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Exposure assessment of selected pesticide residues using occurrence data in foods and serum samples in Pakistan

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Abstract

Pesticides have emerged as a global threat to human health, especially in developing countries. In this study, we examined nine major types of pesticide residues in the food commodities (8 groups) and serum samples (n = 80) of people living in the region of Southern-Punjab, Pakistan. Pesticide residues quantification was performed using gas chromatograph coupled with electron capture detector (GC-ECD). The estimated daily intakes of the selected pesticide residues through consumption of food were also calculated. The concentration of analyzed pesticide residues in food was found below the EU maximum permissible limits except for α-HCH, and β-HCH. The highest risk of cancer was found to be associated with the consumption of HCHs contaminated cereals than other products. The incidence rate of pesticide residues in the serum samples of studied subjects was 96.25%. Pearson correlation test showed no significant correlation between thyroid hormone levels of the studied population group and the analyzed pesticide residues in the food commodities ($p>0.05$). Findings of this study indicate that people of Southern-Punjab, Pakistan are exposed to pesticides via food chain although non-significant health implications were recorded, the levels of a few pesticides need to be curtailed.

Keywords: food; serum; pesticide; estimated daily intake; thyroid.

Practical Application: Prevalence data on pesticide residues in food and serum samples and health risk assessment among Pakistani population.

1 Introduction

Pesticides are the group of heterogeneous chemicals, produced naturally or synthesized by man, widely used in agricultural production to control the harmful pests, to increase the yield as well as to eradicate various disease-causing agents (Li et al., 2018). The use of pesticides particularly in the developing world is consistently increasing due to the rapid growth of intensive agriculture and urbanization (Azandjeme et al., 2014). In the year 2017, the total global pesticide usage was more than 4 million tones with a major portion (52.8%) utilized in Asian countries (Food and Agriculture Organization, 2019). Among South Asian countries, Pakistan ranks second in the total pesticide consumption with major use in the agriculture sector (Khan et al., 2020; Waheed et al., 2017). Even though the usage of pesticides in Pakistan decreased dramatically from 0.99 million tons in the year 2012 to 0.92 thousand tons in 2017 (Food and Agriculture Organization, 2019), some persistent pesticides are still present in various environmental media including air, water, and soil (Ali et al., 2018, 2019; Nakano et al., 2016; Nawab et al., 2020; Sohail, 2019; Sultan et al., 2019; Ullah et al., 2019). The massive use of pesticides is practiced in Punjab Province (88.3%), followed by Sindh (8.20%), Khyber Pakhtunkhwa (2.8%) and Baluchistan Province (0.76%) (Saeed et al., 2017).

The human exposure to pesticides mainly occurs through ingestion, skin contact, and inhalation. However, diet is believed to be the major route of human exposure to pesticide residues contributing more than 90% of the total exposure (Harmouche-Karaki et al., 2019; Zhang et al., 2017). Several studies have reported the levels of pesticide residues in human serum samples from Pakistan (Hayat et al., 2018, 2019; Saeed et al., 2017) with most of the studies focusing on occupational exposure. Analysis of various foodstuffs such as cereals (Ahmad et al., 2008; Mumtaz et al., 2015), fruits and vegetables (Akhtar et al., 2018; Munawar et al., 2013), milk and milk products (Ishaq & Nawaz, 2018; Ul Hassan et al., 2014), poultry and meat (Ahmad et al., 2018; Hamid et al., 2017), and fish (Akhtar et al., 2014; Riaz et al., 2018) for the levels of pesticide residues have also been reported from various regions of the country. However, little information

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regarding the direct association between the dietary intake, the serum levels of pesticide residues and thyroid hormones levels in Pakistan has been reported.

Therefore, the objectives of the present study were (i) to analyze the serum levels of Organochlorine pesticides (OCs) including di-chloro-diphenyl-trichloroethanes (DDTs), hexachlorocyclohexanes (HCHs) and dieldrin, chlorpyrifos (the most widely used organophosphate pesticide) and cypermethrin (the most widely used pyrethroid) among urban and rural adults of Southern Punjab, Pakistan; (ii) to assess the mentioned pesticide residues in various food commodities commonly consumed by the studied population; (iii) to estimate the dietary exposure to the pesticides associated with consumption of the food commodities contaminated with pesticide residues and (iv) and to document the association between the serum levels of pesticide residues and thyroid hormones levels.

