quantification of pesticide residues was performed by Gas Chromatography-Mass spectrometer (Thermo Finnigan Polaris Q type) equipped with Ni Electron Capture Detector. Out of seven pesticides analysed only chlorpyrifos was detected in all the three population groups. Mean concentration of chlorpyrifos in study groups (PG-1 & PG-2) was 0.5194 ± 0.6456 ng/µl and in control group (PG-3) was 0.0008 ± 0.0009 ng/µl. An ANOVA (Analysis of variance) was carried out and a difference was found highly significant in mean concentration of chlorpyrifos between PG-1, PG-2 and PG-3 with F value of 33.39 and P value of 0.000. Tuckey HSD test reveals that PG-1 & PG-2 did not differ (P value, 0.300) in their mean concentration. PG-3 differs significantly with PG-1 & PG-2 (P value, 0.000). This reveals that mean level of pesticide concentration is higher in study population than control group. The findings suggest that chronic low dose exposure to pesticides either directly or indirectly can be a major contributor for presence of pesticide residual levels in human blood.

Keywords: Pesticide, Chlorpyrifos, Blood, Gas Chromatogram Mass Spectrometer.

INTRODUCTION

Pesticides have been used by humans since times immemorial to protect their crops and vectors of human diseases. In spite, of the tremendous uses of pesticides these are also fraught with alarming consequences related to human health and its various components. Rampant and indiscriminate use of pesticides has affected the non target population i.e humans by directly exposing them to serious problems such as teratogenesis, mutagenesis, dermatological, endocrine and neurological dysfunction. Moreover, increased concentration of certain pesticides in the environment are directly related to carcinogenesis. The use of pesticides in large quantities and remarkable biological persistence in environment causes their widespread presence in all elements of food chain, which particularly involves water, vegetation and fish etc. Human beings at the top of food chain are obviously exposed to harmful effects of pesticides from every quarter. Contaminated diet even with a traces of these hazardous chemicals is a chronic unavoidable source of exposure[2,16,18]. Due to their non-polar and lipophilic nature, these are resistant to degradation and persist in the environment and tend to accumulate in lipid rich tissues of the organism and get biomagnified[36]. So therefore, both human life and environment are equally at a high risk due to noxious effect of these chemicals[14,17,19]. Since human health has been a matter of primary concern so many studies which have so far been done arrived at the conclusion that pesticide exposure is associated with chronic health ailments. These primarily include dermatologic, respiratory, memory disorders[1,26],neurodeficits[13,20],birth defects and miscarriages [7, 9, 11, 12, 15, 25, 30,31,34,38]. Moreover, bioaccumulation of pesticide residues show an association to cause cancer, depression, seizure disorder, liver and kidney dysfunction [3,8,10,29,32]. "Dal Lake" one of the most beautiful and famous lakes of India with its pristine glory is second largest lake in state of Jammu and Kashmir. The "Dal" is used for major economic activities relating to tourism, site seeing, recreational activities, fisheries, harvesting of food and fodder plants. The floating gardens of the lake that have originated with time have now assumed a status of biggest vegetable producing bowl of Kashmir. Keeping in view substantial short and long term health risks [40,41] as well as environmental damage/contamination[6], associated with indiscriminate use of pesticide a study was designed with an aim to determine pesticide residual levels in blood serum of both occupationally and non occupationally exposed individuals living within and surrounding areas of Dal Lake which would act as a good indicator to know the quantum of toxic exposure to various pesticides.

MATERIALS AND METHODS

Collection of sample and population description.

The present study was carried out between the years 2008 and 2010 during which 600 blood samples were obtained from three population groups, with 250 blood samples from population living within Dal Lake designated as PG-1, 250 blood samples from population living 1 km from the shore of Dal Lake designated PG-2 and 100 blood samples from population living more than 4 km away from the Dal Lake designated as PG-3 which acted as control group (fig.1). All subjects were asked to fill up a questionnaire to gather detailed information about age, sex, ethnicity, marital status, education, occupation, food habits, economic status, health and disease status. With regard to pesticides, a detailed information was acquired, based on predesigned clinical record form (CRF), like occupational use of pesticides, direct or indirect exposure to pesticides, exposure of pesticides through food chain (water, vegetation, fish), brands /chemical name of pesticides used, frequency of use of pesticides, seasonality of pesticide use, duration for which pesticides have been used, mode of use of pesticides, quantity of pesticide used, area or crop specific pesticide.

