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Current Scenario of Pesticide Residues in Bovine Milk in Punjab and Human Health Risk Assessment

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Abstract

Milk contamination is of extreme concern consideringit's an important component of everybody's diet. In present study 706 milk samples collected from different districts of Punjab were screened for the presence of organophosphate, synthetic pyrethroids and organochlorine pesticides. Pesticide residues were assessed using Gas chromatography with flame thermionic detector and electron capture detector then confirmed by Gas chromatography-Mass Spectrometry. The quantitative estimation of pesticide residues in milk samples revealed the presence of chlorpyrifos, HCH (γ-HCH), DDT, endosulfan and cypermethirn with mean levels of 1.46 ng g^{-1}, 0.43 ng g^{-1}, 0.40 ng g^{-1}, 0.39 ng g^{-1} and 1.23 ng g^{-1}, respectively. Chlorpyrifos was found to be the major contributor with 29.9% contribution, and 3 samples exceeding the maximum residue limit (MRL) followed by HCH with 21.2% contribution and 7 samples exceeded its MRL and cypermethrin 18.1 % contribution and only 2 samples with concentration above MRL. The spatial distribution of residues in Punjab reflected the higher levels in south-west region known as the cotton belt. Risk assessment through consumption of milk in Punjab was evaluated and observed that hazard index for DDT and HCH were within the prescribed ranges.

Keywords: Bovine milk, Pesticide residue, Gas chromatography, Risk assessment, Punjab

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Introduction

Pesticides are one of the most important components of modern agricultural technology and have contributed greatly to the increase of agriculture yields and have also played a significant role in the control of vector borne diseases transmitted by insect vectors and microorganism. However, the inappropriate application of persistent pesticides has led to the adverse environmental and health consequences. Through overuse and misuse, pesticides have affected the whole ecosystem which is indicated by the presence of their residues in the food chain and pollution of the soil, air, and water.[1] The occurrence of pesticide residues in environment and food articles is potentially harmful to the organisms at higher trophic levels in the food chain. Since diet is the main source of chronic exposure to low doses of these chemicals, human beings are mainly exposed to pesticides through ingestion.[2] Increasing incidence of cancer, chronic kidney diseases, suppression of the immune system, sterility among males and females, endocrine disorders, neurological and behavioral disorders have been attributed to chronic pesticide intake.[2] The contamination of food by pesticide residues has become a worldwide public health concern

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and is a leading cause of trade problems internationally. Therefore, the determination of pesticide residues in the environment and in foods is necessary for ensuring that human exposure to contaminants, especially by dietary intake, does not exceed acceptable levels for health.

Milk contamination is of extreme concern since milk and milk products have a very special position in the diet of infants, children and elderly for whom these are considered as a perfect natural food. This point is of particular interest since young children may be highly exposed to pesticides and pesticide residues and they may be at higher risk of adverse health effects because of their physiological characteristics.[3] Lactating cows may be exposed to pesticides from contaminated feed, fodder and water, they can then accumulate residues in different compartments including milk.[4] However, the contamination of milk by pesticides can also be caused by their application to the cow's body, in the cow barn or even in the milk processing areas.[5] Due to the persistence and lipophilicity of the pesticides their residues get magnified in lipid-rich tissues of the organisms and under certain conditions such as lactation, mobilization of deposited contaminants occurs and results in their excretion into milk. Keeping in view the importance of pesticides in humans and their subsequent health hazard and increasing incidence of chronic diseases in the human population of Punjab, the present study was undertaken to assess the current load of pesticides in themilk of this state and calculating the risks associated with it.