2 Materials and methods

2.1 Sampling procedures

A cross-sectional survey was conducted during June 2019-March 2020 to investigate the serum levels of pesticide residues among urban and rural residents of three districts, namely Multan, Bahawalpur, and Vehari, Southern Punjab, Pakistan. Intravenous blood samples (8-10 mL) from a total of 80 subjects (40 urban and 40 rural) of both genders aged between 24-55 years were randomly collected into a vacutainer. The collected blood samples were analyzed for pesticide residues and thyroid hormones according to the method of Lacasaña et al. (2010).

Samples of food items (n = 141) commonly consumed by Pakistani population were purchased from five different areas of three districts (Multan, Bahawalpur, and Vehari) of Southern Punjab, Pakistan. All collected samples were transported to the laboratory under controlled environmental conditions and stored at -18 °C until the experimental analysis.

2.2 Preparation of blood and food samples

The extraction and purification of pesticides from blood were carried out by the procedure as described by Attaullah et al. (2018). For food samples, the extraction of pesticide residues was performed using QuEChERS method according to Association of Official Analytical Chemists (2011).

2.3 Analysis of pesticide residues

The prepared samples of blood serum and food items were analyzed for the following pesticides: p,p′-dichlorodiphenyl dichloroethylene (p,p′-DDE), p,p′-dichlorodiphenyl dichloroethane (p,p′-DDD), p,p′-dichlorodiphenyl trichloroethane (p,p′-DDT), alpha-hexachlorocyclohexane (α-HCH), beta-hexachlorocyclohexane (β-HCH), delta-hexachlorocyclohexane (δ-HCH), gamma-hexachlorocyclohexane (γ-HCH), chlorpyrifos and cypermethrin using gas chromatograph (Shimadzu GC-17A, Japan) equipped with ^{63}Ni Electron Capture Detector attached to a CBM-102 Chromatopak recorder system and ZB-5 capillary column (60 m × 0.53 mm×1.5 µm, J&W Scientific, USA) at HEJ Research Institute of Chemistry, University of Karachi, Pakistan. Nitrogen (N_2) was used as the carrier gas at a flow rate of 40 cm/sec. The temperature of the injector port was maintained at 220 °C. The temperature of the column oven was programmed in between 50 °C to 295 °C ramping with a rate of 3 °C per minute. The column pressure was maintained at 87 Kpa with a flow rate of 11.8 mL per minute. Standard chromatograms were first obtained by injecting the standard mixtures (1µl each) of selected pesticides (0.1%) under split less injector mode. The same procedure was adopted for sample extracts (1µl each). The quantification of detected pesticide residues was carried out with their corresponding peak height and peak areas, while the detected pesticides were identified through their retention times and compared with the standard chromatograms.

2.4 Analytical quality control

Agilent MSD Productivity ChemStation Software was used for system maintenance and data acquirement. To reduce the matrix effect of the method, matrix- matched calibration curves were used. Stock solutions of each pesticide standard (100 mg/kg) were prepared in n-hexane and stored at -18 °C. A mixture of pesticide standard working solutions was also prepared by pipetting 1 mL of each stock solution into a 100 mL volumetric flask and diluting with diethyl ether. For the preparation of matrix-matched calibration standards, mixture of pesticide standard working solutions at 5 different concentrations i.e., 0.05, 0.5, 1.5, 5.0 and 10 mg/L were mixed with the blank sample extracts and the volume of 10µl of TPM in n-hexane was added as an internal standard. To assure the quality of the procedure, blanks were run separately.

The limits of detection (LOD) and the limits of quantification (LOQ) were ranged from 0.01-0.08 ng/g and 0.03-0.24 ng/g, respectively. The recovery percentages of the selected pesticides ranged from 70-98%. Results below the LOQ of the analytical method were taken as zero.