Extraction of Pesticide Residues in Blood Samples

Blood from subjects (5ml), irrespective of age and sex, were collected in a vaccutainer. All the collected blood samples were centrifuged at 3000 rpm for 20 minutes. After centrifugation, serum was separated and transferred into centrifuge tubes. The extraction process was performed

using the method suggested by[5] with some modifications. All the chemicals used were of analytical grade. Ethyl-acetate and hexane (5ml) in the ratio of 1:1 was added to the serum samples. The mixture was shaken vigorously on a vortex mixer for 1 minute. The mixture was centrifuged for 20 minutes at 3000 rpm. The organic layer was separated and collected in a round bottom flask. The elute was completely evaporated with the help of a rotary vacuum evaporator. The dried extract was dissolved in 2 ml of ethyl-acetate. The sample was then ready for analysis by GC-MS/MS. All compounds were identified by their retention times as compared with known standards.

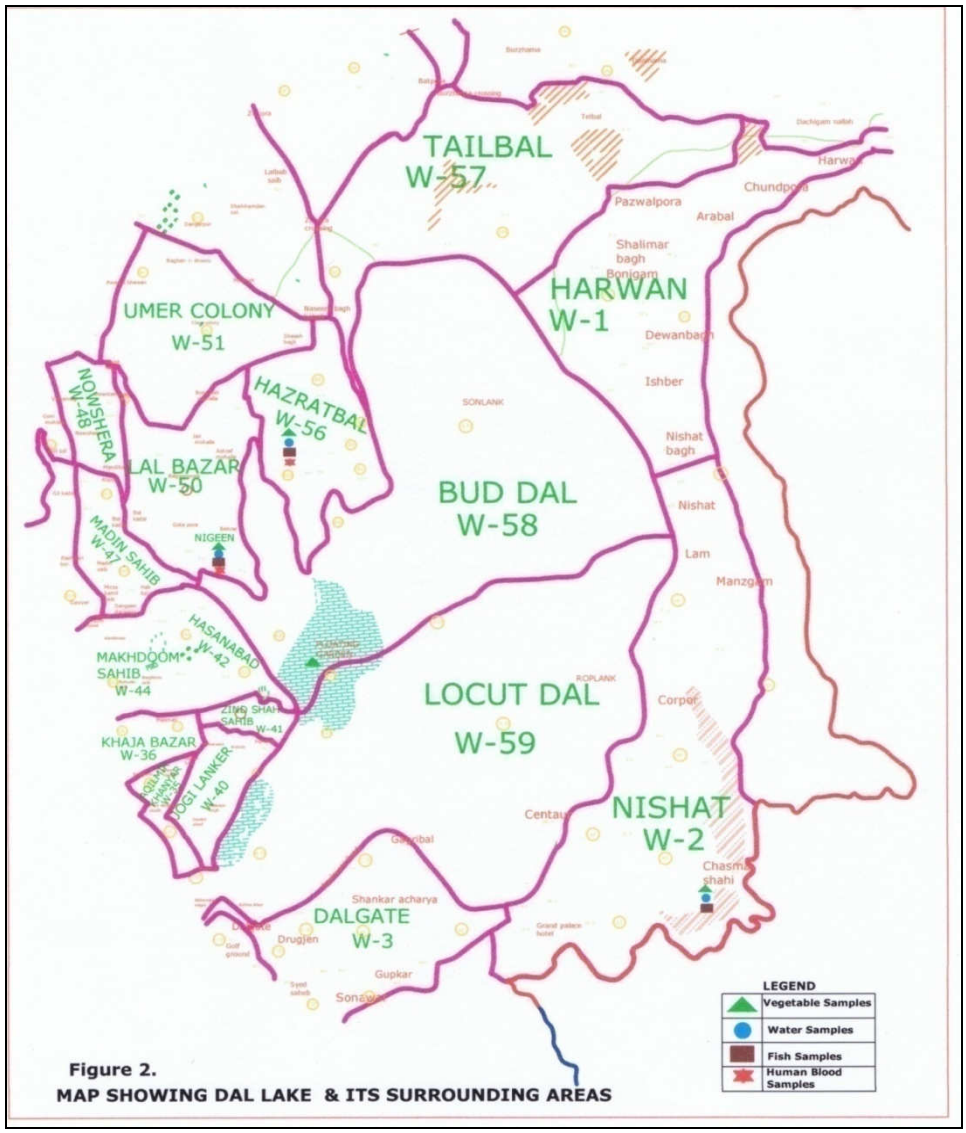

Figure 2.
MAP SHOWING DAL LAKE & ITS SURROUNDING AREAS

Analysis

The analysis includes a qualitative and quantitative estimation of pesticides with the help of Gas Chromatograph Mass Spectrometer (Thermo Finnigan Polaris Q type) equipped with Ni Electron Capture Detector (ECD). The GC operating parameters were: Injector temperature 80 - 270^{0}C, first ramp temperature @ 25^{0}C/min to 200^{0}C, second ramp temperature 200 C/min to 2300C for 1 min and final temperature @ 20^{0}C/min to 280^{0}C used for 10 min. Purified helium gas was used as carrier gas and a known volume (25µl) of the sample was injected in. A column of 30 m length, 0.25mm i.d and 0.25µm film thickness (liquid stationary phase of Dimethylpolysiloxane) was used. Different