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Pesticide Residues in Bovine Milk in Punjab, India: Spatial Variation and Risk Assessment to Human Health

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Abstract In the present study, gas chromatographic analysis of pesticide residues in bovine milk ($n = 312$) from Punjab, India, showed chlorpyrifos, DDT, and γ-HCH as the predominant contaminants. In addition, the presence of β-endosulfan, endosulfan suphate, cypermethrin, cyhalothrin, fenvalerate, deltamethrin, malathion, profenofos, and ethion was reported in milk samples. In this study, it was observed that 12 milk samples exceeded the maximum residue limits (MRLs) for γ-HCH (lindane), 18 for DDT and chlorpyrifos, and 1 sample each for endosulfan, cypermethrin, and profenophos. In India, DDT is still permitted for a malaria control program, which may be the plausible reason for its occurrence in milk samples. The spatial variation for presence of pesticide residues in milk indicated greater levels in cotton-growing areas of Punjab. At current levels of pesticide residues in bovine milk, the human health risk assessment in terms of noncancer and cancer hazard was calculated based on both lower-bound [LB (mean residue levels)] and upper-bound [UP (95th percentile level)] limits. It was noticed that cancer and noncancer risk were within United States Environmental Protection Agency prescribed limits for both adults and children at the LB, but children were being exposed to greater risk for DDT and HCH at the 95th-percentile UB level.

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Bovine milk is an essential component in the diet of infants, children, and the elderly. It is a good source of proteins, fat, and minerals. Therefore, contamination of milk with pesticide residues is extremely alarming. Lactating animals may be exposed to pesticides from ingestion of contaminated feed, fodder, and water and application of pesticides on the animal body, in animal sheds, or in milk-processing areas (Goulart et al. 2008). Due to the persistence and lipophilicity of pesticides, pesticide residues become magnified in lipid-rich tissues of organisms and under certain conditions, such as lactation, mobilization of deposited contaminants, and excretion, into milk occurs (Cajka and Hajslova 2003). Additionally, an increasing number of pesticides is entering into our environment, which is potentially hazardous to human/animal health and to the ecosystem (Gill and Garg 2014). Prolonged exposure to pesticides may results in liver and kidney problems (Peres et al. 2006), disruption of the endocrine system (Colborn et al. 1993), neurological and immune system disorders (Karmaus et al. 2003), and risk of breast, lung, cervix, and prostate cancer (Ahmed et al. 2002).

Punjab is an agrarian state of India and is one of the highest users of pesticides; per-hectare use of pesticides (923 g/ha) is highest in this state compared with other Indian agricultural states (Agnihotri 2000). Organochlorine pesticides (OCPs), particularly dichlorordiphenyl trichloroethane (DDT) and hexachlorocyclohexane (HCH), were the highly used pesticides in this region of India until restrictions on their use were enacted in the late 1990s (Kannan et al. 1997). Nevertheless, a substantial amount of DDT and γ-HCH (lindane) chemicals are being permitted for use in the malaria-control program and in agriculture, respectively [United Nations Industrial Development

Organization (UNIDO) 2006]. However, in recent years, the consumption of organophosphate pesticides (OPs) and synthetic pyrethroid pesticides (SPs) has increased by many fold in agricultural practices (Kumari et al. 2005; States/UTs, Zonal Conference on Inputs 2010), which is reflected by their increasing occurrence in vegetables, fruits, animal feed, and even human breast milk (Blossom and Singh 2004; Bhanti and Taneja 2007; Sinha et al. 2012; Bedi et al. 2013). Although the presence of OCPs residues in milk, meat, and dairy products is well- evidenced in India (Bedi et al. 2006; Nag and Raikwar 2008; Gill et al. 2009), still there is lack of information on the occurrence of OPs and SPs in animal foods, which are one of the essential component of the human diet. Therefore, in view of the increased use of pesticides in Punjab, the changing pesticide consumption patterns, the restrictions on HCH and DDT use, and the lack of reports on the measurement of OP and SP pesticides in bovine milk samples, the present study aims to quantitatively estimate levels of OCPs, SPs, and OPs in bovine milk throughout Punjab and to evaluate their impact on human health through the consumption of milk.

Materials and Methods

Chemicals

All of the reagents used were of analytical grade. Dichloromethane and acetone were glass-distilled before use. Silica gel (60–120 mesh) was purified by washing with dichloromethane and acetone followed by activation at 135° C for 3 h. In a similar manner, anhydrous sodium sulphate was also washed and activated. Analytical technical grade standards with 93–99 % purity for OCPs, OPs, and SPs, were procured from Sigma-Aldrich (United States).