2.5 Thyroid hormone analysis

Levels of triiodothyronine (T_3), thyroxine (T_4) and thyroid-stimulating hormone (TSH) were measured using automatic chemistry analyzer (AU 480; Beckman Coulter) according to the instructions provided by the ELISA kit manufacturer (Helica Biosystems, Santa Ana, CA, USA) of diagnostic kits at the certified City Laboratory, Multan. Laboratory reference values for T_3, T_4, and TSH ranged from 0.8-1.8 ng/mL, 4.6-12 µg/dl, 0.2-6.0 mIU/l, respectively.

2.6 Exposure assessment

Data on volunteers' age, gender, residence status, socioeconomic status, occupation, previous disease history and dietary habits were obtained through interview by trained interviewers. Validated food frequency questionnaire (FFQ) and a 24-hour dietary recall method were administered to obtain data on types and quantities of food consumed. Food items that are consumed on daily basis were categorized into eight groups, namely; i) cereals (wheat, rice, maize); ii) vegetables (green leafy vegetables, root and tuber vegetables, fruiting vegetables, brassica vegetables); iii) fruits (citrus and pome fruits); iv) pulses and legumes (mash, mung, beans. nuts); v) milk and milk products (whole milk, yogurt, cheese); vi) meat (chicken, mutton, beef); vii) fish, and viii) fat-containing foods (eggs, oil, butter, and ghee). The consumption rate (CR) of each food item was calculated by Equation 1.

$$\mathrm{CR} = W \times F \quad (1)$$

where CR is the food consumption rate (kg/day), W is the weight of the food/serving and F is the meal frequency/day (Akoto et al., 2015).

The estimated dietary intake (EDI) of measured pesticide residues in food was calculated by Equation 2.

$$\mathrm{EDI} = \frac{\mathrm{CR} \times \mathrm{C}}{Bw} \quad (2)$$

where EDI is the estimated dietary intake of pesticide (mg/kg bw/day), C is the average concentration of pesticide residue in a food item (mg/kg), CR is the food consumption rate (i.e. quantity of food consumed per day) (kg/day), and Bw is the average body weight of an adult individual (60 kg) (Akhtar et al., 2020; Ismail et al., 2020).

2.7 Statistical analysis

The statistical analyses including descriptive statistics, analysis of variance (ANOVA) and the Pearson Correlation test were performed using IBM SPSS version 26.0 (SPSS Inc., Chicago, Illinois, USA). A probability level of < 0.05 was considered as statistically significant.

3 Results and discussion

3.1 Occurrence of pesticide residues in food commodities

The concentration of analyzed pesticide residues in food items along with their maximum residual limit (MRLs) are summarized in Table 1. Out of the total 141 samples, 83.7% (n = 118) were found to be contaminated with at least one of the tested pesticides, whereas 20.6% (n = 29) samples were found to contain all types of pesticides including OCs, chlorpyrifos, and cypermethrin. There were no significant differences in the level of pesticides in various food commodities between urban and rural areas, therefore, the results are presented conjointly. Pesticide residues among different food items ranged from <LOD-1030 ng/g. The magnitude of levels of pesticides contamination in studied food groups was recorded in the order of meat > fish > vegetables > cereals > fat-containing foods > milk and milk products > fruits > pulses and legumes with the average pesticide levels of 28.54 ng/g, 23.83 ng/g, 23.32 ng/g, 15.50 ng/g, 13.06 ng/g, 11.55 ng/g, 9.0 ng/g, and 3.47 ng/g, respectively. The pesticide prevalence in foodstuff was recorded in the order of cypermethrin > chlorpyrifos > DDTs > HCHs > dieldrin with the average value of 96.70 ng/g, 29.01 ng/g, 20.90 ng/g, 13.39 ng/g, 1.22 ng/g, respectively (data not shown).

