

Screening of Organochlorine Pesticide Residues in Milk Collected from Sahiwal region

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Abstract: Milk and milk products are known to have huge nutritional value with prevalent natural potential. Milk is a source of energy and it contains all required building proteins, bone forming minerals, fats etc. Milk is contaminated with pesticides such as OCPs. Consumption of contaminated food, milk, meat, fish and dairy products is the cause of high level of OCPs in the body. Milk is a good source to dissolve pesticide residues such as OCPs which are fat soluble. The aim of the study is to identify the presence of OCPs in milk and also toxicity level of residues in milk as compared to the MRL values. In the present study seven OCPs are detected by gas chromatography. Milk samples from sahiwal market were analyzed for organochlorine by using gas chromatograph equipped with electron captured detector. Seven organochlorine pesticides were detected. These are DDT, DDE, Dieldrin, γ -HCH, α -Endosulphan, β -Endosulphan, and Endosulphan sulphate. These pesticides were confirmed with GC-MS. The mean values were showed in ug/kg milk fat basis. Analysis of variance (ANOVA) was executed by using Minitab-17 to analyze the data of pesticides in order to find out variation in pesticides residues among all milk samples. The results were non-significant ($p > 0.05$). There was no statistically significant difference among all milk points but a large variations found regarding OCPs among all milk points. The relative corresponding residues level detected in milk samples revealed mean value of DDT was 4.2 ug/kg with the range of 0.05-4.6 ug/kg. The mean value of DDE was 3.13 ug/kg with the range of 0.21-15.61 ug/kg. The mean values of DDT and DDE were less than MRL. However the mean values of Dieldrin, γ -HCH, α -Endosulphan and β -Endosulphan were 11.82 ug/kg, 1.34 ug/kg, 112.69 ug/kg, 107.16 ug/kg and 91.3 ug/kg respectively. The mean values of Dieldrin and γ -HCH, α -Endosulphan and β -Endosulphan were exceeding the MRL values in some of the milk points. However, the mean value of Endosulphan sulphate was below the MRL value. Present study concluded that there is large variation found regarding the contents of DDT, DDE, Dieldrin, γ -HCH, α -Endosulphan, β -Endosulphan, and Endosulphan sulphate. This study showed that humans are exposed to these OCPs through milk. This study provides information regarding contamination of milk so that Pakistan should make strict rules for the use of Organochlorine pesticides.

Key Words: Milk, Organochlorine Pesticides Residues (OCPs), GC Analysis, GC-MC Analysis, Sahiwal, DDT, DDE

1. Introduction:

Milk is considered as a complete food because of its exceptional nutritional value. Milk production is approximately about 42.17 million tons of total Pakistan in top milk producers throughout the world. Buffalo and cow are top milk producing animals in Pakistan that contribute 62 percent and 34 percent milk production respectively [5]. Rural areas produce almost 80% milk with 15% milk is produced in peri-urban areas as well as 5% milk is produced in urban areas. Several authors in different countries have been reporting milk contamination with pesticides for the last few decades [6]. The abuse of pesticides has resulted in economic loss as well as danger to human health and pesticides exposure can be determined by the amount of pesticides residues left in an environment [12-16].

Organochlorine pesticide residues degrade slowly in environment because of this property organochlorine pesticide residues enter in the food chain. The consumption of this contaminated food like meat, milk, fish and dairy products is the cause of elevated level of OCPs in body. The milk producing animals aggregate these OCPs by the use of contaminated feed, grass, corn silage, water etc [27]. A lot of studies had been conducted on aggregation of pesticides more specifically on OCPs in water, soil and food.[29-33].

Organochlorine and organophosphorus pesticides are mostly used as compared to other pesticides.

Soil is contaminated with organochlorine pesticides while application, runoff from plants, when disposed off and incorporation of these pesticide residues with soil particles [35]. Commercial Endosulphan is a mixture of stereo isomers, α -Endosulphan, β -Endosulphan, Endosulphan sulphate and some other compounds. Endosulphan sulphate is derived from Endosulphan. Pakistan is not organochlorine pesticide manufacturer. Hence, these pesticides are imported to fulfill the need of pesticides [46].