Materials & Methods

Extraction of pesticide residues

A total of 706 bovine market milk samples were collected from different districts of Punjab. Around 10 mL of milk was collected in sterilized falcon tubes pre-washed with acetone and brought to the laboratory under chilled conditions. Pesticides residues from milk samples were extracted as per the method of Battu *et al.*[6] Briefly, 5 g of sample was mixed with 20 g each of freshly activated silica gel and anhydrous sodium sulfate to form a free flowing powder. The powder was packed into extraction glass column containing 40 mL of dichloromethane over a plug of glass wool. The mixture was allowed to stand for 90 min. The solvent was then eluted first with dichloromethane and then with 150 mL of dichloromethane: acetone (1:2 v/v) mixture. Anhydrous sodium sulfate was then added to elute to remove the turbidity. The extracted sample was then evaporated to dryness. Fifteen mL of n-hexane was then added and evaporated; thestep was repeated twice to eliminate dichloromethane completely which otherwise will interfere during analysis on GC-ECD. The final volume was made with n-hexane:acetone (1:1) mixture. In this study, the targeted pesticides were HCH isomers, DDT, and its metabolites, heptachlor, fenitrothion, aldrin, fipronil, butachlor, dieldrin, endrin, endosulfan among OCPs. Likewise, monocrotophos, dimethoate, parathion, malathion, chlorpyrifos, fenamiphos, profenophos, ethion, triazophos and phosalone were the OPs tested in the study and L-cyhalothrin, permethrin, cyfluthrin, cypermethrin, fenvalerate, and deltamethrin were the some of the SPs considered in the study.

Estimation of pesticide residues

Estimation of pesticide residues was done using Gas chromatograph (GC) equipped with electron capture detector (Agilent 7890B System) and flame thermionic detector (Shimadzu model 2010). The GC oven temperature for electron capture detector was initially programmed at a temperature of 170 °C for 13 minutes followed by at temperature increase to 270 °C at the rate of 3°C min^{-1} with hold time for 20 minutes. For flame thermionic detector initial oven temperature was set at 150°C with hold time for 5 minutes followed by temperature increase to 220 °C at the rate of 10 °C min^{-1} with hold time for 5 minutes and final temperature ramped to 250 °C at a rate of 5 °C min^{-1} with hold time for 13 minute. The temperature of injection port was set at 280 °C whereas the temperature of both the detectors was adjusted at 310 °C. Nitrogen was used as carrier gas while hydrogen and air were used for flame formation in the flame thermionic detector. Calibration curves for all standards of OCPs, OPs and SPs were drawn for concentration vs. area of the peak and the correlation coefficients (r^2) were determined near to 0.99. Analytical technical grade standards with 93–99% purity for OCPs, OPs, and SPs were procured from Sigma-Aldrich (USA). The confirmation of pesticide residues was done on GC-mass spectrometry (Shimadzu GCMS QP2010 plus). The mass spectrometer was operated in electron ionization mode. The interface, manifold and ion source temperatures were 290°C, 50°C 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^{-1} was used as carrier and collision gas. In this study, selective ion monitoring method was designed for pesticides including retention time windows and base peak ions.

To check the interference of contamination from solvents and glassware while sample processing reagent blanks were run after every six samples. The recovery values were calculated by the processing of spiked bovine whole milk samples with standard pesticides at known concentrations of 50 and 100 μg/kg. The calculated mean recovery values were ranged from 81.2% for δ-HCH to 113.5% for aldrin, which were in accordance with the acceptable recovery range of 70–120% (Association of Analytical Chemists (AOAC) 1999)[7] therefore calculated concentrations of pesticide residues in samples were not corrected for recovery.