The mean concentration of pesticide residues found in the present study was comparable with the studies from China 22.36 ng/g for DDTs (Zhou et al., 2012) and 1.5-674.1 ng/g (Xing et al., 2021), Spain (0.07 ng/g for dieldrin) (Boada et al., 2014), and Tunisia (114.33 ng/g for cypermethrin) (Zarrouk et al., 2020) but higher than that reported earlier from the KPK-Pakistan (11.78 ng/g for DDTs) (Aamir et al., 2018), Romania (5.24 ng/g for HCHs) (Dirtu & Covaci, 2010) and Maryland, USA (0.62 ng/g for chlorpyrifos) (MacIntosh et al., 2001). The presence of pesticide residues in the food commodities indicates the chances of their detection in the blood samples of the residents of Southern Punjab-Pakistan due to likelihood of dietary exposure and consequently the risk of pesticides associated health complications.

3.2 Occurrence of pesticide residues in human blood

The concentration of pesticide residues detected in the blood serum samples of studied subjects are presented in Table 2. Serum levels of chlorpyrifos and cypermethrin were found to be below the detection limit (data not shown). Out of the total 80 participants, 96.25% (n = 77) were found to contain at least one of the OCs in their blood serum. Ten percent (n = 08) of the participants were found to carry all the analyzed types of OCs in their blood serum of which one participant (12.5%) was from urban, while 7(87.5%) were from the rural area. DDTs were found at higher frequency (96.25%) followed by HCHs (82.5%) and then dieldrin (63.75%). Statistical analysis revealed significant differences in the concentration of different types of pesticide residues quantified in the serum samples of the studied population as well as based on residence area (rural/urban). Based on age and gender, non-significant differences were recorded in the serum levels of pesticide residues except for dieldrin (concerning age groups) and DDTs (concerning gender).

It is pertinent to mention that despite the non-significant differences in the concentration of pesticide residues in the food samples of rural and urban areas, significant differences were recorded for the concentration of pesticide residues in the serum samples of both areas (higher in rural areas and much lower in urban areas). This might be due to the fact that the uncontrolled and excessive use of high quantities of pesticides due to the growth of cotton crop in rural areas further increases the chances of environmental contamination with pesticide residues. The serum concentration of total OCs analyzed in the present study ranged from <LOD-12.91 ng/mL. Among the detected OCs, the magnitude of concentrations was recorded in the order of HCHs > DDTs > dieldrin. The earlier data from Mexico (Orta-García et al., 2014), South Africa (Azandjeme et al., 2014), Lebanon (Harmouche-Karaki et al., 2018), Tunisia (Hassine et al., 2014) and Korea (Kim et al., 2018) also have reported the abundance of p,p' DDE, and β-HCH over other congeners of DDT and HCH (data not shown), that is in line with our study. The mean values of DDTs, HCHs and dieldrin found in the present study (Table 2) are comparable to the values reported from district Vehari Punjab, Pakistan (p,p' DDT = 0.30 ng/mL; dieldrin = 0.14 ng/mL) (Saeed et al., 2017), and Beijing, China (dieldrin = 0.52 ng/mL) (Li et al., 2018), lower than that from Mexico (geometric mean; DDTs = 113.3 ng/mL, HCHs = 24.8 ng/mL, dieldrin = 1.6 ng/mL) (Ruiz-Suárez et al., 2014), Jiangsu, China (DDTs (p,p' DDT, p,p'-DDD = 37.24 ng/mL) (Chang et al., 2017) and Beijing, China (DDTs = 8.50 ng/mL; HCHs = 9.91 ng/

Table 1. Descriptive statistics of pesticide concentration (ng/g) in various food items.