Sampling

Punjab is located in Northwestern India and has a landmass area of 50,362 km^2. It extends from latitudes 29.30° to 32.32° North and longitudes 73.55° to 76.50° East. In the present study, bovine milk samples were collected from 11 districts of Punjab (India) representing three different zones (Fig. 1). Zone 1 represents the Northeastern Punjab (Gurdaspur, Hoshiapur, Nawanshehar, and Patiala district); zone 2 belongs to Central Punjab (Amritsar, Jalandhar, and Ludhiana district); and zone 3 is comprised of Southern Punjab, which is commonly referred to as the "cotton belt" (Bathinda, Moga, Muktsar, and Firozepur district). A total of 312 random raw bovine milk samples were collected from dairy farms and local milk vendors/milk shops in summer (May to August) 2011. For each sample, 100 ml of milk was collected in clean, solvent-washed glass bottles. All of the samples were transported to the laboratory on the same day of collection under chilled conditions. The samples were stored at −20 °C and were analyzed within 48 h of collection. All pesticides screened in this analysis are considered highly used in agricultural and veterinary practices along with a few restricted for use.

Extraction of Pesticide Residues

Pesticide residues from milk samples were extracted according to the methods developed by Battu et al. (2004) with minor modifications. Five ml of the milk sample was thoroughly mixed with 20 g each of activated silica gel and anhydrous sodium sulfate and packed into an extraction glass column containing 40 ml of dichloromethane. After 90 min, the solvent in the column was eluted dropwise followed by further elution with 150 ml of a dichloromethane and acetone (1:2 v/v) mixture. The extracted sample was concentrated using a rotary evaporator at 40 °C until dry, and the final reconstitution was performed in 3 ml of n-hexane-and-acetone (1:1) mixture.

Estimation of Pesticide Residues

A gas chromatograph equipped with an electron capture detector (ECD) and flame thermionic detector (FTD) was used for the estimation of pesticide residues (Shimadzu model 2010) using the method described in our previous article (Bedi et al. 2013). OCPs (α-HCH, ß-HCH, γ-HCH, δ-HCH, heptachlor, aldrin, fipronil, butachlor, dieldrin, p,p'DDE, o,p'DDD, p,p'DDD, o,p'DDE, p,p'DDT, endrin, endosulfan sulphate, and ß-endosulfan) and SPs (cypermethrin, permethrin, cyfluthrin, deltamethrin, and fenvalerate) were determined using the ECD, whereas OPs (chorpyrifos, monocrtophos, dimethoate, fenitrothion, parathion-methyl, malathion, fenamiphos, profenophos, ethion, triazophos, and phosalone) determined using the FTD. Calibration curves for all standards of OCPs, OPs, and SPs were drawn for the concentration *versus* area of the peak, and the correlation coefficients (r^2) were determined found to be nearly 0.99.

Method Validation

Reagent blanks were run every six samples to check the interference of contamination from solvents and glassware. The trueness of the method used for extraction and estimation was validated by the processing of spiked bovine whole milk samples with standard pesticides at concentrations of 50 and 100 µg/kg. The mean recovery values of spiked samples ranged from 81.2 % for δ-HCH to 113.5 % for aldrin, which is in accordance with the acceptable

