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Pesticides contamination of lactating mothers' milk in the north-western Himalayan region of India

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Abstract

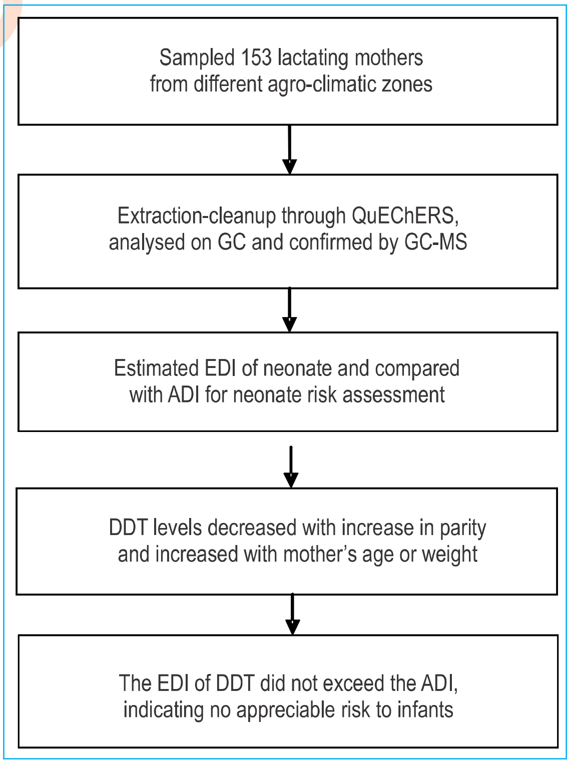
Aim : The study aimed to understand the status of pesticide residues in lactating mother's milk, identify factors involved in the transfer of pesticide residues and health risk to infants.

Methodology : A total of 153 lactating mothers were selected from four agro-climatic zones varying from subtropics to dry temperate high hills for residue analysis. The extraction and cleanup were performed by QuEChERS method. Residue were analysed by Shimadzu 2010 GC equipped with a ^{63}Ni ECD and confirmed by Shimadzu QP 2010 Plus GC-MS through selected ion monitoring (SIM) mode. The EDI was compared with the ADI of Σ-DDT (0.02 mg kg^{-1} body wt d^{-1}) established by FAO/WHO for neonate risk assessment from different pesticides.

Results : The mean DDT levels in breast milk were 0.240 mg kg^{-1} fat (0.011 mg kg^{-1} milk), 0.171 mg kg^{-1} fat (0.010 mg kg^{-1} milk), 0.026 mg kg^{-1} fat (0.001 mg kg^{-1} milk) and below detectable limit (BDL) in Zones I (subtropical), II (sub-humid foothills), III (wet temperate high hills) and IV (dry temperate high hills), respectively. The residue levels decreased with an increase in parity and increased with the age or weight of the mothers. The Σ-DDT residues were higher in rural women (0.011 mg kg^{-1} milk) than urban (0.005 mg kg^{-1} milk), housewives (0.009 mg kg^{-1} milk) than working women (0.007 mg kg^{-1} milk) and lacto-vegetarians (0.007 mg kg^{-1} milk) than omnivorous mothers (0.005 mg kg^{-1} milk). The estimated daily exposure of neonates to Σ-DDT was considerably lower (0.001 mg kg^{-1} body weight) than the ADI (0.02 mg kg^{-1} body weight) indicating no appreciable risk to one-month-old infants.

Interpretation : Mother's rural habitation, demography and primiparous parity seem to be the major cause for transfer of pesticide residues. The study also advocates a constant bio-monitoring of lactating mothers' milk for pesticide residues owing to the continuous changes in the pesticide usage pattern.