Results

Contamination patterns of pesticide residues in bovine milk

The quantitative estimation of pesticide residues in milk samples (706) revealed the occurrence of chlorpyrifos, HCH (γ-HCH), DDT, endosulfan and cypermethirn (Table 1). Among these chlorpyrifos was detected in 38 (5.4%) of the samples with mean levels of 1.46 ng g^{-1} and contributed the highest (29.9%) proportion of the total pesticide residue load in milk samples followed by HCH (21.2%) and cypermethrin (18.1%). The residues of γ-HCH (lindane) were found in 27 milk samples (3.8%) at a mean level of 0.43 ng g^{-1}. The total DDT (sum of pp DDE, op DDD) was detected in 2.5% of total samples at mean levels of 0.40 ng g^{-1} while 2.97 % samples were found positive for endosulfan residues having mean levels of 0.39 ng g^{-1}. Among SPs, only cypermethrin was detected with a mean level of 1.23 ng g^{-1} in 23 (3.25%) samples. It was observed that although mean residue levels of all detectable pesticides were below their respective MRLs (Table 1). However, considering residue levels in individual milk samples, 7 exceeded the MRLs for HCH, 1 for DDT and 9 for endosulfan. However, only 3 samples exceeded the MRLs for chlorpyrifos and 2 for cypermethrin. Considering the contribution of mean levels of OCPs, OPs and SPs to the total pesticide levels, 37.0 % of total mean was contributed by OPs followed by SPs (32.0%) and then by OCPs (31.0%). The spatial distribution results indicated the higher levels of lindane in Ludhiana (1.1 ng g^{-1}) and Faridkot (0.8ng g^{-1}) districts together with more dietary intake of this pesticide by consumer of these districts. Similarly, DDT residues were utmost in Ludhiana (1.1ng g^{-1}) followed by Patiala (0.75 ng g^{-1}) corresponds to the increased dietary intake of DDT by Ludhianvis. Chlorpyrifos residues were estimated more in Fazilka (2.62 ng g^{-1}) followed by Ferozepur (2.44 ng g^{-1}), Ludhiana (1.07 ng g^{-1}) and Faridkot (1.03 ng g^{-1}). Cypermethrin contents were seen higher in Fazilka (2.65ng g^{-1}) as compared to other districts of the Punjab.

Risk assessment to humans by consumption of milk

To assess the risk of pesticide residues in milk, the data was evaluated under two contamination scenarios to handle left-censored data: lower-bound scenario (LB) where undetected results were set to zero and 95th percentile upper-bound (UB) scenario where undetected results were set to the detection limit. The risk assessment for both non-cancer and cancer hazards through consumption of milk in Punjab were evaluated as our already published methodology.[8]The hazard indices (HI) for pesticides were calculated and used to evaluate the potential non-cancer health impact of pesticide residues in milk (Table 3). It was observed that all of the HI values fall below the target value of one in adults in Punjab at both the mean and 95th percentile upper bound levels. However, for children at the 95th upper bound level, the HI for DDT and HCH exceeded one. For the remaining pesticides detected in this study, there is a lower probability of non-cancer risks at both mean and 95th percentile upper bound levels. In a similar study, Pandit *et al.*[9] also reported that HIs fall below the target value of one at mean residue levels of HCH and DDT.

Most of the organochlorine pesticides found in milk and milk products have been identified as potential human carcinogens by USEPA and IARC (International Agency for Research on Cancer). The EPA has traditionally suggested a range of cancer risk 10^{-6} to 10^{-4} as acceptable, with $1x10^{-5}$ as the typically recommended value. Comparing the cancer benchmark concentrations (BMC) derived from this study with those derived by US EPA (Table 4), BMC at mean residue levels for the population of Punjab were found to be in the recommended range of US EPA reference values for both 95th upper bound level and lower bound level.

Table 1: Mean concentrations (ng g^{-1}) of pesticide residues in bovine raw milk samples collected throughout Punjab (India)

Pesticide	Mean±SD (n=706)	Positive samples	% frequency	% Proportion	MRLs by CAC*	Samples above MRL
HCH	0.43±2.39	27	3.80	21.25	10.0	7
DDT	0.40±2.52	18	2.54	14.17	20.0	1
Endosulfan	0.39±2.65	21	3.0	16.53	10.0	9
Cypermethrin	1.23±7.26	23	3.25	18.11	50	2
Chlorpyrifos	1.46±6.83	38	5.4	29.92	20.0	3

*Maximum residue limits recommended by Codex Alimentarius Commission 2008

Table 2: Mean (ng g^{-1}) and estimated daily dietary intake (µg day^{-1})of pesticides in different districts of Punjab, India