peaks of the sample were identified by comparing their retention times with those of standards obtained from pesticide from Pesticides India Limited. Seven commonly used pesticides were chosen and extraction procedures were performed for residual pesticide analysis by GC-MS/MS. The pesticides included were Butachlor (herbicide), Hexachlorocyclohexane, γHCH, chlorpyrifos, Hexaconazole, Endosufan-I , Endosulfan-2 and Dichlorvos (DDVP).

Statistical Analysis

A Z test was carried out to ascertain any significant difference in mean concentration of detected pesticide between study and control population. A "P" (< 0.05) was considered to be statistically significant. An ANOVA (Analysis of variance) was carried to verify the difference in mean level of pesticide residual concentration in three groups viz; PG-1, PG-2, PG-3 respectively.

RESULTS

Percentage occurrence of pesticide residue

In the study population (PG-1 and PG-2) 500 blood samples taken randomly from males and females were analysed for selected pesticides. Out of the seven pesticides analysed, chlorpyrifos was the only pesticide detected in 411 (82.2%) of blood samples with 197 (47.9%) being males and 214 (52.1%) being females. In control population (PG -3) 100 blood samples taken randomly from males and females were also analysed for aforementioned selected groups of pesticides. Chlorpyrifos was the only pesticide to be detected in

49 (49%) samples with 26 (53.1%) being males and 23(46.9%) being females. The number and percentage of subjects in study and control population showing presence of chlorpyrifos residual level in blood samples are presented in (Table 1).

Table 1: Number and percentage of subjects in study and control population showing presence of Chlorpyrifos residual level in blood samples

