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ORGANOCHLORINE AND PYRETHROID PESTICIDES ANALYSIS IN DAIRY MILK SAMPLES COLLECTED FROM COTTON GROWING BELT OF PUNJAB, PAKISTAN

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The present study work was aimed at to monitor the residue level of some selected pesticides (Dichloro diphenyl tricthloro ethane, Dichloro diphenyl dichloro ethylene, endosulfan, aldrin, cypermethrin, deltamethrin, permethrin, and bifenthrin) in milk samples collected from the cotton growing belt of the Punjab province. The residue level of these pesticides in dairy milk samples was analyzed on High Performance Liquid Chromatography (HPLC) system. About 70% of the collected samples were found contaminated with either of the analyzed pesticides. Aldrine was detected in 35% samples with mean concentration level of 0.68µg/ml, ranged between 0.32 µg/ml and 5.19µg/ml. However, DDT, DDE, and endosulfan prevailed comparatively in lower percentage such as 10%, 9%, and 7%, respectively. DDT ranges between .003µg/ml and 0.40µg/ml, with mean concentration level of 0.01 µg/ml. Whereas DDE is present with mean residue level of 0.04µg/ml, ranging from 0.15µg/ml to 1.23µg/ml. Lower level of DDT residues in milk indicates that the further application of DDT has been ceased which is also reported by other monitory studies conducted in most of the countries. Mean concentration level of the Endosulfan in milk was 0.13µg/ml. Among pyrethroids pesticides detected in milk samples, bifentrhin was present with the highest mean concentration level of 1.68µg/ml followed by permethrin with mean residue level of 1.24µg/m. The mean residue level of cypermethrin and deltamethrin was 0.23µg/ml and 0.21µg/ml respectively. The results also indicated that 23%, 21%, 18% and 7% of the milk samples were contaminated with cypermethrin, bifenthrin, permethrin and detlamethrin, respectively.

Keywords: Organochlorin, pyrethroids, pesticides, residue level, dairy milk

INTRODUCTION

Presence of persistence and hazardous agrochemicals in human diet is an emerging problem in developing countries which encounter poor institutional progress and slow financial development. The agrochemicals particularly pesticides enter in our body via food of animal origin, rich with lipid contents. As livestock is a growing business and important sector in Pakistan (Rana *et al*., 2014; Shahzadi *et al.*, 2014) and many studies have indicated that animal origin-food is responsible for more than 90% of the average humane intake of organchlorine compounds and other contaminants (Avancini *et al.*, 2013; Tecles *et al.*, 2013). Of all these products of animal origin-food, milk contamination earned the immediate attention because of its both essential and supplementary nutritional value as well as its consumption in all age-group people. Since milk contains a considerable amount of fat, therefore presence of these lipophilic compounds in it is beyond any suspicions. Presence of pesticides in milk was reported by several authors in different countries over the last few decades, and use of most of these chemicals had been banned in many countries *(*Nag *et al., 2007).*

Organochlorine chemicals have been clinically proven as endocrine disrupting chemicals and carcinogenic substances (Kaushik *et al.*, 2011). Similarly pyrethroid pesticides paralyze the nervous system of living organism. In addition to this, these hazardous chemicals may cause a number of chronic and acute health disorders such as, reproductive dysfunctions and abdominal pain, diarrhea, hypertension, and respiratory diseases respectively (UNEP, 2003).The worth to mention is that although these pollutants are prohibited in many countries but still there application is being practiced and they are readily available in black market (Avancini *et al.*, 2013). As these chemicals are inherently toxic to living organisms, their presence in food items is a matter of serious concern to all consumer groups (Darko and Acquaah, 2008) Pakistan, like other developing countries, has poorly managed its environmental issues, hence, putting largely the health of man and ecosystem at stake. According to Tariq *et al.* (2007) large amount of pesticides are imported and applied to the cotton growing areas of the Punjab every year without following any rules and regulations and adopting the proper safety measures.
Ironically, on the other hand pesticides monitoring activities are very restricted and are without any governmental support. Although, a little information is available about soil

and air contamination in Pakistan but little data is present reporting the milk contamination in Pakistan. Keeping in view the importance of milk with respect to its nutrition value and its consumption, present study was conducted to determine the level of some selected and commonly used pesticides from organochlorine and pyrethroids groups.