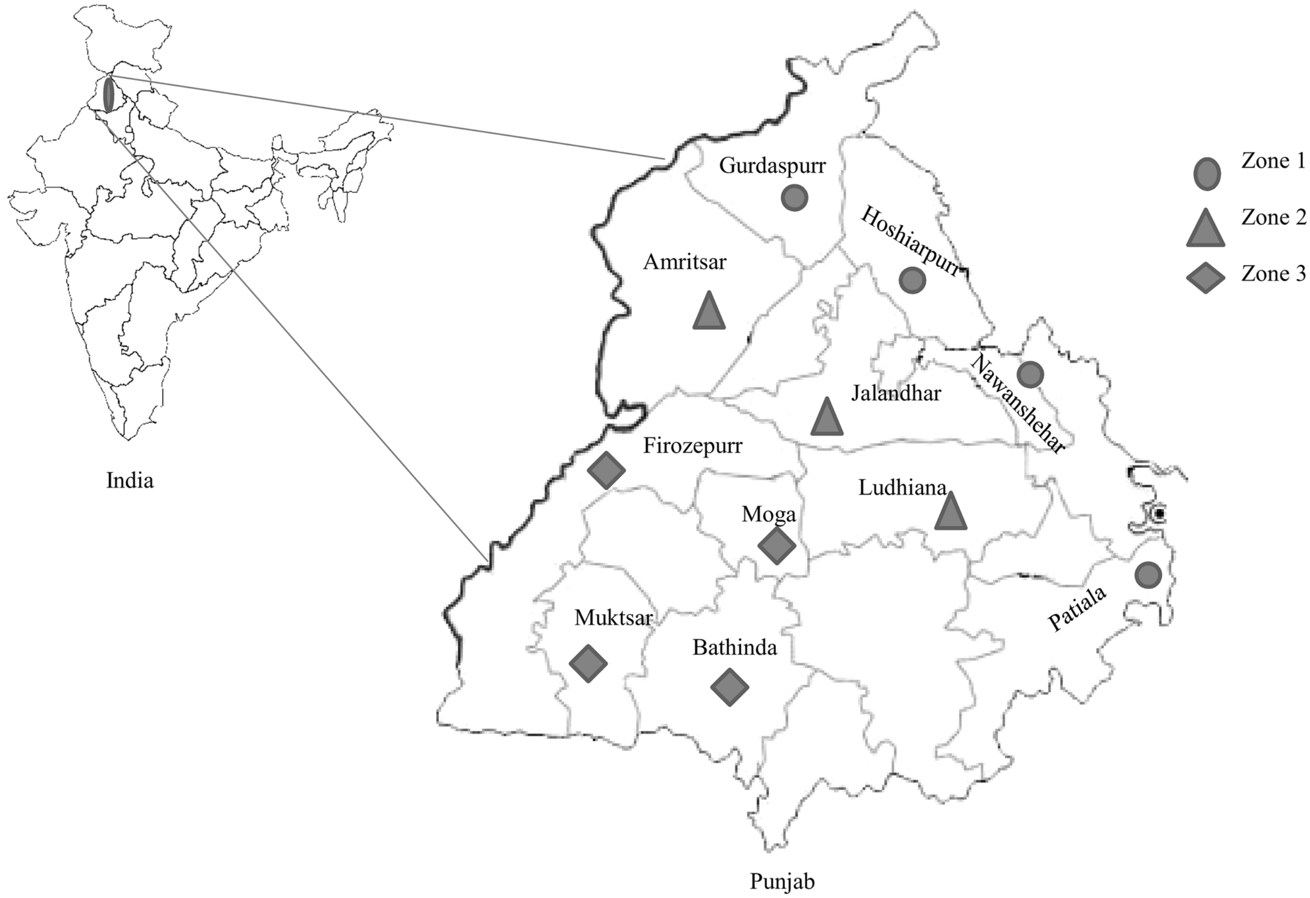

Fig. 1 Milk samples collected from three different zones of Punjab

recovery range of 70–120 % [Association of Analytical Chemists (AOAC) 1999]. Repeatability of the method was aligned with AOAC (1999) estimations by relative SD of the recovery values, which were <10 %. The calculated concentrations of residues in samples were not corrected for recovery. The detection limits of the method ranged from 1.0 µg/kg for α-HCH to 2.0 µg/kg for deltamethrin and chlorpyrifos on a whole-milk basis.

Confirmation of Results by Gas Chromatography–Mass Spectrometry

The confirmation of pesticide residues detected by GC was performed using gas chromatography-mass spectrometry (GC–MS; GCMS QP2010 Plus; Shimadzu). The column temperature was initially set at 80 °C and finally increased to 280 °C. The mass spectrometer was operated in electron ionization mode. The interface, manifold, and ion source temperatures were 290, 50, and 200 °C, respectively. The emission current for the ionization filament was set at 80 µA generating electrons with energy of 70 eV. Helium (99.99 %) at a flow rate of 0.94 ml/min was used as carrier gas. The selective ion-monitoring (SIM) method was designed for OCPs, SPs, and OPs including retention time windows and base peak ions.

All statistical analysis was performed using SPSS software for Windows (Microsoft version 11.0.1 [SPSS, Chicago, IL]). Concentrations of pesticide residues in samples were summarized using arithmetic means and SDs together with minimum and maximum values. One-way analysis of variance (ANOVA) test using Tukey's method was applied to check for significant differences in residue levels of different pesticides in the zones considered in this study; $p < 0.05$ was considered to be statistically significant.

Human Health Risk Assessment

To assess the risk of pesticide residues in milk, data were evaluated under two contamination scenarios to handle left-censored data: the lower-bound (LB) scenario where undetected results were set to zero and the 95th-percentile upper-bound (UB) scenario where undetected results were set to the detection limit. It is widely considered that the

LB scenario generally underestimates contamination and exposure levels, whereas the UB scenario results in overestimation. First, dietary pesticide exposure was assessed by estimating the acceptable daily intake (ADI) values of the pesticides determined in milk samples according to the recommended Guidelines for Predicting Dietary Intake of Pesticide Residues of the Food and Agricultural Organization/World Health Organization [(FAO/WHO) 1997]. The estimated daily intake (EDI) of pesticides was calculated from the following relationship:

$$\mathrm{EDI} = C_{\mathrm{milk}} \times I$$

where C_{milk} is the concentration of pesticide residues in milk, and I is the availability of milk to an individual in units of grams per day. Exposure was estimated on a per-capita basis of the consumption of milk in Punjab, which is 944 g/person/day (Government of India 2010). EDIs were reported in µg of pesticide/kg bw/day.