		Presence							
		Positive Group				Negative Group			
		Study Pop.		Control Pop.		Study Pop.		Control Pop.	
		Number	% age	Number	% age	Number	% age	Number	% age
Sex	Male	197	47.9%	26	53.1%	41	46.1%	30	58.8%
	Female	214	52.1%	23	46.9%	48	53.9%	21	41.2%
Total		411	100.0%	49	100.0%	89	100.0%	51	100.0%
Occupation	Farmworker	160	38.9%	0	0%	38	42.7%	0	.0%
	Non farmworker	251	61.1%	49	100.0%	51	57.3%	51	100.0%
Total		411	100.0%	49	100.0%	89	100.0%	51	100.0%
Smoking	Smoker	211	51.3%	15	30.6%	44	49.4%	19	37.3%
	Non smoker	200	48.7%	34	69.4%	45	50.6%	32	62.7%
Total		411	100.0%	49	100.0%	89	100.0%	51	100.0%
Exposure to pesticides	Direct exposure to pesticides	160	38.9%	0	0%	32	36.0%	0	.0%
	Indirect exposure to pesticides	251	61.1%	49	100.0%	57	64.0%	51	100.0%
Total		411	100.0%	49	100.0%	89	100.0%	51	100.0%
Use of pesticides	Occupational users of pesticides	134	32.6%	0	0%	29	32.6%	0	0%
	Non occupational users of pesticides	277	67.4%	49	100.0%	60	67.4%	51	100.0%
Total		411	100.0%	49	100.0%	89	100.0%	51	100.0%
Hypertension	Hypertensives	116	28.2%	21	42.9%	24	27.0%	15	29.4%
	Normotensives	295	71.8%	28	57.1%	65	73.0%	36	70.6%
Total		411	100.0%	49	100.0%	89	100.0%	51	100.0%

		Presence							
		Positive Group				Negative Group			
		Study Pop.		Control Pop.		Study Pop.		Control Pop.	
		Number	% age	Number	% age	Number	% age	Number	% age
Hypothyroidism	Hypothyroids	27	6.6%	11	22.4%	4	4.5%	11	21.6%
	Euthyroids	384	93.4%	38	77.6%	85	95.5%	40	78.4%
Total		411	100.0%	49	100.0%	89	100.0%	51	100.0%
Dermatologic complications	Dermatological complications	31	7.5%	0	0%	9	10.1%	4	7.8%
	No dermatological complications	380	92.5%	49	100.0%	80	89.9%	47	92.2%
Total		411	100.0%	49	100.0%	89	100.0%	51	100.0%
Psychiatric complications	Psychiatric disorder	70	17.0%	6	12.2%	23	25.8%	9	17.6%
	No significant Psychiatric disorder	341	83.0%	43	87.8%	66	74.2%	42	82.4%
Total		411	100.0%	49	100.0%	89	100.0%	51	100.0%
GIT disorder	GIT disorder	76	18.5%	4	8.2%	22	24.7%	4	7.8%
	No GIT disorder	335	81.5%	45	91.8%	67	75.3%	47	92.2%
Total		411	100.0%	49	100.0%	89	100.0%	51	100.0%
Neurological disorder	Neurodisorder	77	18.7%	8	16.3%	15	16.9%	8	15.7%
	No apparent neurological disorder	334	81.3%	41	83.7%	74	83.1%	43	84.3%
Total		411	100.0%	49	100.0%	89	100.0%	51	100.0%
Kidney problem	kidney disorder	22	5.4%	0	0%	2	2.2%	3	5.9%
	Not any kidney disorder	389	94.6%	49	100.0%	87	97.8%	48	94.1%
Total		411	100.0%	49	100.0%	89	100.0%	51	100.0%
Respiratory diseases	Respiratory disorder	118	28.7%	6	12.2%	24	27.0%	2	3.9%
	No respiratory disorder	293	71.3%	43	87.8%	65	73.0%	49	96.1%
Total		411	100.0%	49	100.0%	89	100.0%	51	100.0%
Anemia	Anemic	42	10.2%	0	0%	10	11.2%	4	7.8%
	Not Anemic	369	89.8%	49	100.0%	79	88.8%	47	92.2%
TOTAL		411	100.0%	49	100.0%	89	100.0%	51	100.0%

Concentration of Chlorpyrifos Residual Levels in serum samples with regard to gender, occupational and disease categories.

The mean concentration of chlorpyrifos in the 500 subjects of the study population (PG-1 and PG-2) as shown in (Table 2) was 0.5194 ± 0.6456 ng/µl whereas in control population (PG-3) the mean concentration was 0.0008 ± 0.0009 ng/µl.