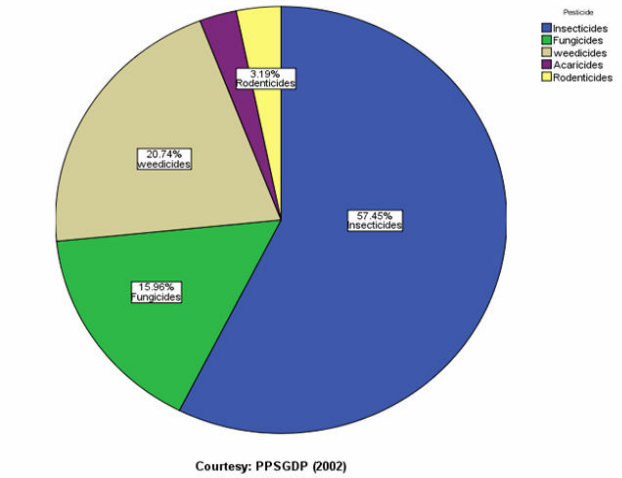

Figure 1. Different types of pesticides used in Pakistan

MATERIALS AND METHODS

***Materials*:** Pyrethriods standards (cypermethrin, permethrin, binfenthtin, and deltamethrin) were granted by Pak China Chemicals, Lahore (Pakistan). Whereas organochlorin Standards (DDT, DDE, endosulfan, and aldrin) were purchased from Sigma Aldrich (Germany) and Chem. Service (West Chester, USA). The purity of these standards varies from 97% to 99%.

Dichloromethane, acetonitrile, and n-hexane were purchased from Tedia (Fairfield, OH, USA), while methanol and anhydrous sodium sulphate were obtained from Merck (Darmstadt, Germany). All the solvents used in the experiment were of HPLC grade. Highly purified LC grade water, obtained through deionization of distilled water, was also used in the experiment.

For organchlorine, stock solutions were prepared by dissolving it in actonitrile and further dilution carried out in acetone to get 10µg/ml concentration of working standard solution.

Stock standard solutions for pyrethroids were prepared by dissolving properly weighed technical standard of each in acetonitrile and diluted them to an appropriate volume for working standard solution to get the concentration of 25µg/ml.

***Milk sampling*:** A total of 150 raw milk samples were collected for the Organochlorine and pyrethroid pesticides analysis. Samples were collected from agricultural areas of South Punjab where cotton is the major cash crop and pesticides are applied in large quantity on that crop. All the samples were collected in pre-cleaned plastic bottles, milked directly from the udders of randomly selected farmer's animals. Five to ten samples per day were collected during the month of November and December, 2012. Samples were stored temporarily in freezers immediately after each collection and were transported fortnightly in iceboxes to the laboratory where they were stored at -20ºC till the further action to be taken.

***Pesticides extraction and clean up*:** Pesticides extraction from milk samples was accomplished by following the method of Darko and Acquaah (2008) but with some modifications. A 10 ml of milk sample was taken in to pestle and mortar and 20gm of Sodium sulphate Anhydrous was added to it. The mixture was ground for 10- 15 minutes until it assumes the powder form and then was transferred to the separating funnel. In separating funnel 15 ml of each n-hexane and acetonitrile was poured and shaken it vigorously for few minutes intermittently. After that 40ml of dichloromethane (DCM) was added to the separating funnel and left as such for 30 minutes. After 30 minutes the sample was eluted out by adding 40 ml of DCM. The volume of the eluent was reduced to 1ml by drying on rotatory evaporator.