Milk is a good source to dissolve pesticide residues such as organochlorine which are fat soluble [53]. One of the study results show that there was contamination milk sample leading to the human health. One study found that there was presence of pesticide residues in milk. Animals whose milk was contaminated were exposed to dust containing pesticides particles and so as air inhaled by animals. One research detected pesticides residues in milk samples collected from local vendors, shop and dairy forms of the relevant target area. This contaminated milk was dangerous for human health so they advised to run monitoring program to control the OCPs level in milk [57].

A study concluded that DDT was higher than HCH in milk. They found traces of OCPs in milk and other dairy products. A study has determined that feeding contaminated food and water to the animals plays a role of source of organochlorine pesticide residues in milk [60].

Objectives of Study

Objective of the study is to screen level of organochlorine residues in milk collected from Sahiwal Region and also to identify the toxicity level of residues in milk by comparison with their Maximum Residues Limit (MRLs).

2. Research Methodology:

2.1 Collection of Milk Samples

Milk samples (250 ml) are collected from local markets of Sahiwal. These samples are collected in sterilized glass bottles which were air tight. The fresh sample so collected placed in cool boxes which consist of ice packets.

2.2 Sample Extraction

The milk sample were defrosted, regimented and mixed thoroughly. Then 100mL sample was taken and divided in 2 equal parts. Then 1g of fat was taken and was mixed anhydrous sodium sulphate in a 250 mL flask. After the mixture got set then petroleum ether was filtered to in another tube containing anhydrous sodium sulphate through glass funnel. This concentrated material added in 3mL of hexane to clean out.

2.3 Cleanup with florisil method:

Glass column of 15cm long plugged with glass wool and washed with hexane. Concentrated fat extract was added to this column and then it was eluted to 100 mL n-hexane to extract organochlorine pesticide residues. The resultant material was added in 250mL round bottom flask and mixed with anhydrous sodium sulphate. The

residue was dissolved in 1mL cyclohexane for GC analysis.

2.4 Gas Chromatography

For Gas chromatography analysis, a variant gas chromatograph equipped with Ni, semi polar and non-polar fused silica capillary column was used. Nitrogen gas was used as electron capture detector.

2.5 Chromatogram of organochlorine pesticides analyzed by GC-ECD

Retention time for DDT, Dde, Dieldrin, γ -HCH, α -Endosulphan, β -Endosulphan and Endosulphan sulphate is 16.05 min, 15.4 min, 16.13 min, 10.87 min, 11.96 min, 12.5 min and 13.9 min. Detector temperature was set to 300°C and the injection volume kept up to 1 μ L (Figure 2.1).

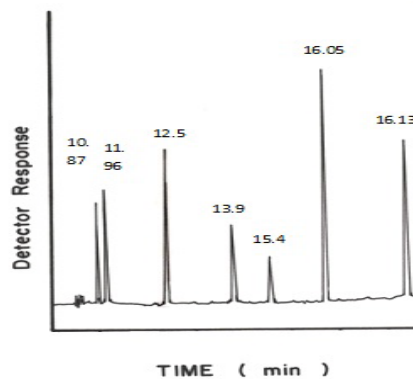


Figure 2.1: Chromatogram of organochlorine pesticides analyzed by GC-ECD. Detector temp. Peaks: 1 γ -HCH (10.87) , α -endosulfan (11.96),

3.βendosulfan (12.54), 4.Endosulfan-sulphate(13.96), 5.DDE (15.44), 6.DDT(16.05), 7.Dieldrin(16.13)

2.6 Statistical Analysis

Analysis of variance (ANOVA) was executed by using Minitab-17 to analyze the data of pesticide residues in order to find out the variation in residues contents among all milk samples collected from different milk points.

