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Pesticide Residues in Total Diet in Punjab, India: Contamination Levels and Human health Risk Assessment

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ABSTRACT

Status of pesticide residues in total diet in Punjab, India, was determined, and human health risks resulting from consumption of contaminated total diet were assessed. One hundred (100) vegetarian and non-vegetarian samples, comprising breakfast, lunch and dinner, were analyzed by gas chromatography. Pesticide residues were detected in 56% of the samples, including malathion, ethion, chlorpyrifos, lindane and permethrin at mean levels of 19.5, 7.4 2.3, 3.1 and 1.5 µgkg^{-1}, respectively. Ethion residues exceeded the Acceptable Daily Intake (ADI) in five samples. There were no significant differences ($p>0.05$) in the levels of contamination of each of the five different pesticides between vegetarian and non-vegetarian diets. For vegetarian total diet, the results showed that Hazard Index (HI) for all pesticides was less than 1 for the lower-bound (LB) scenario. However, in the upper bound (UB) scenario, HI was greater than 1 for ethion (3.2) and lindane (4.1). For non-vegetarian total diet, the results revealed that HIs for all pesticides were less than 1 (H<1) for the LB scenario, except ethion. In the UB scenario, HI was greater than 1 for ethion and lindane. The estimated risk of cancer for lindane at LB level was 1.75 x 10^{-4} and 1.23 x 10^{-4} for vegetarian and non-vegetarian diets, respectively; while at the UB level, the cancer risk for lindane in vegetarian diet was 1.38 x 10^{-3} and for non-vegetarian diet was 3.39 x 10^{-4}. However, cancer risk for lindane from vegetarian diet at UB level was estimated to be 1.38 X 10^{-3}, which is outside the recommended range of 10^{-4} to 10^{-6}. There is a shift to the use of organophosphates and synthetic pyrethroids in Punjab.

Keywords: Pesticide residues, Human health, Total diet, Hazard Index, Cancer risk

INTRODUCTION

Organophosphorus pesticides (OPPs), organochlorine pesticides (OCPs), carbamates, pyrethrins and pyrethroids, and dithiocarbamate (DTC) metabolites have been detected in various table-ready diets [1,2,3,4]. Toxicological studies using animal models and tests of associations in humans have revealed that exposure to pesticides might cause several diseases in humans that affect the endocrine, reproductive, neurological and immunes systems, and also result in cancers [5,6,2]. Regulatory agencies have set maximum limits of pesticide residues for individual food raw materials [7, 8]. As such, foods are usually analyzed unwashed and in the raw state, which does not consider dissipation of pesticides resulting from removal or breakdown of residues due to washing or cooking [9, 3]. Therefore, several total diet studies have been conducted to evaluate actual human health risks as a result of dietary intake of pesticide residues [10, 11, 12]. In India, the occurrence of Dichlorodiphenyltrichloroethane (DDT) in total diet has reduced over the last two decades owing to its usage restrictions in late 1990s [13], except for the control of vector-borne diseases like malaria. However, contamination of total diet with lindane increased during 1999 and 2000, reflecting similar trends in parallel studies of pesticides residues in liquid milk which constitute a substantial part of the diet [14, 15, 16]. In view of the importance and widespread application of pesticides in Punjab and the impact of pesticides on the environment and human health, and in consideration of the paucity of information on pesticide residues in total diet in Punjab, this study was conducted with the aim to monitor the level of contamination of total diet with pesticides and carry out human health risk assessment through dietary exposure to pesticides.

MATERIALS AND METHODS

Sampling

A total of one hundred (100) total diet samples were collected from boys' and girls' hostels of Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab in 2017. Both vegetarian and non-vegetarian total diet samples comprising breakfast, lunch and dinner were collected from the hostels in separately marked polythene bags and transported to the laboratory. The samples were stored in a deep freezer at -15 ^{o}C until analyzed. Prior to residue analysis of total diet, each of the samples was weighed and recorded.