***Clean up*:** Cleaning procedure of analyte was conducted with the help of prepared Extrelut NT3 Cartridges (Merck, K Ga A Darmstadt, Germany).

The SPE cartridges were conditioned by adding 5-10 ml of each DCM and deionized water separately. Sample was loaded in pre-conditioned cartridges at rate of 1-2 drops/seconds or 1 ml/min, to give sufficient retaining time by SPE (sorbent) material.

Once it became adsorbed on SPE material then was washed with 20% methanol and hence remove the impurities from the sample. At the last sample of interest was eluted out by adding hexane to SPE cartridges.

Analyte of interest collected, completely dried and was re-diluted with acetonitrile to inject for detection and separation of pesticides on High Performance Liquid Chromatograpic (HPLC) system.

***HPLC system and conditions*:** The pesticides detection was conducted on HPLC system, Shimadzu, LC 20AT (Japan) with reversed-phase sorbent RP-C_{18} column (250mm×4.6mm ID, pore size,5µm) encapped, Merck (Germany), Quartnary pump, and Photo diod array (PDA) detector. Acetonitrile; water (75:25 v/v) and methanol; water (50:50, v/v) constituted the mobile phase of the HPLC column. Injection volume of the analyte was 20µl. Wavelength and flow rate was 237nm and 1ml/min, respectively.

***Quality assurance*:** Quality measuring parameters i.e., repeatability, reproducibility and linearity for method validation were applied by running a number of pesticides standards of known concentration and spiked milk samples. The recovery values for interested pesticides ranged from

79% to 104%. The linearity studies performed with five samples of known concentration showed the linear relationship, with 'r' value between the calibration curves and concentration of pesticides. Coefficient variations for repeatability studies were below 5% strongly confirm the validity of this method. Limit of detection (LOD) and limit of quantification (LOQ), as shown in table-1, were determined by running at least five samples of known concentration for each pesticide.

***Statistical analysis*:** The statistical analysis was conducted on SPSS Version 16.0. statistical software . The independent t-test was applied to evaluate significance of variation between concentration levels of both pesticides groups. P value was less than 0.05 ($P<0.05$) indicating the significant variation between the concentration level of organochlorine and pyrethroid group of pesticides.

RESULTS AND DISCUSSION

Out of 150 samples analyzed, more than 70% of total samples were contaminated with either of the one or more than one of the pesticides. About 35% of the milk samples were polluted with aldrin (Fig. 2) indicating the highest rate of contamination among other pesticides. The findings are in strong agreement with the study results produced by Avanchi *et al.* (2013) in Brazil, where milk samples indicated the highest percentage (44%) of contamination with aldrin of all the pesticides analyzed. Albeit, mean residue level of the aldrin (0.68µg/ml) was lower than that of the bifenthrin and permethrin which is 1.68µg/ml and 1.24µg/ml respectively. Cypermethrin was present in 21% of the total milk samples, the second highest percentage after aldrin. The mean concentration level of cypermethrin is 0.23 and it ranged from 5.00µg/ml to15.40 µg/ml. The deltamethrin ranges between 2.46µg/ml and 5.03µg/ml and the mean concentration level was 0.21µg/ml, immediately after the concentration of cypermethrin.

The present investigations contradict the result of the study, with respect to pyrethroid pesticides and endosulfan, conducted by Battu *et al.* (2004), while they were monitoring the pesticides residue level in milk and butter in Ludhiana, a district of India. Notwithstanding, presence of DDT in the milk samples in this study is in agreement of the results of their study. The DDT was present in 10% of the samples, ranges between .003µg/ml and 0.40µg/ml, with mean concentration level of 0.01µg/ml. Whereas, DDE prevailed in 9% of samples with mean concentration level of 0.04µg/ml, ranging from 0.15µg/ml to 1.23µg/ml. Gebremichael *et al.* (2013) reported the presence of DDT and DDE in cow milk with approximately the same contamination level and both of these chemicals were present in the same order of percentage level as DDE comes after the DDT with respect to percent contamination in milk samples (Fig. 2). But the concentration level of DDT and DDE in milk samples monitored during the study was lower than mean concentration level of DDT and DDE reported by Kaushik *et al.* (2011). They investigated the changing patterns of organochlorin pesticides residues in raw bovine milk and noted the significant decrease in concentration level with the passage of the time.