3. Results:

3.1Variation of OCPs among all milk points

Organochlorine pesticide residues in the present study were not significantly different among the milk points. However large variations regarding OCPs have been observed among all milk points. The results of each OCP i.e., DDT, DDE, Dieldrin, γ-hexachlorocyclohexane, α-Endosulphan, β-Endosulphan, and Endosulphan sulphate are described under.

3.2Variation of DDT among all milk points

Analysis of variance (ANOVA) showed non-significant differences among the milk points regarding DDT contents in the milk samples ($P > 0.05$). However there were found variations in all milk points. Four milk points' bhola Milk Point (BhMP), Ghauri Milk Point (GhMP), Hassan Milk Point (HsMP) and Ghausia milk point (GosMp) were high in DDT contents as compared to all other milk points. While three milk points Hussain Milk Point (HMP) and Al-Noor milk Point

(AINMP) were low in DDT contents as compared to all other milk points (Figure 3.1).

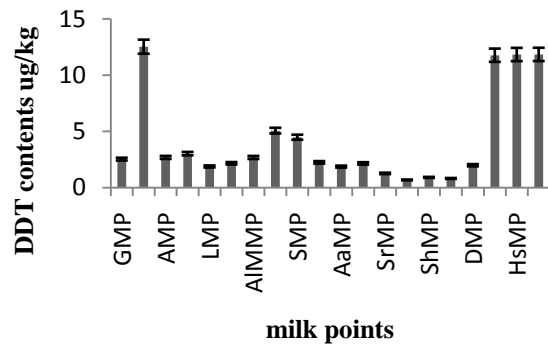


Figure 3.1: Variations among different milk points of Sahiwal regarding contents of DDT in milk samples

3.3Variation of DDE among all milk points

Analysis depicted no big difference among all milk points ($P > 0.05$). Yet analysis indicated significant variations among all milk points. Results exposed high DDE contents in Lasani Milk point (LMP) as compared to all other samples. However, Gujjar Milk Point were low in DDE contents as compared to all other milk points (Figure 3.2).

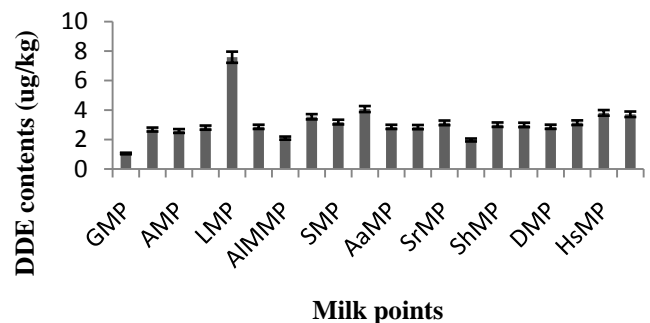


Figure 3.2: Variations among different milk points of Sahiwal regarding contents of DDE in milk samples

3.4 Variation of Dieldrin among all milk points

The presences of pesticide residues contents were statistically analyzed. Four milk points Neeli Bar milk point (NBMP), Ghausia milk point (GosMp), Al-Madina Milk Point (AIMMP), Baloch Milk Point (BIMP) were relatively high in Dieldrin contents as compared to all milk points. Though Al-Noor Milk Point and Azan Milk point were found low in Dieldrin contents as compared to all other milk points (Figure 3.3).

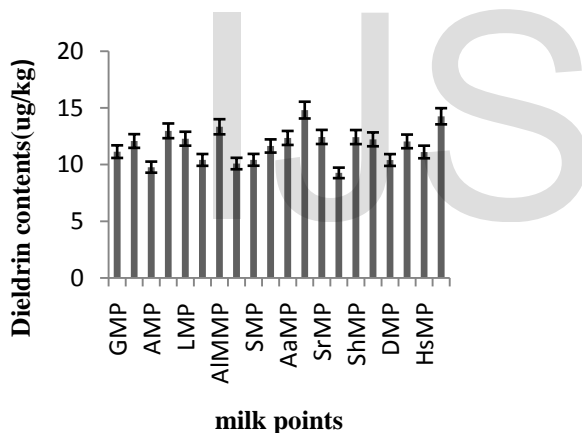


Figure 3.3: Variations among different milk points of Sahiwal regarding contents of Dieldrin in milk samples