The dietary intake estimates of pesticide residues were compared with the recommended guidelines for the ADI formulated by FAO/WHO (2009). Evaluation of the potential risks of estimated exposure based on the dietary intake of pesticides from milk consumption were performed for both noncancer and carcinogenic effects as proposed by USEPA (2009) and as described in other studies (Dougherty et al. 2000; Jiang et al. 2005). For noncancer hazards, hazard ratios (HRs) were estimated by dividing the average daily exposure by the benchmark concentration (BMC) for noncarcinogenic effects based on the reference dose (RfD) determined by USEPA Integrated Risk Information System for each contaminant:

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

For each contaminant, the average daily exposure level for a population was calculated using the following:

$$\begin{aligned}\text{Average daily exposure } (\mu g/\text{kg bw}) \\ = \text{Milk consumption } (\text{g/kg bw}) \\ \times \text{contaminant concentration } (\mu g/\text{kg})\end{aligned}$$

where bw refers to body weight.

A BMC represents a daily concentration below which there is a high probability of no adverse health effects. A hazard ratio >1 indicates that the average exposure level exceeds the BMC and thus poses a potential risk to human health (Dougherty et al. 2000).

$$Benchmark\ concentration = \frac{Risk * Body\ weight}{Milk\ consumption * Cancer\ slope\ factor}$$

For lifetime cancer risk assessment, the average daily exposure was divided by the BMC for carcinogenic effects where risk is the probability of lifetime cancer risk and the cancer slope factor is derived from USEPA Integrated Risk Information System for each contaminant. This approach enabled the estimation of chemical-specific risks for a reasonable maximum exposure using the cancer risk of 1/1,000,000 for a lifetime exposure. To assess potential cancer risk, the HR was estimated at the LB scenario and the 95th-percentile UB scenario (Jiang et al. 2005; USEPA 2009). The average daily doses were calculated by assuming 100 % absorption of pesticides because RfDs and cancer potencies generally do not account for bioavailability.

Results and Discussion

Contamination Patterns of Pesticide Residues in Bovine Milk

The analysis of pesticides in bovine milk samples collected throughout Punjab indicated the predominance of chlorpyrifos, DDT, and endosulfan residues (Table 1). Chlorpyrifos was detected in 6.4 % of the milk samples with mean level of 2.2 µg/kg and contributed the greatest (20.2 %) proportion in the total pesticide residue load in milk samples. The metabolic profile of DDT indicated the presence of p,p' DDE and p,p' DDD in 5.8 and 4.8 % of milk samples at mean levels of 0.97 and 0.63 µg/kg, respectively, resulting in total mean levels of 1.6 µg/kg. Total endosulfan residues comprising of β-endosulfan and endosulfan sulphate were detected in 9.3 % of samples. In this study, out of all four HCH isomers (α-, β-, γ-, and δ-), only γ-HCH was detected in 7.0 % of samples at a mean level of 0.9 µg/kg. Fipronil and butachlor were observed in 3.2 and 2.6 % of milk samples. Among SPs, cypermethrin was detected at a mean level of 0.9 µg/kg followed by cyhalothrin (0.8 µg/kg), fenvalerate (0.7 µg/kg), and deltamethrin (0.5 µg/kg). In addition to chlorpyrifos, malathion, profenophos, and ethion were the other OPs detected in 0.9, 1.6, and 1.3 % of milk samples, respectively. It was observed that although mean residue levels of all detectable pesticides were lower than their respective MRLs. However, considering residue levels in individual milk samples, 12 exceeded the MRLs for HCH, 18 for DDT and chlorpyrifos, and one for each endosulfan, cypermethrin, and profenophos, respectively.

In the comparative results of the pesticide groups detected in milk samples, it was observed that OCP residues contributed a greater proportion (35.0 %) followed by OPs (28.6 %), SPs (27.8 %), fipronil (5.1 %), and butachlor (3.5 %). However, earlier reports from this region and other parts of India reported DDT and HCH (OCPs) as the major contaminants in milk with a much greater detection

Table 1 Mean concentrations (μg/kg) of pesticide residues in bovine raw milk samples collected throughout Punjab (India)

Pesticide	Mean ± SD ($n = 312$)	Positive samples	% Positive[a]	% Proportion[b]	MRLs by CAC[c]	Samples > MRL
HCH	0.9 ± 3.5	22	7.0	8.5	10.0	12
DDT	1.6 ± 3.9	32	10.3	14.9	20.0	1
Endosulfan	1.2 ± 3.8	29	9.3	11.6	10.0	18
Fipronil	0.5 ± 3.0	10	3.2	5.1	20.0	1
Butachlor	0.4 ± 2.5	8	2.6	3.6	–	–
Cyhalothrin	0.8 ± 4.3	14	4.5	8.2	200.0	0
Cypermethrin	0.9 ± 5.0	13	4.1	8.7	50.0	1
Fenvalerate	0.7 ± 3.5	12	3.8	6.5	100.0	0
Deltamethrin	0.5 ± 3.4	7	2.2	4.4	50.0	0
Malathion	0.4 ± 3.9	3	0.9	3.5	–	–
Chlorpyrifos	2.2 ± 8.5	20	6.4	20.2	20.0	18
Profenophos	0.2 ± 1.6	5	1.6	1.8	10.0	1
Ethion	0.3 ± 2.9	4	1.3	3.1	–	–