Table 1. Organochlorin pesticides, mean concentration and standard error of mean

Pesticides	Mean Concentration (µg/ml)	Standard error of mean
DDT	0.0073	0.00291
Endosulfan	0.1326	0.04311
DDE	0.0383	0.01568
Aldrin	0.6771	0.09783

Table 2. Pyrethroid pesticides, mean concentration and standard error of mean

Pesticides	Mean concentration (µg/ml)	Standard error of mean
Bifenthrin	1.7681	0.28683
Cypermethrin	0.2277	0.05158
Deltamethrin	0.2095	0.07343
Permethrin	1.2353	0.20885

Endosulfan was present in 7% of the milk samples analyzed during the study, these results are in accordance with the findings of Kampire *et al.* (2001) reported 7% milk sample contaminated with endosulfan.

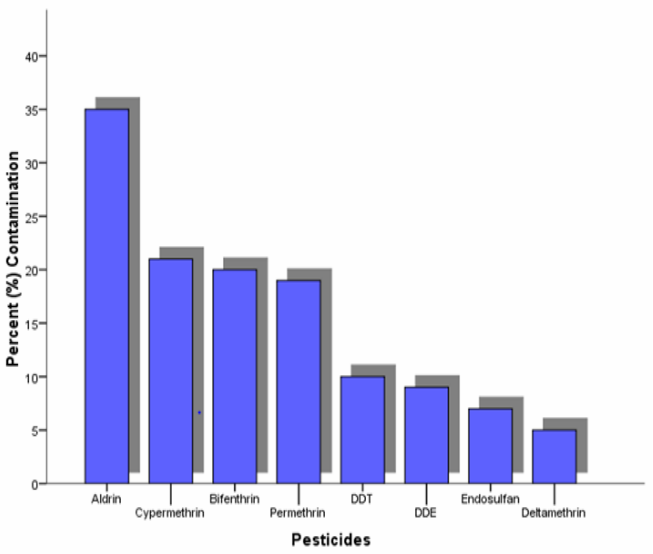

Figure 2. Percentage contaminations of organochlorine and pyrethroid in milk samples

The lower contamination percentage of milk samples with endosulfan pesticides could be attributed to the restricted use of this pesticide as it has been banned in the country

(Luzardo *et al.,* 2012). But Kathpal *et al.* (2001) found 15% milk samples contaminated with endosulfan and Nag and Raikwer (2003) detected the endosulfan pesticide residues in 31% analyzed milk samples. Some authors have reported the more sever situation where milk contamination percentage with endosulfan reached up to 50% (Luzardo *et al.,* 2012).

Cypermethrin presence in the milk samples with the highest concentration level (15.40µm/ml) indicates its continuous use to control the pests at large scale in different areas. Similar study results were obtained by Muhammad *et al.* (2012) as they found highest concentration level of cypermethrin in milk than rest of the investigated pesticides.

Table 3. Level of detection (LOD) and level of quantification (LOQ) and recovery percentage of analyzed pesticide.

Pesticides	LOD (µg/ml)	LOQ (µg/ml)	Recovery %	SD%
Endosulfan	0.03	0.04	89.00	5.43
DDT	0.02	0.03	99.00	5.32
DDE	0.05	0.07	88.00	5.60
Aldrin	0.03	0.09	92.00	3.11
Cypermethrin	0.03	0.05	82.33	5.85
Deltamethrin	50.00	0.09	79.00	10.40
Permethrin	0.04	0.06	101.00	4.20
Bifenthrin	0.05	0.1	104.00	2.12