Food item (n)	DDTs	HCHs	Dieldrin	Chlorpyrifos	Cypermethrin
Cereals (30)					
Mean ± SD	1.34 ± 3.11	4.63 ± 5.74	<LOD	80.33 ± 59.16	93.67 ± 113.33
MRL	50	-----	10	500[a]	2000[a]
%+ve (%>MRL)	36.7(0)	76.67(-)	0(0)	90(6.67)	70(0)
Vegetables (24)					
Mean ± SD	13.53 ± 12.03	12.64 ± 9.33	<LOD	35.38 ± 26.20	192.08 ± 220.7
MRL	50	-----	10	10	2000[b], 50[c], 1000[d], 500[e]
%+Ve (%>MRL)	66.7(0)	76.7(-)	0(0)	90(70.8)	70(0)
Fruits (12)					
Mean ± SD	1.98 ± 6.03	1.51 ± 3.05	<LOD	3.08 ± 3.38	98.0 ± 156.27
MRL	50	-----	10	10, 1500[b]	2000[b], 50[c], 1000[d],
%+Ve (%>MRL)	25(0)	25(-)	-----	58.3(0)	83.3(0)
Pulses and Legumes (18)					
Mean ± SD	6.38 ± 6.89	1.90 ± 2.09	<LOD	2.17 ± 5.40	22.94 ± 17.46
MRL	50	-----	10	10	50
%+Ve (%>MRL)	100(0)	88.89(0)		16.7(5.56)	83.3(0)
Milk and Milk products (12)					
Mean ± SD	24.82 ± 13.99	14.38 ± 17.10	1.59 ± 2.89	12.60 ± 12.94	46.0 ± 29.71
MRL	40	-----	6	10	50
%+Ve (%>MRL)	100(0)	50(-)	50(16.7)	100(50)	100(41.6)
Meat (18)					
Mean ± SD	46.64 ± 26.08	21.94 ± 20.67	1.71 ± 2.28	22.83 ± 8.33	180.8 ± 343.8
MRL	1000	-----	200	10	2000[f]
%+Ve (%>MRL)	83.3(0)	83.3(-)	72.2(0)	100(83.3)	66.7(0)
Fish (09)					
Mean ± SD	78.72 ± 21.46	32.02 ± 26.50	6.10 ± 5.45	4.03 ± 3.96	54.4 ± 54.8
MRL	5000	-----	300	-----	50[g]
%+Ve (%>MRL)	100(0)	100(-)	100(0)	88.9(-)	66.7(55.6)
Fat containing foods (18)					
Mean ± SD	33.17 ± 18.82	29.86 ± 14.73	3.72 ± 6.77	8.71 ± 7.69	18.28 ± 19.81
MRL	50	-----	20	10	50
%+Ve (%>MRL)	83.3(16.7)	100(-)	33.3(0)	94.4(38.9)	88.9(0)
Total (141)					
Mean ± SD/range	20.90 ± 25.92/<LOD-105	13.39 ± 16.61/<LOD-69	1.22 ± 3.44/<LOD-19	29.01 ± 41.41/<LOD-200	96.70 ± 177.65/<LOD-1030

SD = Standard Deviation (a quantity expressing by how much the members of a group differ from the mean value for the group). DDTs = sum of p,p′-DDE, p,p′-DDD, and p,p′-DDT; HCHs = sum of α-HCH, β-HCH, δ-HCH, and γ-HCH; European union maximum residue level (MRL) of pesticides are mentioned (European Union, 2021). [a]for all cereals except maize (that are 50 and 300 ng/g for chlorpyrifos and cypermethrin, respectively); [b]for green leafy vegetables & citrus fruits; [c]for root and tubers vegetables & nuts; [d]for brassica vegetables and pome fruits; [e]for fruiting vegetables; [f]for all types of meat except poultry (that is 100 ng/g) for chicken; [g]EU MRLs of cypermethrin for wild animals.

mL) (Li et al., 2018) and higher than that from Jiangsu, China (HCHs = 5.41 ng/mL) (Chang et al., 2017) and Bolivia (geometric mean; DDTs = 1.3 ng/mL) (Arrebola et al., 2013). Higher concentration of pesticide residues in the serum samples of the people of Southern Punjab – Pakistan is linked with prevalence of pesticide residues in the food commodities available in the region as indicated in Table 1.

3.3 Thyroid hormones levels

The mean levels of T_3, T_4, and TSH measured in the present study were 1.47 ng/mL, 9.82 µg/dl, and 3.93 mUl/mL, respectively (data not shown). Non-significant differences were recorded for the serum levels of thyroid hormones across both gender and residence area. The laboratory reference values of T_3, T_4 and TSH were 0.8-1.8 ng/mL, 4.6-12 µg/dl and 0.5-6.0 mUl/mL, respectively. The results of the study indicated that T_4 and TSH levels in 12.5% (n = 10) participants and T_3 levels in 8.75% (n = 7) of the participants were higher than their respective reference ranges. Thyroid hormone levels in all other participants were found within the reference ranges.