[a] Percentage of positive samples from total samples (312) analyzed

[b] Based on the mean residue levels, the contribution of a particular pesticide among total pesticide residue load detected in milk samples

[c] Maximum residue limits recommended by Codex Alimentarius Commission (2008)

frequency and concentrations (Kalra et al. 1999; Battu et al. 2004; Nag and Raikwar 2008). In an another study, total DDT, HCH, endosulfan, and aldrin were present in 100, 97, 43, and 12 % of milk samples with a mean value of 36.7, 29.2, 2.2, and 3.6 μg/kg, respectively (Sharma et al. 2007). In present study, the possible reason for the decreased occurrence of total HCH may be related to the application of technical HCH (α, β, γ and δ) being banned in India since 1997 and only lindane being permitted for use in agriculture including fodder crops [Central Insecticide Board and Registration Committee (2012)]. However, the presence of lindane at greater than its MRL in 12 milk samples in the present study is still regarded with concern because lindane is carcinogenic (Vettorazzi 1975).

Regarding the occurrence of DDT in the present study, p,p' DDE was the leading metabolite imitated its long-term bioaccumulation in living beings. But the presence of traces of p,p′ DDD (decreased metabolites of DDT in living beings) can be interpreted as evidence of recent exposure to DDT. This may be related with that, although DDT use was banned in India; however, due to the lack of a suitable alternative for malaria control, DDT is still permitted for use up to 10,000 tons for vector control programs only (UNIDO 2006). In our earlier study, p,p′ DDE, p,p′ DDT, and p,p′ DDD residues have been reported in human breast milk samples from this region (Bedi et al. 2013). In this study, the occurrence of endosulfan residues could be due to the presence of endosulfan residues in animal feed and fodder because contamination of animal feed has been reported earlier by many investigators (Deka et al. 2004; Nag and Raikwar 2011).

The consumption of herbicides, such as butachlor, in India has increased from 380 metric tonnes in 2005–2006 to 1291 metric tonnes in 2009–2010 (States/UTs, Zonal Conference on Inputs 2010). In the present study, we also reported the presence of butachlor in 2.6 % of milk samples at mean levels of 0.4 μg/kg. In an earlier article, residues of butachlor were also detected in rice grains and rice straw, which are used as animal feed and may lead to their excretion in animal milk (Sondhia et al. 2006). Similarly, the insecticide fipronil is used for the control of many soil and foliar insects on a variety of crops and can also be formulated as flea and tick sprays for domestic animals. It has the potential for excretion in milk if animals are fed with fipronil-contaminated feed or fodder (Faouder et al. 2007). Regarding the other OCP residues analyzed, aldrin, dieldrin, endrin, and heptachlor were not detected in any milk sample, and this is likely the result of their being banned for use in crop protection in India since two decades back.

Synthetic pyrethroids are hydrophobic compounds with log octanol–water partition coefficient (Kow) values near to 6 and short environmental persistence varying between 12 and 197 days (Feo et al. 2010). In present study, the occurrence of SP pesticide residues, such as cyhalothrin, cypermethrin, fenvalerate, and deltamethrin was observed in milk samples. SPs are widely used pesticides to control pests in agriculture, homes, communities, restaurants, hospitals, schools, and dairy farms. In India, residues of cypermethrin, deltamethrin, and fenvalerate have been reported in food products in a few recent studies (Misra et al. 2005; Chandra et al. 2010; Sun et al. 2011). Thus, results of the present study regarding the occurrence of SPs in milk samples can be related to the increasing use of SPs as a replacement for persistent organochlorine pesticides (States/UTs, Zonal Conference on Inputs 2010). Presence of pyrethroid residues has also been reported in human breast milk samples in recent studies (Sereda et al. 2009; Corcellas et al. 2012; Feo et al. 2012; Bedi et al. 2013). Among the various milk contaminants, OPs are among the most important due to their high toxicity at very low residual concentrations. The presence of OP residues in milk has already been reported by researchers (Salas et al. 2003; Pagliuca et al. 2006) and is mainly attributed to the ability of OPs to covalently link with milk proteins (Deiana and Fatichenti 1992). Cheema et al. (2004) have also reported the presence of chlorpyrifos in 6.7 % of milk samples collected from Punjab, and concentrations in all of these samples exceeded MRL values. Similarly, Kathpal et al. (2001) reported the presence of chlorpyrifos residues in three milk samples that exceeded the MRL.