Key words: Human milk, Infant exposure, Pesticide residue, Risk assessment



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Introduction

Mother's milk is known to have numerous beneficial health effects and now is vastly considered as the best food for the baby (Hornell *et al.,* 2013). It is thought that pesticide residues accumulated throughout a woman's lifetime are mobilized during lactation and excreted in her milk (Ashby *et al.,* 1999). Schutz *et al.* (1998) reported that 90 percent of chlorinated hydrocarbons ingested by a mother are excreted in her milk. The most vulnerable time of exposure to pesticides appears to be during the embryonic and fetal periods (Toft *et al.,* 2010). High correlations of organochlorines exist between maternal adipose tissue, plasma, cord blood and human milk, demonstrating both placental and lactation transfer (Eik Anda *et al.,* 2007). A substantial elimination of organochlorine compounds from the maternal body occurs during human feeding (Uemura *et al.,* 2008). For this reason, women who have fed one or more children will have lower concentrations of these compounds than women who have not fed. Human milk is a sensitive matrix for monitoring the concentration of pesticides in the maternal body. Furthermore, the sampling of milk is non invasive and is, therefore, greatly accepted by mothers.

Primiparous women have the potential to excrete greater amounts of pesticide residues in their milk than multiparous woman, who likely have excreted the majority of their accumulated residues during previous lactations (Ennaceur *et al.,* 2007). High concentrations of pesticides in human milk have been reported in women working in agricultural fields (Kumar *et al.,* 2006) and living in malaria-affected areas (Bouwman *et al.,* 2006). The focus of agriculture in the Himalayan region has been shifting very fast from traditional cereal crops to high-value monoculture-dominated cash crops, leading to excessive and indiscriminate use of agrochemicals. This changing agricultural scenario has increased the mothers' exposure to different pesticides in the region. The present study was, therefore, carried out to determine the pesticide (OCs, OPs and SPs) residue status in milk of lactating mothers, transfer of pesticide residues from mother to infant and estimated daily intake (EDI) from different environments in Himachal Pradesh, India.

Materials and Methods

Study population and sample collection: The delivering mothers were approached at regional government hospitals located at Bilaspur, Hamirpur, Mandi, Kangra and Una (Zone I); Chamba, Nahan and Solan (Zone II); Kullu and Shimla (Zone III) and Keylong and Rekong Peo (Zone IV) of Himachal Pradesh. The sampling locations were all within the four major agro-climatic zones: the sub-mountainous subtropics of Zone I (365-914 m above mean sea level), the sub-humid foothills of Zone II (914-1523 m amsl), the wet, temperate high hills of Zone III (1523-2742 m amsl) and the dry temperate and high hills of Zone IV (2742-3656 m amsl). Zone I represents the 40 per cent of the total cultivated area followed by Zones II (37%), Zone III (21%) and Zone IV (2%). The milk samples were obtained within 4 days of delivery from 153 lactating mothers inhabiting for at least five years in their respective locations. A sample of 10-15 ml was collected from each mother thrice a day at an interval of four hours by manual expression directly into a well-labelled glass vials with Teflon-lined caps under supervision of a qualified nurse. Three replicates from each mother were mixed together and a representative sample was transported in an ice box to the Pesticide Residue Laboratory of YSP University of Horticulture and Forestry, Nauni, Solan and analyzed immediately.

Ethical clearance: The objectives of the study were explained clearly to each of the consulted lactating mother and/or her family attendant. Samples were only collected from mothers who had completed and signed the consent form (or whose family attendant did). A questionnaire was used to collect data about the socio-demographic characteristics of participating lactating mothers adopted from different studies (Bassil *et al.,* 2018).

Analysis of pesticides: The pesticide standards having 97 to 99 percent purity were purchased from Dr. Ehrenstorfer, Germany and stock solutions were prepared using pesticide-grade solvents (99.999% pure).