In this study, a very weak inverse correlation was found between serum levels of OCs and T_3 ($r = -0.005$; $p = 0.96$), T_4 ($r = -0.06$; $p = 0.59$), and TSH ($r = -0.15$; $p = 0.19$) levels. Several studies have investigated the association between OCs levels in the body and the levels of thyroid hormones (Alvarez-Pedrerol et al., 2009; Bloom et al., 2003; Hagmar et al., 2001; Kim et al., 2013; Meeker et al., 2007; Schell et al., 2004; Takser et al., 2005; Teeyapant et al., 2014). Though, the results have not been consistent across the studies. The findings of our study are in agreement with the study of Teeyapant et al. (2014) who reported a non-significant association of thyroid hormones with serum levels of DDTs (p,p'-DDE, and p,p'-DDT) but inconsistent with

Kim et al. (2013) who reported a negative association of DDTs with thyroid hormones (free T_4 and total T_3) and a positive association between DDTs and TSH. Alvarez-Pedrerol et al. (2009) also reported a positive association between TSH levels and levels of β-HCH. Though the associations in both studies were statistically non-significant (P>0.05).

3.4 Estimation of pesticide exposure

The estimated daily dietary intake (EDIs) values of pesticide residues from 8 different food groups are presented in Table 3. The daily dietary exposure of total pesticides from different food groups was in the order of cereals > vegetables > meat > milk and milk products > fruits > fat-containing foods > pulses and legumes > fish. With respect to the type of pesticides, the EDI was recorded in the order of cypermethrin > chlorpyrifos > DDTs > HCHs > dieldrin. DDTs exposure was found in milk and milk products (32.6%), meat (25.4%) and vegetables (17.4%), while in case of HCHs the major contributors were vegetables (21.5%), cereals (20.2%) and meat (16.08%). Dieldrin exposure was recorded due to milk and milk products (44.01%), fat-containing foods (28.02%), meat (19.56%) and fish (18.39%). For cypermethrin and chlorpyrifos, cereals (76.5% and 38.9%, respectively) and vegetables (13.3% and 31.5%, respectively) were the major contributors to dietary exposure.

The EDI values of the pesticide residues obtained in this study are much lower than the reference values (Table 3).

Table 2. Descriptive statistics of pesticides concentration (ng/mL) in blood.

Variables	DDTs	HCHs	Dieldrin
All Participants (n = 80)			
Mean (CI 95%)	3.46(3.95)	7.34(7.93)	0.20(0.24)
SD	2.23	2.63	0.20
Range	0.59-8.20	2.82-12.91	<LOD-0.67
95th percentile	2.34	1.131	0.54
p-value*	<0.01	<0.01	<0.01
Age in years (n)			
24-31 (27)	3.18(4.13)	6.13(7.59)	0.19(0.26)
32-39 (18)	3.00(4.09)	4.25(6.30)	0.13(0.22)
40-47 (22)	4.01(5.02)	7.42(8.98)	0.17(0.26)
48-55 (13)	3.54(4.83)	6.33(8.31)	0.37(0.48)
p-value**	0.51	0.07	0.01
Gender (n)			
Male (40)	2.91(3.45)	5.91(7.05)	0.23(0.30)
Female (40)	3.94(4.79)	6.28(7.57)	0.17(0.22)
p-value*	0.043	0.672	0.123
Residence area (n)			
Rural (40)	5.11(5.73)	8.54(9.58)	0.26(0.32)
Urban (40)	1.74(2.05)	3.65(4.44)	0.14(0.20)
p-value*	<0.01	<0.01	<0.01

*p-value obtained from Student's T-test; **p-value obtained from ANOVA; DDTs = sum of p,p′-DDE, p,p′-DDD, and p,p′-DDT; HCHs = sum of α-HCH, β-HCH, δ-HCH, and γ-HCH. SD= Standard Deviation (a quantity expressing by how much the members of a group differ from the mean value for the group). CI= Confidence Interval (a range of values so defined that there is a specified probability that the value of a parameter lies within it).