Pesticide Standards

High-purity analytical standards of organochlorines (OCs), organophosphorus (OPs) and synthetic pyrethroids pesticides (SPs)) were purchased from Sigma-Aldrich, USA and Rankem Ltd, India, and used for recovery tests and quantification of residues in samples.

Extraction and cleanup of residues in total diet samples

The total diet samples were analysed following the methodology established by Battu et al. [16]. The vegetarian dietary components were amalgamated and titrated in a blender to create a smooth paste. Likewise, non-vegetarian ingredients were amalgamated and pulverized in a blender to create a smooth mixture. Subsequently, 10 g of the pulp paste was combined with 20 g of silica gel (60–120 mesh), 20 g of anhydrous sodium sulphate, and 1 g of activated charcoal in a mortar to produce a free-flowing powder. The powdered material was subsequently combined with dichloromethane and placed into an extraction glass column (60 x 2 cm i.d.) containing about 40 ml of dichloromethane above a plug of pre-washed cotton. The column was sealed and allowed to sit for approximately 90 minutes. Dichloromethane was eluted gradually from the column. As dichloromethane approached the adsorbent level, the column was subsequently eluted with 150 ml of a dichloromethane-acetone combination (1:2, v/v). Anhydrous sodium sulphate was added to the eluate to eliminate turbidity. The volume was reduced to around 2–3 ml under vacuum at temperatures below 30 °C. Subsequently, 10–15 ml of hexane was introduced, and the liquid was concentrated once more to approximately 5 ml. The technique was repeated to entirely eliminate dichloromethane, and the final volume was adjusted with an n-hexane: acetone (1:1) mixture.

Estimation of residues

The residues in purified extracts were measured via gas chromatography (GC). The Electron Capture Detector (ECD) was employed for organochlorine compounds, while the Flame Thermionic Detector (FTD) was utilised for organophosphorus compounds. ECD exploits the high electron affinity of chlorine in organochlorine pesticides, while FTD exploits the selective flame ionization behavior of phosphorus in organophosphorus pesticides, delivering maximum sensitivity and selectivity for each class. A 2 µl sample was put into the gas chromatograph (GC). The GC Solution software on the PC was utilised for the integration and calculation of signals. The chemicals were identified and quantified by comparing the retention times and peak heights/areas of the sample chromatograph with those of standards analysed under identical operating conditions. The formula employed for residue quantification was:

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

Recovery studies for pesticide residues in total diet samples

The method used for extraction and estimation of pesticide residues in total diet samples was validated by calculating the recovery from diet samples spiked with known concentrations of pesticide standards at 0.1mgkg$^{-1.}$ The mean recovery values of spiked samples ranged from 89.0 to % to 92.8% for vegetarian total diet, and 89.5 to 91.3% for non-vegetarian total diet. Repeatability of the method was also estimated by calculating the relative standard deviation of the recovery values, which were found to be below 10%. The limit of detection ranged from 1.0 ng g^{-1} for OCs and SPs to 5.0 ng g^{-1} for OPs. Data were subjected to one-way analysis of variance (ANOVA) and Duncan post hoc test using SPSS version 16.0. Mean group differences at $p<0.05$ were considered significant.

Human Health Risk Assessment from Exposure to Pesticides

The data were evaluated under two contaminations scenarios. In the first scenario, the lower-bound (UB) scenario, results from samples in which pesticides were not detected but considered as zero; the second scenario, the 95th-percentile upper-bound (UB) scenario entailed the setting of undetected results to the detection limit for the particular pesticide. The LB tends to underestimate contamination and exposure levels, while the UB results in overestimation [17]. The assessment of dietary pesticide exposure involved estimating the estimated daily intake (EDI) values of pesticides identified in dietary samples, in accordance with the Guidelines for Predicting Dietary Intake of Pesticide Residues established by the Food and Agricultural Organization/World Health Organization [18]. The estimated daily intake (EDI) of pesticides was derived from the subsequent equation:

$$EDI = C \times I$$

Where C represents the concentration of pesticide residues in the food, while I denotes the average total dietary intake per individual, both vegetarian and non-vegetarian, quantified at 2850 grammes per day. EDIs were expressed in µ/kg body weight/day of pesticide

The estimations of food intake of pesticide residues were compared with the Acceptable Daily Intake (ADI) levels established by FAO/WHO [19].