Districts	HCH		DDT		Endosulfan		Chlorpyrifos		Cypermethrin	
	Mean	EDDI	Mean	EDDI	Mean	EDDI	Mean	EDDI	Mean	EDDI
Faridkot (n=57)	0.8±3.1	0.75	0.7±3.1	0.66	0.36±1.96	0.34	1.03±4.4	1.02	1.1±6.1	1.01
Fathehgarh Sahib(n=47)	-	-	-	-	-	-	0.87±2.16	0.86	0.31±2.16	0.9
Fazilka (n=54)	0.51±2.1	0.48	-	-	0.22±1.66	0.21	2.62±10.3	2.60	2.65±13.7	2.50
Ferozepur (n=37)	-	-	-	-	0.39±2.38	0.37	2.44±8.8	2.42	0	0
Gurdaspur (n=62)	0.43±1.9	0.40	0.63±2.9	0.59	0	0	1.01±5.7	1.00	1.15±6.5	1.08
Jallandhar (n=43)	0.25±1.7	0.24	0	0	0.64±3.13	0.60	0	0	0	0
Kapurthala (n=48)	0.6±2.3	0.57	0	0	0.55±2.83	0.52	0.57±2.7	0.57	1.84±8.83	1.73
Ludhiana (n=71)	1.1±4.2	1.01	1.55±4.9	1.46	1.04±3.7	0.89	1.07±4.24	1.06	2.61±8.9	2.46
Mansa (n=63)	0	0	0	0	0.87±5.8	0,82	0	0	2.29±10.4	2.16
Moga (n=48)	0.39±1.9	0.37	0.73±3.9	0.69	0.2±1.4	0.19	0.39±1.9	0.39	0.92±4.48	0.87
Rpoar (n=61)	0.41±1.85	0.39	0	0	0	0	0.96±7.52	0.95	0	0
Patiala(n=62)	0.76±3.9	0.72	0.75±3.5	0.71	0.4±2.2	0.38	0.95±5.3	0.94	1.24±6.9	1.17
Sangrur (n=55)	0	0	0.22±1.64	0.21	0.19±1.0	0.18	0.96±5.18	0.95	0.83±6.2	0.79

EDDI (estimated daily dietary intake)= Meanx944 (availability of milk/capita/day in Punjab)/1000 (constant for change in units from ng to µg)

Discussion

The present study reported the predominance of OPs (chlorpyrifos) and SPs (cypermethrin) pesticides in milk samples. 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 higher detection frequency and concentrations.[10,6] In a study, total DDT, HCH, endosulfan, and aldrin were present in 100%, 97%, 43% and 12% milk samples, with a mean value of 36.7, 29.2, 2.2 and 3.6 ng g^{-1}, respectively.[9] Thus the present results may indicate thechanging trends in use of OPs and SPs in as replacement for OCPs which is also evident in some recent studies conducted in this region.[11, 12] Nevertheless, technical HCH (α, β,γ & δ) has been banned in India since 1997, still in 7 milk samples exceeded the MRLs for γ-HCH which could be corroborated to the exception of γ-HCH isomer use in agriculture including fodder crops.[13] Further considering the individual pesticides detected in this study, DDT was present in only 2.54 % of total samples with minimum contribution in total load of pesticides being observed in

Table 3: Hazard indices for pesticide residues from milk in adults and children of Punjab at deterministic (mean LB) and probabilistic (P95_UB) approaches

Pesticides	RfDa/µg Kg^{-1} per day [a]	ADI (µg/Kg bw/d)[b]	TADI adults (µg/d)[c]	TADI children (µg/d)[d]	Hazard indices for pesticide residues			
					Adults		Children	
					Mean LB	P95_UB	Mean LB	P95_UB
HCH	0.3	5	300	50	0.022	0.665	0.133	3.990
DDT	0.5	10	600	100	0.011	0.336	0.070	2.018
Endosulfan	6.0	6	360	60	0.001	0.028	0.006	0.168
Chlorpyrifos	-	10	600	100	-	-	-	-
Cypermethrin	10.0	20	1200	200	-	-	-	-

aRfD values (Guidance values and) obtained from USEPA
bAcceptable daily intake (ADI) obtained from Codex Alimentarius Commission
cTADI (total acceptable daily intake) value for adults was multiplied by average body weight of 60 Kg
dTADI value for children was multiplied by average body weight of 10 Kg