The levels of organophosphate pesticide residue viz. chlorpyrifos for all the subjects are shown in (Table 3).The mean concentration of

this pesticide in the study population in males was 0.5048 ± 0.6475ng/µl with concentration ranging from undetected to 1.9830 ng/ µl whereas in females it was 0.5326 ± 0.6448 ng/ µl ranging from undetected to 0.6448 ng/µl. In the control population the mean concentration of chlorpyrifos in males was 0.0008 ± 0.0009 ng/µl with concentration ranging from undetected to 0.0030ng/µl whereas in females it was 0.0007 ± 0.0008ng/µl with concentration ranging from undetected to 0.0030 ng/µl.

Table 2: Mean concentration (ng/µl) and standard deviation of Chlorpyrifos residual levels quantified in human serum samples in study and control population

		Group			
		Study pop. Pesticide level ng/µL		Control pop. Pesticide level ng/µL	
		Mean	**S.D**	**Mean**	**S.D**
Sex	Male	.5048	.6475	.0008	.0010
	Female	.5326	.6448	.0007	.0008
Total		**.5194**	**.6456**	**.0008**	**.0009**

***** *Standard Deviation*

Table 3: Mean concentration (ng/µl), standard deviation (SD) and ranges of Chlorpyrifos residual level quantified in human blood samples in study & control population from subjects of selected occupational and disease categories.

		Group							
		Study population				Control population			
		Pesticide level ng/µL				Pesticide level ng/µL			
		Mean	**SD***	**Min.**	**Max.**	**Mean**	**S.D.**	**Min.**	**Max.**
Sex	Male	.5048	.6475	.0000	1.9830	.0008	.0010	.0000	.0030
	Female	.5326	.6448	.0000	1.9830	.0007	.0008	.0000	.0030
Occupation	Farmworker	.5296	.6455	.0000	1.9830	N.A.	N.A.	N.A.	N.A.
	Non farmworker	.5127	.6466	.0000	1.9830	.0008	.0009	.0000	.0030
Smoking	Smoker	.5039	.6452	.0000	1.9830	.0008	.0011	.0000	.0030
	Non smoker	.5355	.6469	.0000	1.9830	.0007	.0009	.0000	.0030
Exposure to pesticides	Direct exposure to pesticides	.5142	.6736	.0000	1.9830	N.A.	N.A.	N.A.	N.A.
	Indirect exposure to pesticides	.5226	.6285	.0000	1.9830	.0008	.0009	.0000	.0030
Use of pesticides	Occupational use of pesticides	.5213	.6705	.0000	1.9830	N.A.	N.A.	N.A.	N.A.
	Non occupational use of pesticides	.5185	.6342	.0000	1.9830	.0008	.0009	.0000	.0030
Hypertension	Hypertensives	.5763	.6674	.0000	1.9830	.0010	.0010	.0000	.0030
	Nomotensives	.4972	.6364	.0000	1.9830	.0006	.0009	.0000	.0030
Hypothyroidism	Hypothyroids	.5650	.6324	.0000	1.9750	.0007	.0008	.0000	.0020
	Euthyroids	.5164	.6470	.0000	1.9830	.0008	.0010	.0000	.0030
Dermatologic complications	Dermatological complication	.4409	.6390	.0000	1.9580	.0000	.0000	.0000	.0000
	No dermatological complication	.5262	.6464	.0000	1.9830	.0008	.0009	.0000	.0030
Psychiatric complications	Psychiatric disorder	.4683	.6207	.0000	1.9830	.0007	.0010	.0000	.0030
	No significant Psychiatric disorder	.5310	.6513	.0000	1.9830	.0008	.0009	.0000	.0030
GIT disorder	GIT disorder	.4837	.6903	.0000	1.9750	.0006	.0007	.0000	.0020
	No GIT disorder	.5281	.6348	.0000	1.9830	.0008	.0010	.0000	.0030
Neurological disorder	Neuro disorder	.4977	.6106	.0000	1.9750	.0010	.0012	.0000	.0030
	No apparent neurological disorder	.5243	.6538	.0000	1.9830	.0007	.0009	.0000	.0030
Kidney problem	kidney disorder	.5438	.7040	.0000	1.9830	.0000	.0000	.0000	.0000
	Not any kidney disorder	.5181	.6433	.0000	1.9830	.0008	.0009	.0000	.0030
Respiratory diseases	Respiratory disorder	.6157	.7001	.0000	1.9830	.0016	.0013	.0000	.0030
	No respiratory disorder	.4812	.6195	.0000	1.9830	.0007	.0009	.0000	.0030
Anaemia	Anaemic	.5158	.6240	.0000	1.6570	.0000	.0000	.0000	.0000
	Not Anaemic	.5198	.6487	.0000	1.9830	.0008	.0009	.0000	.0030