The extraction and cleanup of samples were performed according to standard QuEChERS method (Anastassiades *et al.,* 2003) with slight modifications. A 10 ml of milk sample was put into a 50 ml centrifuge tube. Twenty millilitre of acetonitrile (an extraction solvent), 4 g of anhydrous magnesium sulfate and 1.5 g of anhydrous sodium chloride was added to it and shaken vigorously for 1 min in a shaker. The tube was centrifuged at 3000 rpm for 1 min. Six millilitre of acetonitrile extract was transferred to 15 ml centrifuge tube, avoiding the upper oily layer for dispersive solid phase extraction (SPE) using 50 mg of PSA, 50 mg of C18 and 750 mg of magnesium sulfate. The extract was mixed for 20 sec and the tube was centrifuged again at 6000 rpm for 1 min. A 4 ml fraction was transferred to a 30 ml turbo tube and evaporated to dryness in turbo-evaporator in presence of a nitrogen current at 45°C. The residues were dissolved in 2 ml of n-hexane for gas chromatographic (GC) analysis.

The analysis was performed on a Shimadzu 2010 gas chromatograph equipped with a 63Ni electron capture detector (GC-ECD). One µl of extract was injected in split mode into a DB-1 (30m x 0.25 mm ID, 0.25 µm film thickness) capillary column, using nitrogen carrier gas with a 30 ml min^{-1} flow rate. The oven temperature varied over time: an initial temperature of 170 °C was held for 5 min, and then the temperature was increased to 220°C at 2°C min^{-1} and was kept for 10 min. The temperature was again increased to 280°C at 4°C min^{-1} and was held there for 7 min. The temperature of the detector and injector were kept at 300°C and 280°C, respectively. The GC estimation of pesticide residues were confirmed by gas chromatography mass spectrometry (Shimadzu QP 2010 Plus GC-MS) analysis through selected ion monitoring (SIM) mode. Chromatographic separation was achieved using a DB-5 capillary column (30m x 0.25 mm id with the film thickness of 0.25 µm). Ultra-pure helium gas was used as a carrier gas with split-less injection mode. The injection temperature was kept at 250°C, while the oven initial temperature of 80°C was increased to 280°C at the rate of 5°C min^{-1}. The MS detector was operated at 70 eV in EI auto-ionization mode.

Recovery studies: To determine the quality of the method, the recovery study was conducted in triplicate at various fortification levels. A number of blank and control samples were analyzed together with the samples to verify the accuracy and precision of the measurements. Recoveries for organochlorines and chlorpyrifos were between 91.33 to 120 percent whereas, for synthetic pyrethroids, recoveries varied from 85.6 to 100 percent. The limit of quantification (LOQ) was determined by the lowest calibration level, which ranged from 0.005 to 0.05 mg kg^{-1} for OCs, 0.01 mg kg^{-1} for chlorpyrifos and 0.02 to 0.04 mg kg^{-1} for SPs.

Estimation of daily intake of pesticides (EDI): The estimated daily intake (EDI) of Σ-DDT was calculated by the formula: EDI = Cmilk 700 g/5, where EDI is the estimated daily intake (mg kg^{-1} body weight day^{-1}) and Cmilk is the concentration of total DDT (Σ-DDT; p, p'-DDD + p, p'-DDE + p, p'-DDT + dicofol) in mg kg^{-1} milk (Sharma *et al.,* 2014). The variables i.e. average body weight (5 kg $neonate^{-1}$) and average milk consumption (700 ml $neonate^{-1}$ day^{-1}) were used as suggested by Azeredo *et al.* (2008). The EDI was compared with the acceptable daily intake (ADI) of Σ-DDT (0.02 mg kg^{-1} body weight d^{-1}) established by FAO/WHO (2008) for neonate risk assessment from different pesticides.