Spatial Variation of Pesticide Residues in Milk from Punjab

The increasing number of human cancer cases in cotton belt of Punjab (zone 3) has become a matter of serious concern at both the national and international level (Thakur et al. 2008). Bearing in mind different cropping patterns and agroclimatic zones, we analysed pesticide residues separately in three zones of Punjab (Table 2). The occurrence of pesticide residues in these three zones of Punjab showed zone 3 to be more contaminated than zones 1 and 2. In all three zones, chlorpyrifos was the leading contaminant, but comparatively greater levels were observed in zone 3 (2.6 μg/kg) followed by zone 2 (2.3 μg/kg) and then zone 1 (1.3 μg/kg). Similarly, lindane residues were detected 2.7 and 1.6 times greater in zone 3 than in zones 1 and 2, respectively. In zone 3, significantly greater ($p = 0.026$) residues of endosulfan were observed than in the other two zones. Although statistically insignificant, residue levels of fipronil, butachlor, cyhalothrin, cypermethrin, malathion, profenophos, and ethion were greater in zone 3 than zone 2 by a factor of 2.3, 2.1, 1.3, 3.6, 2.1, 5.4, and 3.3, respectively. Cotton cultivation employs high use of a dangerous cocktail of pesticides such as chlorpyrifos, acephate, ethion, triazophos, fenvalerate, cyhalothrin, cypermetharin, etc. which may lead to the contamination of animal feed, fodder, and environment (Singh and Kaur 2012). In addition, cotton seed cake is frequently used as a protein-rich animal feed, and cotton seed samples harvested in five locations in India showed that 26 % were found to be contaminated with chlorpyrifos, 22 % with endosulfan, and 16 % with ethion (Blossom and Singh 2004). Furthermore, the cropping intensity of different districts of Punjab showed that the zone 3 has maximum area under cultivation (92.8–95.2 %) compared with the other two regions. The cropping patter in zone 2 suggests that rice and wheat are the major crops; pesticides are also regularly sprayed on these, which may be responsible for the occurrence of pesticide residues in fodder, animal feed, and ultimately in milk.

Estimation of Daily Dietary Intake of Pesticide Residues

In recent years, the evidence for increased residues of chemical compounds in food as a consequence of environmental pollution has driven the need to screen for the potential public health significance of residue concentrations and exposures. Risk assessment for persistent compounds, such as OCPs, plays an important role in consumer and animal health. In this study, potential health risks were assessed based on the dietary intake of pesticide residues from milk consumption (Table 3). The estimated ADI values of all of the pesticides included in this analysis were observed to be lower than the recommended ADI values in three zones. However, the 95th-percentile UB level for the intake of fipronil exceeded the ADI by 7.02 times in zone 3. Furthermore, the comparative dietary intake value of pesticides in the different zones indicated more pesticide exposure in zone 3 followed by zone 2 then zone 1. Assuming the same dietary consumption of milk in children and adults, it can be interpreted that children are at greater risk than adults. In the region of present study, during the period 1999–2001, the estimated dietary intake of lindane based on mean residues levels in milk was 1.8 times more than the ADI values; for maximum detected levels, the estimated dietary intake increased to 3.67 times of the ADI value (Battu et al. 2004). Thus, the present findings indicate that although the dietary intake of lindane has decreased during the past decades, the concomitant increase in the intake of other pesticides (such as endosulfan, fipronil, chlorpyrifos, cypermethrin) may offset any gains due to the decrease of lindane residues and thus warrants serious attention. Moreover, the ingestion of a cocktail of pesticide residues may lead to more severe hazards as a result of the synergistic effect of pesticides.

Risk Assessment to Humans by Consumption of Milk

Noncancer Risk Assessment

The HIs for pesticides were calculated and used to evaluate the potential noncancer health impact of pesticide residues in milk (Table 4). It was observed that all of the HI values fall below the target value of one in adults in all three zones

Table 2 Spatial distribution of pesticide residue levels (μg/kg) in milk from three different zones of Punjab