3.5 Variation of γ -hexachlorocyclohexane among all milk points

The residual analysis (ANOVA) revealed that all milk samples were found contaminated with pesticide residues. However, there was no significant difference among all milk points. But all

milk points showed large variations in γ -hexachlorocyclohexane contents. Residues of γ -hexachlorocyclohexane were found high in Ravi milk point (RvMP), Hussain Milk point (HMP) and Shama Milk Point (ShMP), while γ -hexachlorocyclohexane residue were found low in all milk points, though Neeli Bar Milk Point (NBMP), Al-Madina Milk Point (AIMMP) were found relatively lowest in γ -hexachlorocyclohexane residues (Figure 3.4).

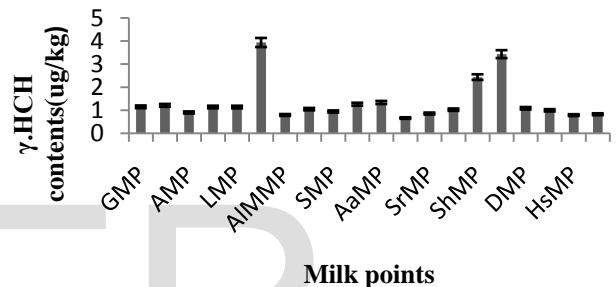


Figure 3.4: Variations among different milk points of Sahiwal regarding contents of γ .HCH in milk samples

3.6 Variation of α -Endosulphan among all milk points

After statistical analysis (ANOVA) contents of α -Endosulphan were noticed in all milk points. There is no big difference in all milk points, although there were variations in all milk points ($P > 0.05$). Sadiq milk Point (SMP), Rai Milk Point (RMP) and Dilbahar Milk Point (DMP) were observed higher in α -Endosulphan contents. Bholia Milk Point (BhMP), Gujjar Milk Point (GMP) and Ravi Milk Point (RvMP) were observed lower in α -Endosulphan contents as compared to all other milk points (Figure 3.5).

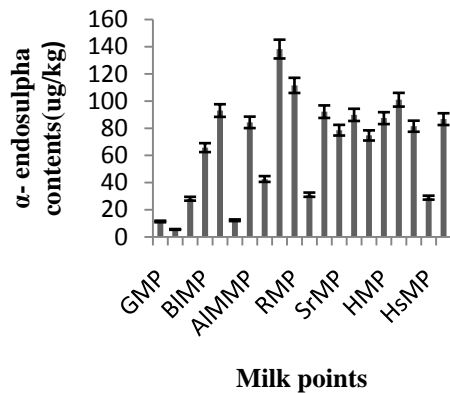


Figure 3.5: Variations among different milk points of Sahiwal regarding contents of α -Endosulphan in milk samples

3.7 Variation of β -Endosulphan among all milk points

Considerable variations were found in all milk samples, after applying analysis of variance (ANOVA), regarding β -Endosulphan contents ($P > 0.05$). All milk points revealed variations among them such as Sardar Milk point (SrMP), Gujjar Milk point (GMP), Shama Milk Point (ShMP), Dilbahar Milk point (DMP) and Hassan Milk Point (HsMP) exhibited relatively high β -Endosulphan contents as compared to all milk points. However, Bhola Milk Point (BhMP) and Ghausia Milk Point (GosMP) showed comparatively low contents (Figure 3.6).