Table 3. Estimated dietary intake (EDI) (mg/kg bw/day) of pesticide residues in food groups.

Food group	DDTs	HCHs	Dieldrin	Chlorpyrifos	Cypermethrin	CEDI
Cereals	1.05E-05	3.63E-05	----	6.31E-04	7.36E-04	1.41E-03
Vegetables	4.19E-05	3.85E-05	----	1.10E-04	5.96E-04	7.86E-04
Fruits	2.66E-06	2.03E-06	----	4.14E-06	1.32E-04	1.41E-04
Pulses and legumes	5.41E-06	1.61E-06	----	1.83E-06	1.94E-05	2.82E-05
Milk and milk products	7.89E-05	4.15E-05	5.05E-06	4.0E-05	1.46E-04	3.12E-04
Meat	6.14E-05	2.89E-05	2.25E-06	3.0021E-05	2.38E-04	3.60E-04
Fish	1.24E-05	4.70E-06	9.64E-07	6.3674E-07	8.6E-06	2.73E-05
Fat-containing foods	2.87E-05	2.58E-05	3.22E-06	7.5356E-06	1.58E-05	8.11E-05

DDTs = sum of p,p′-DDE, p,p′-DDD, and p,p′-DDT; HCHs = sum of α-HCH, β-HCH, and γ-HCH; CEDI= cumulative estimated daily intake; Toxicological reference dose (RfD) values of p,p′-DDE, p,p′-DDD, p,p′-DDT, α-HCH, β-HCH, γ-HCH, dieldrin, chlorpyrifos, and cypermethrin are 3×10^{-4}, 3×10^{-5}, 3×10^{-4}, 8×10^{-3}, 6×10^{-4}, 3×10^{-4}, 5×10^{-5}, 1.0×10^{-3}, and 2×10^{-2} mg/kg/day, respectively (Agency for Toxic Substances and Disease Registry, 2020; U.S. Environmental Protection Agency, 2001).

The findings of dietary exposure estimation obtained in this study are in line with the estimated exposure reported from the Russia (3.10×10^{-5} mg/kg bw/day for DDTs) (Polder et al., 2010), India (6.2×10^{-6} mg/kg bw/day for dieldrin) (Betsy et al., 2014) and China (1.71×10^{-5} for HCHs) (Zhang et al., 2017). However, the levels are comparatively higher than that from KPK-Pakistan (2.0×10^{-5} mg/kg bw/day for DDTs) (Aamir et al., 2018), Cameron (2.7×10^{-5} to 1.2×10^{-5}mg/kg bw/day for cypermethrin) (Gimou et al., 2008) and Indonesia (4.0×10^{-8} mg/kg bw/day for DDTs) (Shoiful et al., 2013) indicating higher health risks associated with the consumption of pesticides contaminated food available in the markets of Southern-Punjab, Pakistan.

4 Conclusion

The concentration of pesticide residues in food items was found lower than the EU MRLs except for α-HCH, and β-HCH in certain food items. The dietary exposures to all the analyzed pesticides were below the toxicological reference values set by the regulatory authorities. However, this study had several limitations. Firstly, the study investigated only the serum level of pesticide residues including the level of organophosphates and pyrethroids that are excreted out through urine in the form of conjugates within 4-48 hrs of exposure and their minute concentration remains in the blood (Huen et al., 2012; Li & Kannan, 2018). Therefore, further investigation based on urinary biomarker approach is recommended to assess the exposure of organophosphates and pyrethroids in general population of Pakistan. Secondly, the population group studied in the present investigation was only the adults (24-55 years). Thus, future total diet studies focusing on vulnerable population groups of Pakistan (children and elderly) are recommended. Additionally, dietary modifications, and implementation of good agricultural practices such as proper washing of foodstuff prior to use and the use of eco-friendly bio-pesticides and good manufacturing practices must be ensured to remarkably reduce the dietary exposure to pesticides and associated health risks (Hussnain et al., 2021; Rembischevski & Caldas, 2020).

Conflict of interest

The authors declared no conflict of interest.

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