***** Standard Deviation

N.A. Not Applicable

Comparison of mean concentration of chlorpyrifos level (ng/µl) in males and females, farm workers and non farmworkers, occupational and non-occupational users of pesticides, hypertensives and normotensives, hypothyroid and euthyroid, those with respiratory and no respiratory diseases, and mean concentration of chlorpyrifos in hypertensive and normotensives, hypothyroids and euthyroids, those with respiratory and without respiratory diseases reveal that females had high level of chlorpyrifos 0.5326 ± 0.6448ng/µl than males 0.5048 ± 0.6475ng/µl, farmworkers had high level 0.5296 ± 0.6455ng/µl than non farmworkers 0.5127 ± 0.6466ng/µl, occupational workers of pesticides had high level 0.5213 ± 0.6705ng/µl than non-occupational workers 0.5185 ± 0.6342 . Likewise hypertensives 0.5763 ± 0.6674ng/µl, hypothyroid 0.5650 ± 0.6324ng/µl and those with respiratory diseases 0.6517 ± 0.7001ng/µl had higher levels than normotensives (0.4972 ± 0.6364ng/µl), euthyroids (0.5164 ± 0.6470) and without respiratory disorders 0.4812 ± 0.6195ng/µl.The highest concentration of chlorpyrifos level was found in individuals with respiratory diseases, followed by hypertensives and hypothyroids respectively. In control population chlorpyrifos residual level quantified in blood samples was substantially low in all categories of subjects as depicted in (Table 3).Comparing mean levels of chlorpyrifos in PG-1, PG-2 and PG-3 it was observed that mean concentration of chlorpyrifos in PG-1, PG-2 and PG-3 was 0.5583 ± 0.6893 ng/µl, 0.4803 ± 0.598 and 0.0007± 0.005 respectively. Moreover, in PG-1 females had higher level 0.5910 ± 0.6890 ng /µl than males 0.5226 ± 0.0.6886ng/µl. In PG-2, males had slightly higher level (0.4871 ± 0.6060ng/µl) than females

0.4741 ± 0.5933ng/µl and in PG-3 level of chlorpyrifos in males and females are 0.0008 ± 0.0010ng/µl and 0.0007 ± 0.0008ng/µl respectively.

Concentration of chlorpyrifos in different age groups

While comparing mean concentration of chlorpyrifos in different age groups as shown in (Table. 4).It was observed that mean concentration was highest in males 0.5602 ± 0.6985ng/µl in age group 21- 40 years and among females it was found highest 0.5911 ± 0.6907 ng/µl in age group 41- 60 years in study population. In occupational workers it was found highest 0.6627 ± 0.7266ng/µl in age group 21-40 years, in hypertensives it was highest 0.6162 ± 0.7118ng/µl in age group 21-40 years, in individuals with respiratory disease it was found highest 0.8212 ± 0.8195ng/µl in 61-80 years age group in the study population. In control population the values are very low in all the categories.

Table 4: Comparison of mean concentration of chlorpyrifos residue level analysed in serum samples of study and control population of different age groups.