Potential risks of exposure were evaluated for both cancer and non-cancer effects based on the estimated dietary intake of pesticide residues as proposed by United States Environmental Protection Agency (USEPA) [21] and described by Bedi et al. [18]. For non-cancer risk, Hazard Ratio (HR) was estimated by dividing the average daily exposure by the benchmark concentration (BMC) for non-carcinogenic effects which are based on the reference dose (RfD) determined by USEPA Integrated Risk Information System for each contaminant. The oral Reference Dose (RfD) is predicated on the premise that thresholds exist for specific toxic effects, including cellular necrosis. It was quantified in milligrammes per kilogramme per day. The RfD is an estimation of daily exposure for the human population, including sensitive subgroups, that is expected to pose negligible risk of adverse effects over a lifetime [20].

$$\text{Hazard Ratio (HR)} = \frac{\text{Average daily exposure}}{\text{Benchmark concentration}}$$

The average daily exposure level for each pollutant in a population was determined using the following method:

Average daily exposure (μg/kg bw) = total diet consumption (g/kg bw) x contaminant concentration (μg/kg),

where bw denotes body weight. A Benchmark Concentration (BMC) denotes a daily concentration threshold beneath which there is a significant likelihood of no detrimental health consequences. A hazard ratio greater than 1 signifies that the mean daily exposure level is above the BMC, hence presenting a possible risk to human health [21].

The cancer risk was determined by multiplying the lifetime average exposure to a chemical by its carcinogenic potency factor. The cancer potencies for this analysis were sourced from USEPA. The Environmental Protection Agency (EPA) has historically recommended an acceptable cancer risk range of 10^{-6} to 10^{-4}, with 1 x 10^{-5} being the standard recommendation. The carcinogenic risk was determined using the subsequent calculation, assuming a 70-year lifespan for adults.

$$\text{Cancer risk} = \text{ADD X ED X (1/70) X CPF X } 10^{-6}$$

Where ADD is the average daily dose in ng kg^{-1}bw per day, ED is the exposure duration which is equal to the age of the exposed person (in this study, 60 years was used for adults). CPF is the cancer potency factor (in mgkg^{-1}bw per d)$^{-1}$.

3. **RESULTS AND DISCUSSION**

Levels of pesticide residues in total diet samples

Fifty-six percent (56%) of the 100 total diet samples analyzed were found to be contaminated with at least one of the following five different pesticides: malathion, ethion, lindane (γ-HCH) and permethrin. Malathion was detected at a mean level of 19.5 µgkg^{-1}, followed by ethion (7.4 µgkg^{-1}), lindane (3.1 µgkg$^{-1)}$, chlorpyrifos (2.3 µgkg$^{-1)}$ and the lowest mean residue level was found in permethrin (1.5 µgkg^{-1}).

In terms of frequency of occurrence (percentage positive), ethion recorded the highest (21%), followed by malathion (17%), permethrin (10%)), lindane (9%)) and chlorpyrifos (7%). However, the most predominant pesticide in terms of contribution to the overall pesticide load (percentage proportion) was malathion which accounted for 57.7% of the pesticide load, followed by Ethion (21.9%), lindane (9.2%), chlorpyrifos (6.8%) and the least contribution was recorded for permethrin (4.4%) (Table 1).

