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Assessment of the dietary transfer of pesticides to dairy milk and its effect on human health

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The transfer of organophosphate and pyrethroid pesticides in dairy cattle's milk when fed on agro-industrial by-product diet was assessed in this study. The transfer and accumulation of such pesticide in cattle fat tissue and milk was also assessed and the adverse effect on cattle's and human health was also studied. For that purpose, about 80 milk and 30 diet samples were collected from various dairy farms. All samples were extracted with acids using "quick, easy, cheap, effective, rugged, and safe" (QuEChERS) method and analyzed through gas chromatography-mass spectrometry (GC-MS). The results show that about 40 and 20% of milk samples had greater content of cypermethrin+chloropyrifos and porfenofos than their maximum residue limits as suggested by World Health Organisation (WHO). Cypermethrin, chloropyrifos and profenofos were present at concentration greater than their maximum residue limits in mixed diet whereas profenofos was completely absent in sugarcane khal and was present in traces in cotton khal but remained within the maximum residue limit. Nonetheless, transfer of residue of parent cypermethrin, chloropyrifos and profenofos to milk was not consistent with diet in all dairy milk samples. This revealed the contention that some other sources such as drinking or inhaling contaminated water or dust are also contributing to pesticide contamination in milk. Cancer potency factor for cypermethrine in children and adults remained within the recommended value. Generally, although pesticides residue in milk was not high enough to cause cancer risk in human, they might cause adverse health effect and delayed toxicity due to their long term accumulation and persistence within cattle's body. Therefore, there was an urgent need to estimate their contents in dairy cattle milk in order to provide a baseline for the health ministry to make safety regulations.

Key words: Pesticides, contamination, milk, cattle diet, risk assessment.

INTRODUCTION

Agro industrial by-products are commonly used to feed dairy cattle's over the last several years (Hassan et al., 2008). These products are prepared according to the body requirement of the cattle for producing milk and have high nutritive value and energy content. These by-products are highly beneficial and economical for the dairy farmers. However, such by-products become more effective when cattle's were fed in combination rather than be fed on single by-product more likely because of the difference in their nutritive value and energy such

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Abbreviations: TCP, 3,5,6-Trichloro-2-pyridinol; **QuEChERS,** Quick, Easy, Cheap, Effective, Rugged, and Safe; **GC-MS,** gas chromatography-mass spectrometry; **LOQ,** quantification limits; **BCF,** bio-concentration factor; **TIC,** total ion chromatogram; **CPF,** cancer potency factor; **MRLs,** maximum residue limit; **DDE,** dicchloro-diflouro ethane; **OPPs,** organophosphorus pesticides; **ChE,** cholinesterase.

as wheat bran contains more flour and energy than silage (Gebremedhin et al., 2009). There are evidences in previous literature that feeding such by-products to cattle can increased the milk production (Sutton, 1989; Singh et al., 2010). Khan et al (2000) carried out a study to evaluate the difference in milk production in two groups of lactating cow's when one group was fed on fishmeal and other was on wheat bran diets. They observed that high milk production was obtained from wheat bran due to low cost to benefit ratio. In another study, Tahir et al. (2002) reported that milk production increased when dairy cattle was fed on maize bran rather than when fed on wheat bran and rice bran. Singh et al. (2000) reported that milk production increased in dairy cattle when fed on straw rather than when fed on deoiled rice bran. The adverse health effect on cattle while feeding industrial by-products have not been reported yet but there are evidences in previous literature that improper storage of such products in the farm can cause several infections among cattle's because of eating molded feed.

By-products are usually obtained from their respective crops and such crops are heavily sprayed with organophosphate and pyrethoids in the field to protect them from pest and insect attack. The most commonly used organophosphate and pyrethroids are cypermethrin, chloropyrifos and carbamates which are sprayed over wheat, maize and cotton crops regularly (Muhammad et al., 2012). By-products obtained from such crops will carry some portion of pesticides which remained there even after processing of these crops. The transfer of such pesticide from eating contaminated by-products to cattle fat tissue or in milk has not been studied till now. Nevertheless there are evidences in previous literature that pesticide contamination in milk has been observed more likely because of drinking or inhalation of contaminated water and soil (Ivie and Dorough, 1977). However once contaminated by-products which are previously heavily sprayed with pesticides are added in the daily diet of dairy cattle, a considerable portion of such pesticide residue may penetrate inside the cattle's body or may enter into the food chain and accumulate in the fat tissues that may later appear in the milk and meat products (Nag and Raikwar, 2010). McKellar et al. (1976) reported 0.01 to 0.02 mg/L of chloropyrifos and metabolite residue of 3,5,6, trichloro-2-pyridinol (TCP) in milk and milk products of cows when fed on 30 mg/kg of chloropyrifos for 14 days. Dishburger et al. (1977) noted that addition of 3 mg/kg of chloropyrifos in cattle diet for 30 days results into accumulation of chloropyrifos and metabolite residue of TCP was <0.01, 0.08, 0.23, and 0.015 mg/kg in muscle, fat, liver and kidney, respectively. MacLachlan and Bhula (2007) estimated the transfer of pesticide residue from diet to milk, hence the quality and quantity of milk is slowly deteriorating and indirectly drinking milk of low quality causes health risk to human beings. Therefore, there was an urgent need to estimate their content in dairy cattle milk in order to provide a baseline for the health ministry to prepare guidelines for dairy farms owners.