		(Age Years)															
		1-20				21-40				41-60				61-80			
		Study Pop.		Control Pop.		Study Pop.		Control Pop.		Study Pop.		Control Pop.		Study Pop.		Control Pop.	
		Pesticide level ng/µl		Pesticide level ng/µl		Pesticide level ng/µl		Pesticide level ng/µl		Pesticide level ng/µl		Pesticide level ng/µl		Pesticide level ng/µl		Pesticide level ng/µl	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Sex	Male	.4997	.5461	.0000	.0000	.5602	.6985	.0004	.0005	.4149	.5828	.0010	.0012	.5490	.7121	.0009	.0009
	Female	.5418	.6346	.0000	.0000	.5081	.6167	.0003	.0005	.5911	.6907	.0009	.0010	.4536	.6424	.0008	.0004
Occupation	Farmworker	.6167	.6472	NA.	NA.	.3986	.5774	NA.	NA.	.5980	.6801	NA.	NA.	.5750	.6953	NA..	NA..
	Non Farmworker	.4680	.5674	.0000	.0000	.6065	.6885	.0003	.0005	.4215	.6023	.0010	.0011	.4584	.6666	.0009	.0008
Smoking	Smoker	.5066	.5800	NA.	NA.	.5162	.6554	NA.	NA.	.5162	.6715	.0008	.0012	.4521	.6528	.0009	.0009
	Non smoker	.5457	.6264	.0000	.0000	.5550	.6641	.0003	.0005	.4966	.6258	.0011	.0010	.5808	.7144	.0010	.0000
Direct Exposure to pesticides	Direct exposure to pesticides	.4875	.5128	NA.	NA.	.5645	.7474	NA.	NA.	.4608	.6379	NA.	NA.	.5336	.6807	NA.	NA.
	Indirect exposure to pesticides	.5414	.6345	.0000	.0000	.5149	.5958	.0003	.0005	.5415	.6511	.0010	.0011	.4842	.6781	.0009	.0008
Use of pesticides	Occupational users of pesticides	.5288	.6171	NA.	NA.	.6627	.7266	NA.	NA	.4051	.6295	NA.	NA.	.4975	.6904	NA.	NA.
	Non occupational users of pesticides	.5249	.5968	.0000	.0000	.4893	.6287	.0003	.0005	.5662	.6490	.0010	.0011	.5023	.6735	.0009	.0008
Hypertension	Hypertensives	.5557	.5265	.0000	.0000	.6162	.7118	NA.	NA.	.5794	.6935	.0012	.0010	.5124	.6750	.0010	.0009
	Nomotensives	.5150	.6306	.0000	.0000	.5079	.6400	.0003	.0005	.4752	.6242	.0008	.0011	.4940	.6817	.0008	.0007

DISCUSSION

The results of the present study reveals that out of seven pesticide residues analysed only chlorpyrifos (o,o-diethyl-o-(3,5,6-trichloro-2-pyridyl phosphorothioate), an organophosphate pesticide was detected in human serum samples. The results demonstrated an indiscriminate availability and use of organophosphate pesticide chlorpyrifos in vegetable fields. There was also a policy shift towards substituting organochlorines with organophosphates and carbamates, which are considered less persistant and cumulative. Chlorpyrifos is one of about 100 organophosphate (OP) insecticides on the market today. It is used to kill insect pests by disrupting their nervous system. It is a broad spectrum insecticide used on a wide variety of crop types, for the control of locusts and is present in some cattle dips for the control of ticks and lice, besides being registered for use in domestic gardens, as indoor insect control, termiticide, for pest products and commercial and industrial insect control. Diet is recognized to be a pathway of human exposure to chlorpyrifos yet the relationship between chlorpyrifos residues in food and personal exposure to chlorpyrifos is not well understood[23].This information would be valuable for evaluating the relationship between personal exposure and possible health effects as low levels of exposure to chlorpyrifos could cause subtle neurological effects[27,28]but methods for estimating chlorpyrifos exposure from food intake have not been evaluated.