Table 1. Levels of pesticide residues in total diet samples from Punjab

Pesticide	**No. of positive samples out of 100**	**Mean residue level ± s.e. (µgkg^{-1})**	**% contribution to pesticide residue burden in total diet**	**ADI** (µgkg^{-1})	**Sample with residue level above ADI**
Malathion	17	19.5± 55.6	57.7	0-300	0
Ethion	21	7.4±17.2	21.9	0-2	5
Chlorpyrifos	7	2.3 ± 10.2	6.8	0-10	0
Lindane	9	3.1 ± 11.8	9.2	0-5	0
Permethrin	10	1.5 ± 7.4	4.4	0-50	0

n=100; > greater than; s.e. = standard error; ADI = Acceptable Daily Intake recommended by Codex Alimentarius Commission (CAC)

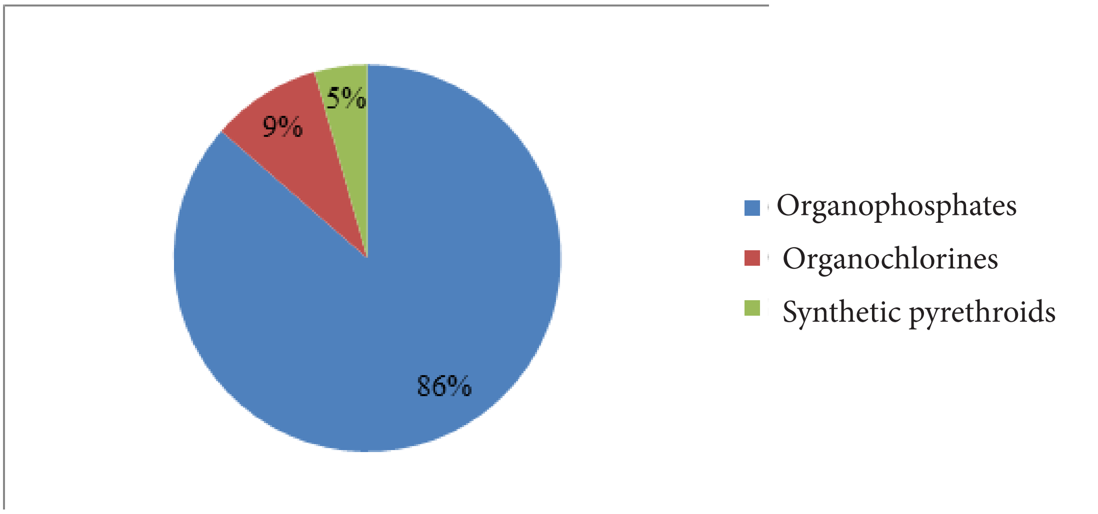

Fig. 1. Percentage contribution of different pesticides classes to pesticide load in total diet

Fig.1 shows the contribution of the three major pesticides classes to the total residue load, corroborating a paradigm shift in the usage of pesticide in Punjab toward the OPs as reported earlier by Bedi et al [17].

Levels of pesticide residues in vegetarian and non-vegetarian diet

A comparison of pesticides contamination patterns of vegetarian and non-vegetarian total diet samples are presented (Table 2). Malathion was detected in vegetarian diet at a mean level of 14.6 μgkg^{-1}, while it was found in non-vegetarian diet at a mean level of 38.1 $\mu gkg^{-1.}$ However, there was no significant difference in the levels of malathion in vegetarian and non-vegetarian total diet ($p=0.304$). Ethion was also detected in both vegetarian and non-vegetarian total diet at mean levels of 5.2 μgkg^{-1} and 15.5 μgkg^{-1}, respectively; but the two means did not differ significantly ($p=0.069$). The mean concentrations of chlorpyrifos in vegetarian and non-vegetarian total diet were 1.2 μgkg^{-1} and 6.3 μgkg^{-1}, respectively, but this difference was not statistically significant ($p=0.133$). Lindane was detected in vegetarian diet at a mean level of 3.3 μgkg^{-1}, while it was found in non-vegetarian diet at a mean level of 2.3 $\mu gkg^{-1.}$ However, there was no significant difference in the levels of lindane in vegetarian and non-vegetarian total diet ($p=0.729$). Lastly, the mean concentrations of permethrin in vegetarian and non-vegetarian total diet were 1.9

μgkg^{-1} and 0.2 μgkg^{-1} respectively, but this difference was not statistically significant (p=0.349).