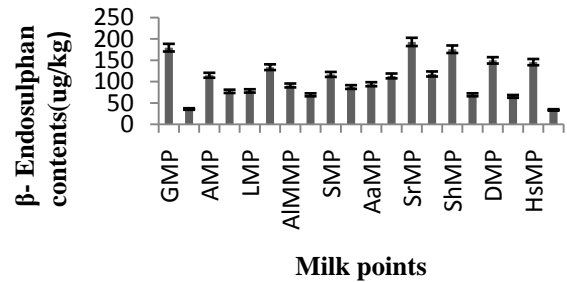


Figure 3.6: Variations among different milk points of Sahiwal regarding contents of β -Endosulphan in milk samples

3.8 Variation of Endosulphan sulphate among all milk points

As a result of statistical analysis (ANOVA), all examined milk samples do not show significant difference in milk samples. Although results showed variation regarding Endosulphan sulphate contents ($P > 0.05$). It was found that Bloch Milk Point (BIMP), Neeli Bar Milk Point (NBMP), Dilbahar milk Point (DMP), Al-Latif milk point (AILMP), Al-Madina Milk Point (AIMMP) had the highest contents. On the other hand Hassan Milk point (HsMP), Sadiq milk point (SMP) and Azan Milk Point (AMP) had low amount of Endosulphan sulphate contents as compared to all other milk points (Figure 3.7).

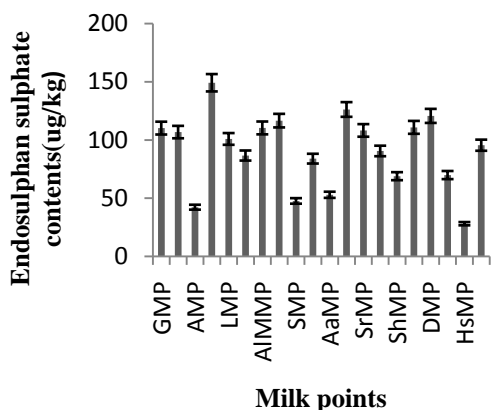


Figure 3.7: Variations among different milk points of Sahiwal regarding contents of Endosulphan sulphate in milk samples

3.9 Comparison of Mean values of OCPs with their MRL values

The analysis of milk samples showed presence of DDT, DDE, Dieldrin, γ -HCH, α -Endosulphan, β -Endosulphan, Endosulphan sulphate in milk presented in (Table 3.1). The relative corresponding residues level detected in milk samples revealed mean value of all OCPs. However the mean values and ranges of Dieldrin, γ -HCH, α -Endosulphan and β -Endosulphan were exceeding the MRL values in all milk points (Table 3.1).

Table 3.1: concentration of pesticide residues (ug/kg) in all milk points

Variable	Mean	range	MRL ug/kg
DDT	4.2	0.05-4.6	40
DDE	3.13	0.21-15.61	40
Dieldrin	11.82	2.08-18.45	6
γ -HCH	1.34	0.19-10.18	1
α -Endo	112.69	0.75-228.6	100
β -Endo	107.16	2.4-297.1	100
Endosulphan sulphate	91.3	1.5-19.6	100

4. Conclusion and Discussion

Many pesticides are widely utilized by the farmers. These pesticides used in agriculture are mostly organochlorine compounds. The results from current study have shown that milk samples from Sahiwal region has contamination of with OCPs i.e. DDT, DDE, Dieldrin, γ -HCH, α -Endosulphan, β -Endosulphan, and Endosulphan sulphate. There were eight OCPs were found in milk samples. The purpose of this study is to analyze the level of Organochlorine pesticide residues in milk samples collected from different milk points of Sahiwal region. The main source of OCPs is animal feed. Being a fat rich food, milk is a great source of OCP accumulation. .