In the present study chlorpyrifos was detected in 82% of blood samples of study population with concentration mean of 0.5194 ± 0.6456ng / µl and in control population with a concentration mean of 0.0008±0.009 ± ng/ µl. The higher level of chlorpyrifos in study group than control group can be primarily attributed to the dietary and occupational factors, as the population living within and around the areas of Dal Lake are exclusively dependent on food produced within the Dal Lake in the form of vegetables and fish, as well as dermal exposure by occupational use of pesticides. Some studies suggest that in addition to diet, other potential routes of exposure to

chlorpyrifos include inhalation, dermal absorption, and incidental ingestion of soil and settled dust [39,42].

Mean concentration of females (0.5326 ± 0.644 ng/µl) was found slightly higher than males 0.5048±0.647 ng/µl. In the valley of Jammu & Kashmir females are aggressively involved in all sort of agricultural practices and males have taken up other occupations besides agribusiness. Similar results have also observed with some organochlorine pesticide in which ∑DDT concentration was found higher in female populations in Coimbra,Portugal[21]. Likewise, highest concentration of DDT was found in women from Canary Island [43] .

It was observed that mean concentration of farmworkers 0.5296 ± 0.6455 ng/µl and commercial pesticide applicators 0.5213 ± 0.6705ng/µl were slightly more than non farm workers 0.5127 ± 0.6466 ng/µl and non occupational users 0.5185 ± 0.6342 ng/µl. It is quite obvious that both farmworkers and occupational workers of pesticides are directly as well as indirectly exposed to the pesticide application than those not involved directly in agribusiness. Similar kind of studies have been conducted in India in different villages of Punjab[23] where chlorpyrifos was detected in 85% of blood samples with a mean level of 0.0622 mg/L and ranged from not detected to 0.4965mg/L. the results correlate with the results obtained in our study.

With regards to study population (SPG1 and SPG2) and control population (SPG3) the difference in the chlorpyrifos residual levels in blood samples between the three groups was determined statistically. ANOVA with regard to chlorpyrifos residual levels of the three groups viz; SPG1, SPG2, and SPG3 revealed that the difference was highly significant with F value of 33.39 and P value of 0.000. Further, Tukey HSD reveals that SPG1 and SPG2 does not differ (P value 0.300) in their mean concentration. SPG3 differs significantly with SPG1 and SPG2 (P value 0.000). The test revealed that mean level of pesticide contamination was higher in study population than control group. A P-value less than 0.05 was considered to be statistically significant.

Study related to health effects was also carried out on Dal Lake population which are vulnerable to chronic low dose non-target pesticide exposure. The results reveal that certain diseases in study population are present with a higher percentage than control population. These include hypertension, hypothyroidism, dermatological conditions, respiratory problems, psychiatric disorders and neurological disorders. Chronic low dose exposure of organophosphate pesticide like chlorpyrifos both directly and indirectly can pose a great amount of health risk to the population in long term. Organophosphates are associated with well known acute health problems such as nausea, dizziness, vomiting, headaches, abdominal pain and skin and eye problems.

Organophosphate insecticide exposure has been reported to be associated with affective disorders such as depression[22].Termite applicators exposed to Chlorpyrifos reported more neurological symptoms including fatigue, loss of muscle strength and depression[33].

Currently the direct estimation of pesticide residual level in biological and environmental samples is a good indicator for assessing the total body burden of pesticide exposure in human and in environment. Alternatively, dietary exposure to chlorpyrifos and estimation of 3,5,6-trichloro-2-pyridinol (TCP y) in urine of healthy volunteers suggests that intake of chlorpyrifos from food is a minor contributor to TCPy in urine **.** Studies related to health outcomes in a population associated with chronic low level exposure to pesticides are possible now by use of latest biological and genetic techniques falling into three areas viz; markers of DNA and RNA damage or repair, indicators of oxidative stress and markers of changes in gene expression related to exposure to pesticide have been developed4,35.The biomarkers assays are in a developmental stages and have not so far been used in populations extensively exposed to low dose pesticide. Though they lack in concrete evidence of an association between the biomarker and specific health outcomes, yet they provide potential to increase our understanding of the biological mechanism associated with the health outcomes that have been associated with pesticide exposure in multiple epidemiological investigations.

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