The frequency of occurrence of each of the five pesticides in vegetarian and non-vegetarian total diet were also subjected to test of association with a view to determining the presence or absence of significant association between diet type and any of the pesticides detected. Statistically there was no association between presence of malathion and diet type (p=0.343), although malathion was detected in 15.2% of vegetarian diet samples compared with 23.8% of non-vegetarian diet samples. Likewise, despite the apparent difference in the frequencies of occurrence of ethion in vegetarian (17.7%) and non-vegetarian total diet (33.3), there was no association between ethion and diet type (p=0.137). The frequency of occurrence of chlorpyrifos in vegetarian diet (5.1%) was lower than in non-vegetarian diet (14.3%), but there was no statistically significant association between chlorpyrifos and diet (p=0.159). Lindane was detected in vegetarian and non-vegetarian total diet in 8.9% and 9.5% of the samples respectively, but the difference in diets was not statistically significant (p=1.00). The frequency of occurrence of permethrin in vegetarian diet (11.4%) was higher than in non-vegetarian diet (4.8%), but this difference was found to be non-significant (p=0.684). When the levels of residues detected in vegetarian and non-vegetarian total diet in this study was compared with the residue levels in earlier studies in Punjab [22,16]; and in USA, Germany, France, Australia and Hong Kong [22, 23, 24, 25, 26, 27], a declining trend of HCH and DDT residues in total diet was observed which is due to the decline in the use of these two pesticides. However, the preponderance of organophosphate pesticide residues in this study is due to the increase in the usage of these pesticides.

Table 2. Levels of pesticide residues in vegetarian and non-vegetarian diet

Pesticide	Vegetarian diet		Non-vegetarian diet	
	% frequency	**Mean pesticide residue level (µgkg^{-1}± s.e.)**	**% frequency**	**Mean pesticide residue level (µgkg^{-1} ± s.e.)**
Malathion	15.2	14.6 ± 41.8	23.8	38.1 ± 89.6
Ethion	17.7	5.2 ± 13.4	33.3	15.5 ± 26.1
Chlorpyrifos	5.1	1.2 ± 7.0	13.6	6.3 ± 18.9
Lindane	8.9	3.3 ± 12.4	9.0	2.3 ± 9.2
Permethrin	11.4	1.9 ± 8.3	4.8	0.2 ± 0.9

s.e.= standard error; vegetarian diet samples: n =78; Non-vegetarian diet samples: n =22;

Human health risk assessment from consumption of total diet

Out of the 78 vegetarian total diet samples analyzed, only one contained ethion residues above the ADI specification by CAC. There were no total diet samples that contained residues of chorpyrifos, malathion, lindane and permethrin above the ADI. Similarly, in the 22 non-vegetarian diet samples analyzed, only four samples had residues of ethion which exceeded the ADI (Table 3).

Table 3. Total dietary intake of pesticides (μgkg^{-1} person^{-1} day^{-1}) in vegetarian and non-vegetarian total diet