Deltamethrin showed least presence in the analyzed milk samples (7% only) with lowest mean concentration level with in the other pyrethroid pesticides. This could be due to its short period of half life, hence deplete rapidly in the milk (Bouwman *et al.*, 2006; Kan and Meijer, 2007; Feo *et al.*, 2010; Ahmad *et al*., 2012). The similar results were obtained by Feo *et al.* (2010) where the cypermethrin was investigated more frequently in analyzed water samples than the deltamethrin. However the deltamethrin may prove more toxic than cypermethtrin as Shen *et al.* (2012) conducted the comparative study of both deltamethrin and cypermethtrin pesticides on their chronic and acute toxic effects to aquatic organism, *Ceriodephnia dubia.* The study results with respect to the presence of permethrin in milk samples are similar to that of Bouwman *et al.* (2006) detected the permethrin in human breast milk in South Africa. But the permethrin is detected more frequently than cypermthrin, reverse of our results, where permethrin comes after the cypermethrin regarding to its contamination percentage (Fig. 2).

Bifenthrin presence in milk samples is reported for the first time in the current study however the previous studies have monitored this toxicant in water (Zhou *et al.*, 2010) and lypophilized coconut water (Santos *et al.*, 2012).

***Conclusion*:** Pesticides presence in analyzed milk samples indicates the application of chlorinated pesticides in the past and its sparse application to some extents up to dates, as well as recent use of pyrethroid pesticides for agricultural purposes. Contamination of milk samples with pesticides is an obvious risk to human health particularly for children. Although the organochlorine pesticides are present in minor quantity but the bioaccumulation of these compounds may lead to deleterious health effects for regular consumers. Additionally, pyrethroids concentration level exceeds to maximum residue limit (MRL) in number of milk samples which is also matter of concern for consumer health. The current study provides some basic information regarding the pesticide residues level in milk in the areas where huge amount of pesticides are applied every year but the pesticides monitoring activities are very limited. The demand for comprehensive and well planned monitoring activities, launch of public awareness campaign, and adopting the alternative clean approach to control the pests and other disease spreading vectors in the best interest of the public health seems plausible to manage the risk of milk contamination in future.

REFERENCES

Ahmad, L., A. Khan and M.Z. Khan. 2012. Pyrethroid-induced reproductive toxico-pathology in non-target species. Pak. Vet. J. 32: 1-9.

Avancini, R.M., I.S. Silva, A.C.S. Rosa, P.D.N. Sarcinelli and S.A. de Mesquita. 2013. Organochlorine compounds in bovine milk from the state of Mato Grosso do Sul—Brazil. Chemosphere 90: 2408-2413.

Battu, R.S., B. Singh and B.K. Kang. 2004. Contamination of liquid milk and butter with pesticide residues in the Ludhiana district of Punjab state, India. Ecotoxic. Environ. Safetly 59: 324-331.

Bouwman, H., B. Sereda and H.M. Meinhardt. 2006. Simultaneous presence of DDT and pyrethroid residue in human breast milk from a malaria endemic area in South Africa. Environ. Poll. 144: 902-917.

Darko, G. and S.O. Acquaah. 2008. Level of organochlorine pesticides residues in dairy products in Kumasi, Ghana. Chemosphere 71: 294-298.

Feo, M.L., A. Ginebreda, E. Eljarrat and D.Barceló. 2010. Presence of pyrethrpoid pesticides in water and sediments of Ebro River Delta. J. Hydro. 393: 156-162.

Gebremichael, S., T. Birhanu and T.A. Tessema. 2013. Analysis of organochlorin pesticide residues in human and cow's milk in towns of Asendabo, Serbo and Jimma in South-Western Ethiopia. Chemosphere 90: 1652-1675.

Kampire, E., B.T. Kiremire, S.A. Nyanzi and M. Kishimba. 2011. Organochlorine pesticide in fresh and pasteurized cow's milk from Kampala markets. Chemosphere 84: 923-927.

Kan, C. A. and G. A. L. Meijer. 2007. The risk of contamination of food with toxic substances present in animal feed. Ani. Feed Sci. Tech. 133: 84-108.