Statistical analysis: Statistical analysis was performed using SAS ver. 9.4 to calculate arithmetic means, standard deviations and minimum and maximum values. Samples with pesticide residues below 0.01 mg kg^{-1} were considered to be below detection limit (BDL) and were treated as zero in calculations. Pesticide concentrations were estimated on milk fat basis, assuming fat levels of 4.32 percent in human milk (Bergkvist *et al.,* 2012). Chi-Square test through PROC FREQ in SAS software was performed to determine the difference in proportion of contaminated lactating mothers in different zones. Variations in DDT residues in different cases, like habitation, diet delivery etc. were tested by Wilcoxon test. Tukey's Honest Significant Difference (HSD) test was used to differentiate the distribution of p, p'-DDE and p, p'-DDT. Age, weight and parity of the mother influence the residue contamination in milk (Sharma *et al.,* 2014), hence Pearson's correlations coefficient between these parameters and pesticide residue was calculated.

Results and Discussion

The maximum number of mothers (26.80%) in Zones I, II and III and in Zones II and III were found with residues of p, p'-DDE and p, p'-DDT, respectively. Earlier studies have also screened the presence of pesticide residues in breast milk, especially organochlorine pesticides (Zhou *et al.,* 2012; Rojas-Squella *et al.,* 2013; Muller *et al.*, 2017). The single highest concentration of p, p'-DDE (0.064 mg kg^{-1} milk) occurred in ZoneI. The presence of chlorpyrifos was detected in one sample only from zone II (1.96% of zone II samples tested); however, this concentration was within the range of BDL-0.011 mg kg^{-1} milk. The lactating women from Zone IV showed no sign of pesticide contamination in their milk (Table 1).

In the present study, the concentration of p, p'-DDE in mothers' milk was higher than that of p, p'-DDT in all agro-climatic zones. Due to high solubility and tendency of DDT and its metabolites to accumulate and reside in fatty tissues under natural conditions in a living organism, DDT is usually transformed into its more stable metabolite, DDE. According to Subramanian and Solomon (2006), the presence of high levels of DDE in human milk is an indicator of chronic and historical exposure of the mothers to DDT, which is subsequently metabolized to DDE and retained in the body (Qu *et al.,* 2010; Zhou *et al.,* 2011). In the foothills and intermediate hills, agriculture is the dominant occupation, and pesticides are thus, frequently and abundantly used to control crop pests. Furthermore, consumers in the mountains imported various cereals, fruits, vegetables, milk and milk products from adjoining states, where contamination levels have been reported to be higher than in other parts of the county (Sharma *et al.,* 2014). In dry temperate zone, farmers generally grow traditional crops such as barley, oats, buckwheat and wheat having less pest problems. The minimal application of pesticides in this Zone seems to be the major reason for mothers' safety from pesticide contamination. Rai *et al.* (2012) has reported varied patterns of pesticides residues in mother's milk at different altitudes in Uttarkhand State, India.

Chi-Square test inferred statistically non-significant difference in the proportion of residue contamination in lactating mothers from Zone I and Zone II. Whereas, the proportion of contaminated nursing mothers of Zone I differed significantly from Zone III, and Zone II from Zone III. The Wilcoxon test revealed that the Σ-DDT residues were independent of mothers' habitation, demography, primiparous parity and infant's weight. Tukey's HSD test was applied to check the significant differences in the amount of p, p'-DDE and p, p'-DDT between the zones. Zone 1 and II were different to Zone III and IV. The higher pesticide load in milk samples from Zone I and Zone II owes to the fact that these zones occupy more than $3/4^{th}$ of the total cultivated area under study.

The environment of a mother seemed to affect the residue concentration in her milk. Mothers residing in agro-climatic Zones I and II had higher Σ-DDT residues (0.011 and 0.010 mg kg^{-1} milk, respectively) than did mothers in Zone III (0.001 mg kg^{-1} milk). However, all the insecticides were found to be below detection limit in cold, dry zone (Zone IV) . The Σ-DDT residue levels were found to be higher in women from rural areas (0.011 mg kg^{-1} milk) than urban areas (0.005 mg kg^{-1} milk). Lactating housewives carried more residues (0.009 mg kg^{-1} milk) than working women (0.007 mg kg^{-1} milk). Diet was also found to affect pesticide residues in human milk. Σ-DDT residue concentrations were higher in lacto-vegetarians (0.007 mg kg^{-1} milk) than in omnivorous mothers (0.005 mg kg^{-1} milk) (Table 2).