Pesticide	Vegetarian diet (Mean ± SD, Range)	Non-vegetarian diet (Mean ± SD, Range)
Malathion	0.7 ± 2.0 (0–11.7)	1.8 ± 4.3 (0–15.0)
Ethion	0.3 ± 0.6 (0–3.8)	0.7 ± 1.2 (0–3.7)
Chlorpyrifos	0.1 ± 0.3 (0–2.7)	0.3 ± 0.9 (0–3.7)
Lindane	0.2 ± 0.6 (0–3.1)	0.1 ± 0.4 (0–2.0)
Permethrin	0.1 ± 0.4 (0–3.2)	0.009 ± 0.04 (0–0.19)
ADI (μg kg^{-1} bw)	Malathion 0–300; Ethion 0–2; Chlorpyrifos 0–10; Lindane 0–5; Permethrin 0–50	Same as vegetarian
No. ≥ ADI	1 (Ethion)	4 (Ethion)

[a]Acceptable Daily Intake (ADI) recommended by Codex Alimentarius Commission (CAC (μgkg^{-1} body weight))

[b]Average total diet consumed by each vegetarian and non-vegetarian individual = 2850g

Non-cancer risk from consumption of total diet

Tables 4 and 5 show the estimated average daily dietary intake (EADDI) (μg/d) of pesticide residues through consumption of total diet at LB (mean) and UB (P95) approaches for vegetarian and non-vegetarian total diet, respectively. For all the pesticides, estimated average daily intakes of pesticide residues fell far below the acceptable daily intake (ADI) in both the lower-bound and upper-bound scenarios.

Table 4. Estimated average daily dietary intake (EADDI) (μg/d) of pesticide residues through consumption of vegetarian total diet at LB (mean) and UB (P95) approaches

Pesticide	ADI	TADI[b] Adults (60 kg) (μg/day)	EADDI Mean LB (μg/day)	EADDI P95_UB (μg/day)	EMADDI 60 kg Mean LB	EMADDI 60 kg P95_UB
Malathion	300	18000	41.6	328	0.69	5.46
Ethion	2	120	14.8	97.2	0.25	1.62
Chlorpyrifos	10	600	3.4	14.3	0.06	0.24
Lindane	5	300	9.4	74.1	0.16	1.24
Permethrin	50	3000	5.4	23.1	0.09	0.39

ADI = Acceptable Daily Intake recommended by Codex Alimentarius Commission (μgkg^{-1} body weight)

TADI = Total ADI value for adults multiplied by average body weight of 60 kg

EADDI= Estimated average daily dietary intake

P95= 95th percentile

Table 5: Average daily dietary intake (EADDI) (μg/d) of pesticide residues through consumption of non-vegetarian total diet at LB (mean) and UB (P95) approaches

Pesticide	**ADI[a]**	**TADI[b] Adults (μg/day)**	**EADDI Mean LB (μg/day)**	**EADDI P95_UB (μg/day)**	**EMADDI 60kg Mean LB**	**EMADDI 60kg P95_UB**
Malathion	300	18000	108.6	761.0	1.81	12.68
Ethion	2	120	44.2	216.6	0.74	3.61
Chlorpyrifos	10	600	18.0	119.7	0.30	2.00
Lindane	5	300	6.6	18.24	0.11	0.30
Permethrin	50	3000	0.57	2.85	0.01	0.05

[a]Acceptable Daily Intake (ADI) recommended by Codex Alimentarius Commission (CAC (μgkg^{-1} body weight))

[b] TADI (total ADI) value for adults was multiplied by average body weight of 60 kg

EADDI= estimated average daily dietary intake

P95= 95th percentile

Table 6 showed the hazard index (HI) for pesticide residues through consumption of vegetarian and non-vegetarian total diet at LB (mean) and UB (P95). For vegetarian total diet, the results showed that HIs for all pesticides were less than 1 (H<1) for the lower-bound scenario. However, in the upper bound scenario, HI was greater than 1 (>1) for ethion (3.2) and lindane (4.1). For non-vegetarian total diet, the results reveals that HIs for all pesticides were less than 1 (H<1) for the lower-bound scenario, except ethion (1.5). In the upper bound scenario, HI was greater than 1 (>1) for ethion having a value of 7.2 while lindane was 1.01.