Kaushik, C.P., H.R. Sharma, D. Gulati and A. Kaushik. 2011. changing pattern of organochlorine pesticides in raw bovine milk from Haryana, India. Environ. Monit. Assess. 182: 467-475.

Kathpal, T.S. , Kumari, B., S. Singh and J. Singh. 2001. Multi-residue analysis of bovine and human milk in cotton growing belt of Haryana. Proceedings of the International Conference on Pesticides, Environment, Food Security organized by Pesticide Science; New Delhi, India.

Luzardo, O.P., M.A. González, L.A.H. Hernández, M. Zumbedo, E.E.A. León and L.D. Boada. 2012. Polychlorobiphinyles and organochlorine pesticides in conventional and organic brands of milk: Occurance and dietry intake of milk in the population of Canary Islands (Spain). Chemosphere 88: 307-315.

Muhammad, F., I. Javed, M. Akhter, Z. Rahman, M. M.Awais, M.K. Saleemi and M.I. Anwar. 2012. Quantitative Structure activity relationship and risk analysis of some pesticides in the cattle milk. Pak. Vet. J. 32: 589-592.

Nag, S.K. and S.K. Raikwar. 2003. Monitoring of bovine milk for organochlorine pesticide residues in some parts of Bundelkhand region. In: proceedings of 40th Annual Convention of Chemists. Ind. Chem. Society p.D4.

Nag, S.K., S.K. Mahanta, M.K. Raikwar and B.K. Bhadoria. 2007. Residue in milk and production performance of goats following the intake of pesticides (Endosulfan). Small Ruminants Res. 67: 235-242.

Rana, A.S., A-u-H. Ahmad, N. Saleem, A. Nawaz, T. Hussain and M. Saad. Differential response of sorghumcultivars for fodder yield and quality. J. Glob. Innov. Agric. Soc. Sci. 2: 6-10.

Santos, L.F.S., N.R.D.S. Souza, J.A. Ferreira and S. Navickiene. 2012. A reverse-phase high performance liquid chromatography method combined with matrix solid-phase dispersion extraction for the determination of teflubenzuron, lufenuron, and bifenthrin residues in lyophilized coconut water; Short communication. J. Food Comp. Analysis 26: 183-188.

Shahzadi, F., F. Iqbal, T. Aziz, R. Saleem, M. N. Abbas. 2014. Iron status and haematological profile of lactating and non-lactating buffaloes. J. Glob. Innov. Agri. Soc. Sci. 2: 28-30.

Shen, M.F., A. Kumar, S. Ding and S. Groke. 2012. Comparative study on the toxicity of pyrethroids, cypemethrin and deltramethrin to *Ceriodephnia dubia*. Ecotoxic. Environ. Safety 78: 9-13.

Taiq, M.I., S. Afzal, I. Hussain and N. Sultana. 2007. Pesticides exposure in Pakistan: A review. Environ. Int. 33: 1107-1122.

Tecles, F., A. Tvarijonaviciute and J.J. Ceron. 2013. A new assay for measurement of acetylcholinesterase and butyrylcholinesterase in canine whole blood combining specific substrates and ethopropazine hydrochloride as a selective butyrylcholinesterase inhibitor. Pak. Vet. J. 33: 458-461.

UNEP. 2003. Global report on regionally based assessment of persistent toxic substances. UNEP chemicals, Geneva, Switzerland; cited in K.P. Singh, A. Malik, D. Mohan and S. Sinha. 2005. Persistent organochlorine pesticide residues in alluvial groundwater aquifers of Gangetic plains, India. Bull. Environ. Cont. Toxicol. 74: 162-169.

Zhou, Q., Y. Gao, H. Bai and G. Xie. 2010. Preconcentration sensitive determination of pyrethroid insecticides in environmental water samples with solid phase extraction with SiO_2 microsphere catridge prior to high performance liquid chromatography. J. Chromatog. 1217: 5021-5025.