Pesticide	Zone 1 (n = 133)				Zone 2 (n = 101)				Zone 3 (n = 78)			
	Mean ± SD	Maximum	% Positive[a]	% Proportion[b]	Mean ± SD	Maximum	% Positive[a]	% Proportion[b]	Mean ± SD	Maximum	% Positive[a]	% Proportion[b]
HCH	0.5 ± 2.4	14.4	3.8	10.6	0.8 ± 3.2	19.7	6.8	8.1	1.3 ± 3.7	23.1	9.9	8.3
p,p’ DDE	1.0 ± 3.8	17.5	6.1	21.7	0.9 ± 3.8	21.5	5.3	8.6	1.1 ± 3.8	19.7	5.9	6.7
p,p’ DDD	0.3 ± 1.9	13.3	2.6	6.8	0.6 ± 2.7	14.7	5.3	6.3	0.8 ± 2.9	17.4	5.9	5.2
β-Endosulfan	ND	–	–	–	0.3 ± 1.8	12.3	3.0	3.1	0.3 ± 1.5	13.2	3.0	2.2
Endo. sulfate	0.6 ± 2.5	14.7	5.1	12.6	0.6 ± 2.8	17.0	4.5	5.8	1.8 ± 4.7	27.3	11.9	11.6
Fipronil	ND	–	–	–	0.5 ± 2.6	16.5	3.0	4.4	1.1 ± 4.4	26.1	5.9	6.8
Butachlor	ND	–	–	–	0.3 ± 2.7	22.8	1.5	3.3	0.7 ± 2.6	16.8	5.9	4.6
Cyhalothrin	ND	–	–	–	1.0 ± 4.5	31.7	5.3	9.9	1.4 ± 4.7	30.4	6.9	8.6
Cypermethrin	0.7 ± 3.4	21.6	3.8	14.8	0.5 ± 2.7	18.6	3.0	4.6	1.7 ± 5.9	50.0	6.9	10.7
Fenvalerate	ND	–	–	–	1.0 ± 4.3	27.2	5.3	9.5	0.8 ± 3.1	18.4	4.9	5.2
Deltamethrin	0.2 ± 1.6	14.4	1.3	3.9	0.8 ± 4.4	38.7	3.8	7.7	0.3 ± 5.5	27.4	4.0	1.7
Malathion	ND	–		–	0.3 ± 3.9	45.3	0.8	3.3	0.7 ± 3.2	39.8	2.0	4.5
Chlorpyrifos	1.3 ± 5.8	31.5	5.1	29.2	2.3 ± 8.8	51.3	6.8	22.4	2.6 ± 8.7	46.3	6.9	16.4
Profenophos	ND	–		–	0.1 ± 1.0	12.0	0.8	0.9	0.5 ± 1.7	18.1	4.0	3.1
Ethion	ND	–	–		0.2 ± 2.5	29.0	0.8	2.1	0.7 ± 3.5	23.6	3.0	4.6

n number of milk samples, *ND* not detected

[a] Percentage of positive samples from total samples analyzed

[b] Based on the mean residue levels, contribution of a particular pesticides among total pesticide residue load detected in milk samples

Table 3 Estimated average daily dietary intake (EADDI) (μg/d) of pesticide residues through consumption of milk from three zones of Punjab at LB (mean) and UB (P95) approaches

Pesticides	ADI (μg/kg bw/day)[a]	TADI adults (μg/day)[b]	TADI children (μg/day)[c]	Zone 1		Zone 2		Zone 3	
				EADDI Mean LB (μg/day)	EADDI P95_UB (μg/day)	EADDI Mean LB (μg/day)	EADDI P95_UB (μg/day)	EADDI Mean LB (μg/day)	EADDI P95_UB (μg/day)
Lindane	5	300	50	0.5	0.9	0.8	9.4	1.3	9.4
DDT	10	600	100	1.2	13.6	1.4	12.7	1.8	16.4
Endosulfan	6	360	60	0.3	2.2	0.9	9.4	2.1	15.1
Fipronil	0.2	12	2	0	0.9	0.4	0.9	1.0	14.2
Cyhalothrin	20	1200	200	0	1.9	1.0	5.7	1.3	10.4
Cypermethrin	20	1200	200	0.3	1.9	0.4	1.9	1.6	14.6
Fenvalerate	20	1200	200	0	1.9	0.9	5.7	0.8	1.9
Deltamethrin	10	600	100	0.2	1.9	0.7	1.9	0.2	1.9
Malathion	30	1800	300	0	1.9	0.3	1.9	0.7	1.9
Chlorpyrifos	10	600	100	1.2	4.3	2.2	28.3	2.5	29.7
Profenophos	30	180	300	0	1.9	0.1	1.9	0.5	1.9
Ethion	2	120	20	0	1.9	0.2	1.9	0.7	1.9

EADDI estimated average daily dietary intake, *P95* 95th percentile

[a] Acceptable daily intake (ADI) obtained from Codex Alimentarius Commission

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

[c] TADI value for children was multiplied by average body weight of 10 kg

Table 4 HIs for pesticide residues from milk in adults and children from three zones of Punjab at LB (mean) and UB (P95) approaches