Table 6. Hazard Index (HI) for pesticide residues through consumption of vegetarian and non-vegetarian total diet at LB (mean) and UB (P95)

Pesticide	RfD (µg/kg/day)	HI Vegetarian		HI Non-Vegetarian	
		Mean LB	P95_UB	Mean LB	P95_UB
Malathion	20	0.03	0.27	0.09	0.63
Ethion	0.5	0.49	3.24	1.47	7.22
Chlorpyrifos	5	0.01	0.05	0.06	0.40
Lindane	0.3	0.52	4.11	0.37	1.01
Permethrin	50	0.00	0.01	0.00	0.00

RfD = Reference dose values obtained from USEPA; P95= 95th percentile; HI= Hazard index

Cancer risk from consumption of total diet

The cancer risk from consumption of total diet contaminated with lindane for adults at the lower-bound (LB) scenario was found to be within the safe range. The estimated risk of cancer for lindane from vegetarian total diet at LB level was 1.75 x 10^{-4}, and for the non-vegetarian diet, the cancer risk was estimated to be 1.23 x 10^{-4}. In the upper-bound (UB) scenario, the cancer risk for adults for lindane residue from non-vegetarian diet was 3.39 x 10^{-4} which falls within the safe limit. However, cancer risk for lindane from vegetarian at UB level was estimated to be 1.38 x10^{-3}, which is outside the USEPA recommended range of 10^{-4} to 10^{-6} (Table 7).

Table 7. Estimated cancer risk associated with exposure to lindane from vegetarian and non-vegetarian total diet at LB (mean) and UB (P95) approaches

Pesticide	USEPA[a] classification	CSF[b]	Vegetarian		Non vegetarian	
			Mean LB	P95_UB	Mean LB	P95_UB
Lindane	B2	1.3	1.75E-4	1.38E-3[c]	1.23E-04	3.39E-04

[a] USEPA classification: A=known human carcinogen; B2=probable human carcinogen; C= possible human carcinogen; [b] Cancer Potency Factor (mgkg^{-1}bw per d) as per USEPA; [c] Outside the USEPA recommended range of 10^{-4} to 10^{-6}; P95= 95th percentile

The total absence of DDT residues in this study and levels of lindane that are much lower in this study than values reported in previous studies conducted in India are indicative of the decline in the residues of DDT and HCH in total diet as a result of the ban on the use of these two persistent organochlorine pesticides except in the limited area of mosquito control. There were no significant differences between the mean levels of malathion, ethion, chlorpyrifos, lindane and permethrin between vegetarian and non-vegetarian diet. There was also no association between any of the five different pesticides detected in total diet samples and diet type, despite apparent differences in the frequencies of occurrence. The occurrence of ethion residues exceeding the ADI in five samples is a serious cause for concern. Potential risks of exposure evaluated for both cancer and non-cancer effects based on the estimated dietary intake of pesticide residues revealed that cancer risk for lindane at UB scenario is too high, and Hazard Index (HI) for ethion and lindane at the UB level exceeds one, reflecting the need for concerted efforts by the regulatory authorities to promote measures geared at reducing the burden of these pesticide in foodstuff.

In conclusion, the findings from this study reveals the widespread presence of pesticide residues in both vegetarian and non-vegetarian total diets in Punjab, India, with over half of the analyzed samples testing positive for at least one pesticide. Although the majority of detected levels were within acceptable limits, ethion exceeded the Acceptable Daily Intake in several cases, and the upper-bound Hazard Index values for ethion and lindane indicated potential health risks, particularly for vegetarians. Importantly, the estimated cancer risk associated with lindane in the vegetarian diet at upper-bound exposure levels surpassed the recommended safety thresholds, underscoring the need for continued monitoring and stricter regulatory oversight. The observed shift towards greater use of organophosphates and synthetic pyrethroids further emphasizes the importance of proactive risk assessment and the adoption of safer pest management practices to safeguard public health.

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