Table 1: Pesticide residues detected in mothers' milk in different agro-climatic zones

Pesticides reporting status	Pesticides detected	Zone I (n=53)		Zone II (n=51)		Zone III (n=29)		Zone IV (n=20)	
		Sample (%)	Range[a]	Sample (%)	Range[a]	Sample (%)	Range[a]	Sample (%)	Range[a]
Pesticides in use	Chlorpyrifos	0	BDL	1 (1.96)	BDL 0.011	0	BDL	0	BDL
α-cypermethrin, bifenthrin, chlorpyrifos, deltamethrin dicofol, fenpropathrin, fenvalerate fluvelinate, λ- cyhalothrin									
Not in use in past fifteen years: HCHs, DDTs	p, p'-DDE	23 (43.40)	BDL (0.064)	17 (33.33)	BDL 0.050	1 (3.45)	BDL 0.022	0	BDL
	p, p'-DDT	0	BDL	1 (1.96)	BDL 0.018	1 (3.45)	BDL 0.010	0	BDL

– number of samples at each zone; BDL- below detection limit; [a]Range is concentration of pesticides expressed in mg kg^{-1} milk

Fruits and vegetables have already been reported as an important source of pesticide residues to nursing mothers (Elgueta *et al.,* 2017). A significant positive correlation was found between Σ-DDT residues and the age (r=0.99, p<0.05) and weight (r=0.96, p<0.05) of primiparous mothers. Σ-DDT residues were found to increase with the age and weight of primiparous mothers. The milk of mothers delivering smaller children was found to carry more DDT residues (0.007 mg kg^{-1} milk) than that of average weight infants (0.004 mg kg^{-1} milk) (Table 2).

Parity may be taken as an important factor in the prediction of OCs concentration in breast milk, although a better indicator may be the number and length of previous lactations. A significant negative correlation was observed between concentrations of Σ-DDT and parity in Zones I ®= -0.9467), Zone II (r= -0.9894) and Zone III (r= -0.7981). In all agro-climatic zones, pesticide residues decreased with increasing parity. The study found higher levels of DDT residues in primiparous mothers (0.010 mg kg^{-1} milk) and lower levels of DDT residues in multiparous women (0.007 mg kg^{-1} milk). A lower DDT contamination rate in colostrum (0.007 mg kg^{-1} milk) than in mature milk (0.004 mg kg^{-1} milk) was also observed, which indicated a correlation between residue concentrations and milk fat content (Table 2). Higher pesticide residues in the milk of rural women and housewives can be attributed to differences in factors such as occupation, food habits, education and other resources (income status, etc.) between the individuals of each lifestyle. Most women in rural mountainous areas were housewives who also engaged in various farm operations and thus were exposed to pesticides. Similar findings of high concentrations of pesticides in rural women have been reported by Zeinab *et al.* (2011). However, Limon-Miroa *et al.* (2017) reported higher concentrations of *p,p'*- DDT and cypermethrin in breast milk of urban mothers than those residing in agricultural areas in Mexico. The Σ-DDT residues were found to be higher in vegetarians than in omnivorous women. For Indian vegetarians, whole milk is a source of animal proteins. The application of pesticides to forage and cereal crops carry residues to the cattle feed. Due to the lipophilic properties of OCs, their residues are stored in the fat-rich tissues of milch animals and are subsequently excreted through milk fat. Reports of pesticide residues in milk and milk products have been reported by various authors (Tsiplakou *et al.,*

Table 2: Extent of pesticide residues in mothers' milk on the basis of different factors and lactation