Pesticide	RfD (μg/kg bw/day)	Zone 1				Zone2				Zone 3			
		Adult		Children		Adult		Children		Adult		Children	
		Mean LB	P95_UB	Mean LB	P95_UB	Mean LB	P95_UB	Mean LB	P95_UB	Mean LB	P95_UB	Mean LB	P95_UB
γ-HCH	0.3	0.03	0.2	0.05	0.31	0.04	0.26	0.52	3.15	0.07	0.42	0.52	3.15
DDT	0.5	0.04	0.2	0.45	2.73	0.05	0.29	0.43	2.59	0.06	0.36	0.55	3.28
Endosulfan	6.0	0.0	0.0	0.01	0.04	0.0	0.01	0.03	0.16	0.01	0.03	0.04	0.25
Cyhalothrin	5.0	0.0	0.0	0.01	0.04	0.0	0.02	0.02	0.11	0.0	0.03	0.03	0.21
Cypermethrin	10.0	0.0	0.0	0.0	0.02	0.00	0.0	0.0	0.02	0.0	0.02	0.02	0.15
Fenvalerate	25.0	0.0	0.0	0.0	0.01	0.0	0.0	0.0	0.02	0.0	0.0	0.0	0.01
Malathion	20.0	0.0	0.0	0.0	0.01	0.0	0.0	0.0	0.01	0.0	0.0	0.0	0.01

P95 95th percentile

[a] RfD values (guidance values and) obtained from USEPA

of Punjab at both the LB and 95th-percentile UB levels. However, for children at the 95th-UB level, the HI for DDT exceeded value of one in all three zones, and for HCH it exceeded the value of one in zones 2 and 3. Therefore, it can be interpreted that pesticide exposure in these two zones may pose noncancer risks to children at 95th-percentile UB levels. For the remaining pesticides detected in this study, there is a lower probability of noncancer risks at both the LB and 95th-percentile UB levels. In a similar study, Pandit and Sahu (2002) also reported that HIs fell below the target value of one at mean residue levels of HCH and DDT.

Cancer Risk assessment

Most of the OPs found in milk and milk products have been identified as potential human carcinogens by USEPA and International Agency for Research on Cancer. USEPA has

Table 5 Estimated cancer risk associated with exposure to pesticides from milk in adults and children from three zones of Punjab at LB (mean) and UB (P95) approaches

Pesticides	USEPA classification	CSF	Zone 1		Zone 2		Zone 3	
			Mean LB	P95_UB	Mean LB	P95_UB	Mean LB	P95_UB
Lindane	B2	1300	2.55E−06	5.32E−06	4.427E−06	5.35E−05	7.08E−06	5.35E−05
DDT	B2	340	6.91E−06	7.72E−05	8.22E−06	7.34E−05	1.01E−05	9.30E−05

P95 95th percentile

[a] Relevant USEPA classifications: A = known human carcinogen; B2 = probable human carcinogen; C = possible human carcinogen

[b] Cancer potency factor (µg/kg days)$^{-1}$ per USEPA

traditionally suggested a range of cancer risk 10^{-6} to 10^{-4} as acceptable with 1×10^{-5} as the typically recommended value.

Comparing the BMC derived from this study with those derived by USEPA (Table 5), BMC at mean residue levels for the population of Punjab were found to be in the recommended range of USEPA reference values. However, at the 95th percentile, cancer risks were found to be greater for both DDT and HCH in zones 2 and 3. The comparative cancer risk values in the different regions of Punjab reflect the greater probability of risk in zone 3 than the other two zones. However, in such analyses, considerable uncertainty exists arising from a number of potential sources, e.g., (1) the potential health impact due to the intake of milk and milk products containing chemicals in excess of the guidance value; and (2) guidance values include an extrapolation from animals to humans for the identification of a "no-observed adverse effect." In addition, toxicological effects were assessed on a single-compound basis; potential effects of the mixture of chemicals were not considered; and certain chemicals detected in milk and milk products do not have published guidance values and have not been evaluated for carcinogenicity, thus rendering the assessment of these chemicals highly uncertain (Pandit and Sahu 2002). The findings of the present study suggest that children are at comparatively greater risk from exposure to pesticides than are adults, particularly in sectors of the population where pesticide use is greater.

Conclusions

From the results of this study, it can be concluded that after the imposition of bans or restrictions on the use of HCH and DDT, only few bovine milk samples contained residues of both substances along with some violations of MRLs. Here, it is worth mentioning that the benefits of antimalaria application of DDT may considerably outweigh the minor risks of pesticide intake with milk. However, the occurrence of less persistent OPs and SPs residues in milk reflect the improper application of pesticides and poor animal husbandry–management practices in dairy farms. Therefore, the development and use of appropriate management strategies should be a priority in the near future because accumulation of pesticides in the food chain and the consequent population exposure to pesticides through food consumption poses serious public health risks. The spatial variation of residues showed greater pesticide exposure in the cotton-growing belt compared with other regions of Punjab. In addition, children were observed to be at comparatively greater risk than adults at UB levels of pesticide residues in bovine milk in this region of Punjab. On the basis of these findings, we recommend the need for continuous monitoring of pesticide residues in milk and its products along with formulation of more stringent standards for the application of pesticides. Furthermore, a risk benefit assessment should be performed before suggesting that a particular chemical treatment is to be stopped due to the risk of contaminating the food chain.

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