Table 4: Estimated cancer risk associated with exposure to pesticides from milk in adults and children of Punjab at deterministic (mean LB) and probabilistic (P95_UB) approaches

			Cancer Risk	
Pesticides	US EPA classification	CSF[b]	Mean LB	P95_UB
Lindane	B2	1300	0.492E-06	1.470E-06
DDT	B2	340	0.113E-06	3.241E-06

[a]Relevant US EPA classifications: A, known human carcinogen; B2, probable human carcinogen; C, possible human carcinogen
bCancer Potency Factor (ng g-1 per d) -1 as per USEPA

milk samples. The present results observed much lower levels of DDT than reported in the previous studies in the region and studies from other parts of the country.[8] Among DDT metabolites, p,p' DDE was the leading metabolite imitated its long-term bioaccumulation in living beings. In one of 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.[11] In present study, the occurrence of endosulfan residues could be due to the presence of endosulfan residues in animal feed and fodder since contamination of animal feed has been reported earlier by many authors.[14] With regard to the other OCP residues analysed, aldrin, dieldrin, endrin, butachlor, fipronil and heptachlor were not detected in any milk sample and is likely the result their being banned for use in crop protection in India since two decades back.

Synthetic pyrethroids are widely used pesticides to control pests in agriculture, homes, communities, restaurants, hospitals, schools and dairy farms.). In the present study, the occurrence of cypermethrin has been observed in milk samples with a comparatively higher mean concentration than previous studies.[8] In India, residues of cypermethrin, deltamethrin, and fenvalerate have been reported in various food products.[15, 16] and even in human breast milk samples in recent studies.[11,12]Presence of pyrethroid residues has also been reported, similarly, chlorpyriofs was the only OPs detected in milk samples and the presence of OP residues in milk has already been reported by many researchers.[17,18]. In an earlier study, Cheema *et al.*[19] have also reported the presence of chlorpyrifos in 6.71% milk samples collected in Punjab with all samples exceeded MRLs. Similarly, Kathpal *et al.*[20] reported the presence of chlorpyrifos residues in three milk samples which exceeded the MRLs.

The spatial distribution of residues in Punjab reflected the higher levels in Ludhiana, Faridkot and Fazilka. The Faridkot and Fazilka region belongs to

the South-western Punjab (cotton belt) similar results have also been indicated by Bedi *et al.*[13] where relatively more pesticide residues were detected in south-western Punjab. The results may be justified with the fact higher pesticides are used in cotton crops as compared to others. However, in such type of analyses, high uncertainty exists because of intake of milk and milk products containing chemicals in excess of the guidance value is unclear, guidance values include an extrapolation from animals to humans for the identification of a "No Observed Adverse Effect". Further, toxicological effects were assessed on a single compound basis and potential effects of the mixture of chemicals were not considered and certain chemicals detected in milk do not have published guidance values and have not been evaluated for carcinogenicity rendering the assessment of these chemicals highly uncertain. The findings of the present study suggest that a mild risk from exposure to pesticides may occur in children.

Conclusions

The present study indicated the strict compliance of bans/ restrictions on the usage of HCH and DDT, with few milk samples exceeded the MRLs. However, the presence of OPs and SPs residues in milk reflect the changing pattern of pesticide residues occurrence in milk. Therefore monitoring of pesticide residues in milk and milk products should be conducted in future also. The risk assessment data indicated that at current levels of pesticide residues in milk, these do not pose any significant hazard to consumers.

Conflict of Interest

None Declared

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