Factor	∑-DDT residues (mg kg^{-1} milk)	
External factors		
Habitation		
Rural	0.011±0.012	0.152±0.282
Urban	0.005±0.012	0.100±0.252
Occupation		
Housewives	0.009±0.014	0.140±0.291
Working	0.007±0.012	0.134±0.276
Diet		
Vegetarian	0.007±0.013	0.158±0.295
Omnivorous	0.005±0.011	0.119±0.256
Physiological factors		
Infants' weight		
1-3 kg	0.007±0.012	0.165±0.270
3-5 kg	0.004±0.013	0.097±0.287
Effect of lactation Parity		
Primiparous	0.010±0.014	0.212±0.323
Multiparous	0.007±0.013	0.140±0.285
Mothers' milk type		
Colostrum	0.007±0.013	0.152±0.290
Mature milk	0.004±0.010	0.084±0.213

Values are mean ±SD

Table 3: Estimated daily intake (EDI) of ∑-DDT residues in infants

Infant age (n)	Residues recorded (mg kg^{-1} milk)	Estimated daily intake[a]	EDI over ADIs (Fold increase[b])
<10 days (121)	0.0076	0.0011	0.0550
10-20 days (19)	0.0065	0.0010	0.0500
20-30 days (13)	0.0021	0.0003	0.0150

[a]Estimated daily intake- residues recorded x milk requirement /body weight (mg kg^{-1} body wt day^{-1}); [b] Fold increase- EDI/ADI of DDT

2010; Bulut *et al.,* 2011; Fagnani *et al.,* 2011). The Σ-DDT residues increased with the weight and age of primiparous mothers. The lipophilic and hydrophobic nature of DDT together with its extreme persistence in the environment gives it considerable potential to accumulate in human tissues, particularly fat tissues. Other studies have reported similar results (Sudaryanto *et al.,* 2006; Ennaceur *et al.,* 2007).

The Σ-DDT residues may impair the anthropometric development of the fetus, reducing the birth weight. A negative correlation has been found between birth weight and Σ-DDT residue levels. The low-birth-weight infants are more vulnerable to milk contaminants due to their slow elimination mechanism and immature metabolism that cannot eliminate pollutants. Dewan *et al.* (2013) reported a negative correlation between infant birth weight and levels of OCs. Lactation is the most significant mechanism to reduce concentrations of pesticides stored in the human body and parity predicts pesticides concentrations in human milk. In this study, DDT residues in human milk of multiparous mothers were lower than the levels in primiparous mothers. This is because of the loss of pesticides from the mother's body during each period of lactation due to residues mobilization into the milk. Declining levels of POPs with parity were also reported by Al-Rahman (2010). A decrease in contamination was observed as milk progressed from colostrum to mature milk. This trend suggests a correlation between the residue concentration and the fat content of the human milk, as the milk produced immediately after delivering birth carries more fat than the milk in later periods (Waliszewski *et al.,* 2009).

The presence of DDT in breast milk indicates that breastfed neonates risk exposure to these chemicals. However, this study, found the EDI of Σ-DDT for neonates (Table 3) to be lower than the established ADI value (0.02 mg kg^{-1} b.wt. d^{-1}). As the EDI for various pesticides in mothers' milk was lower than the ADI, the infants did not pose any appreciable risk while kept on continuous feeding by mothers. Recent studies reported that most of the mothers' milk samples were free from pesticide residues; where ever detected were below ADI (Limon-Miroa *et al.,* 2017; Smadi *et al.,* 2019) and suggested breast feeding to be a safe practice. Mothers' age, weight, parity, fat mobilization, diet, and occupation and age of infants appeared as the major factors affecting the transfer of pesticide residues into the mothers' milk, thus require special consideration in future studies. Regular changes in pesticide usage pattern advocate a constant bio-monitoring of lactating mothers' milk for pesticide residues to minimize the health risks to infants and thereby to the future generation.

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