DDT, DDE, Dieldrin, α -Endosulphan, β -Endosulphan, γ -Endosulphan and Endosulphan sulphate were present in all milk samples which were collected from Sahiwal region. Restricted use of these pesticides might be the reason for this lower contamination rate with DDT and DDE [10]. The present study showed the contents of Dieldrin, α -Endosulphan, β -Endosulphan, γ -HCH and

Endosulphan sulphate with the ranges of 2.08-18.45 ug/kg, 0.75-228.6 ug/kg, 2.4-297.1 ug/kg, 0.19-10.18 ug/kg and 1.5-19.6 ug/kg respectively. Results in present studies are in agreement with the previous studies. All OCPs were present in milk samples although some of them did not exceed the MRLs. There can be following reasons for highest contamination in few milk points. One of the reasons for milk contamination is high fats contents in milk or due to dietary habit of animals like different fodder. The major source of residues contamination in milk is the animal feed stuff. Milk contamination can be avoided and controlled by preventing contamination of feed stuff [64].

A study conducted by Sosina *et al.* [65] on cow's milk who reported somewhat similar results as compared to current study. Current study showed that some of the milk points showed relatively high DDT contents as compared to other milk points. This study analyzed cheese contamination with OCPs produced in Brazil. Whereas another study indicated contamination of milk fat with DDT contents slightly above the MRL value [67]. On the other hand the present study is showing the results of DDE contamination that all milk samples collected from milk points were contaminated with DDE. Current study detected concentration of DDE contents with the range of 0.21-15.61 ug/kg. That study revealed somehow similar results in relation to DDE contents with 9% of their samples were contaminated with the DDE contents ranging from 0.15ug/ml to 1.23 ug/ml [55]. Another study conducted by Gebremichael *et al.* [69] reported

milk contamination with DDT and DDE were found same percentage level of contamination because DDE is the metabolite of DDT. But our study showed high DDT contents with respect to the DDE which are lower than DDT contents.

It is concluded from the results that all milk points showed contamination with Dieldrin. Although variations were observed in all milk samples. Our study revealed lower Dieldrin contents as compared to this study with 11.82 ug/kg [71].

From the study it is calculated that all milk samples were contaminated with γ -HCH and 15% samples were highly contaminated with γ -HCH contents at mean level of 1.34 ug/kg with the range of 0.19-10.18 ug/kg as compared to all other milk samples. Current study reported mean value of γ -HCH (1.34 ug/kg) lower than those reported by a study which was conducted on Organochlorine pesticide on milk samples in Uganda. This study reported γ -HCH contents at mean level of 26/ug/kg [54]. Our results were also found lower than another study conducted on children exposure to Organochlorine through milk. This study revealed γ -HCH at mean level of 7 ug/kg [72]. From the graphs it has been indicated few milk samples were contaminated with α -Endosulphan with the range of 0.75 228.6 ug/kg. Few samples showed contamination with β -Endosulphan and there were high contents of Endosulphan sulphate in most of the milk samples among all milk samples. However there was no considerable difference among all milk samples

but analysis revealed large variation among all milk samples. In that study Endosulphan and its isomers α -Endosulphan, β -Endosulphan and Endosulphan sulphate were major contents in animal feed. One of the studies conducted by Tsiplakou *et al.* [74] showed that Endosulphan was the main pesticide that was detected in milk samples with the average of 100 ug/kg. Current study is in contrast to this average value that shows 112.69 ug/kg and 107.16 ug/kg average values for α -Endosulphan and β -Endosulphan. Three major groups were formed on the basis of similarity of all OCPs present among all milk samples. The reason for these higher values may be still usage of Endosulphan pesticide residues on fodder crops.

(Table 3.2) is showing the detection rate of all OCPs in milk samples. There were large variations found among all milk points. 60% samples were detected with DDT, DDE, α -Endosulphan, 80% samples were contaminated with γ -HCH, 50% were contaminated with β -Endosulphan and 80% showed Endosulphan sulphate contamination. However, few milk points showed relatively high OCP contents as compared to other milk points.

By the use of these contaminated feed OCPs integrated in milk producing animal body.

In most cases, the value of OCPs exceeds the set MRLs showing OCPs usage in Pakistan. DDT, DDE, Dieldrin, α -Endosulphan, β -Endosulphan, γ -Endosulphan and Endosulphan sulphate were

detected in all samples collected from sahiwal region.

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