The main objective of this study was to evaluate and assess the presence of pesticides in industrial by-products which is used as a feed for dairy cattle and then to understand the accumulation of such pesticide in cattle body and later their appearance in the milk. The adverse health effect on human after drinking contaminated milk was also explored.

MATERIALS AND METHODS

Study Participation/Dairy cattle selection

Dairy farming is a rapidly growing industry in Pakistan. The mixed farming system, are urban and peri-urban dairy farming. Mixed farming is commonly used in rural areas where small numbers of cattle (up to 10 in numbers) are kept in herds. Around 57% of cattle are kept in this system (Sarwar et al., 2002). Cattle's are fed on fodder, crop residue, weeds and agro industrial waste products after harvesting. Feeding and grazing requirement of cattle is low and this is a resource poor system which needs further modification. Urban and peri-urban dairy farming is a system which is developed in and around big city and in towns in order to meet the demand for milk within the city. Mainly cattle are kept in small herds located within the centre of town. The cattle are fed on agro industrial by-products such as cotton khal, sugarcane khal, maize khal or wheat grains purchased commercially. Around 32 to 47% of cattle are kept in herds in this system (Livestock Census Report, 1996). Cattle are taken out for grazing to the open field surrounding the dairy farms. Sugarcane khal is obtained from sugarcane industry. Wheat bran is purchased from flour industry whereas cotton khal is obtained from the farmers. All cattle in urban and peri-urban dairy farms are on minimum grazing whereas in rural farms, they are on high grazing. Urban and peri-urban farms are located either in the densely populated towns, near highways or industrial processing units hence cattle in the farm are vulnerable to environmental pollution. The size of the dairy farms is not more than 3 kanal and water and food containers are corrosive and unhygienic.

Agro industrial by-products and milk sampling and extraction for gas chromatography-mass spectrometry (GCMS) analysis

Around 80 and 30 milk and cattle diet samples were collected from the dairy farms.

Sample analytical procedure and information (reagents and standards, calibration curve, precision, recovery, LOD/LOQ)

Sample analytical procedure

All by-products such as cotton and sugarcane khal, wheat and maize bran and mixed diet samples were analyzed for pesticides by liquid extraction using the "Quick, Easy, Cheap, Effective, Rugged, and Safe" (QuEChERs) method as described by Anastassiades et al. (2003) and Schenck and Wong (2008).

Commercially available cattle feed was collected and 5 g of chopped diet was placed in 100 mL of glass beaker and was thoroughly mixed with hands with 15 g of anhydrous sodium sulfate and then was soaked with 30 mL of acetonitrile and incubated in the laboratory for 24 h. The soaked sample was chopped with blender for 2 min and allowed to settle for 5 min. Thereafter, the

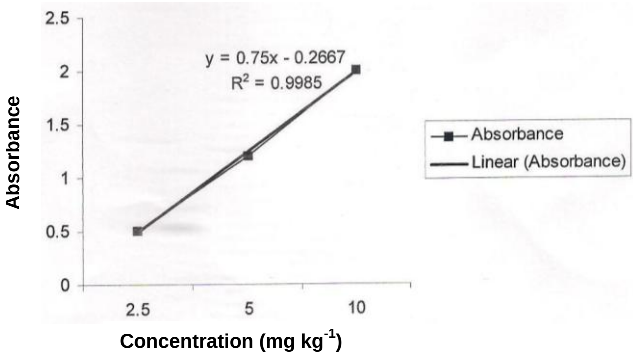

Figure 1. Calibration curve of standards vs absorbance .

Table 1. Detection limit of profenofos, cypermethrin and chloropyrifos through GCMS.

Pesticide	Value (mg/L)
Profenofos	0.0005
Cypermethrin	0.01
Chloropyrifos	0.01

supernatant was passed through the filter paper and placed in the glass beaker (100 mL) and diluted further with 30 mL of acetonitrile and was placed in the refrigerator for further analysis. Fifteen milliliter (15 mL) of supernatant was then digested with 15 mL of acetonitrile and was added to a 50 mL polypropylene centrifuge tube named ECMSSC50CT containing 6 g anhydrous $MgSO_4$ and 1.0 g of NaCl and was well shaken and then centrifuged for a minute at 3700 rpm. The supernatant was added to the dispersive solid-phase cleanup tube: UCT ECMPSC1815CT which is a 15 mL centrifuge tube with 900 mg anhydrous magnesium sulfate, 300 mg PSA and 150 mg endcapped C18 and was shaken for 1 min, centrifuged again for a minute at 3700 rpm. The extract was filtered and then was placed in 15 mL air tight glass vial and was analyzed through GC-MS. The analysis was performed using Perkin Elmer Clarus Series 600. USA equipped with capillary injector and autosampler. The injector temperature was held at 250°C for 1.5 min during injection and then programmed at 200°C per min to 300°C and was held for 20 min. One microliter (1 µL) of sample was injected through. Similar procedure of extraction was followed for extraction of milk samples.

Response factor of each peak = Area of the peak/concentration of the sample injected

Reagents and standards

All reagents and standard were purchased from Fluka (Germany) and United Chemists Bristol USA.

Calibration curve

The calibration curve is determined by the analysis of 5 calibration levels, that is, 0.0005, 0.00, 0.01, 0.1 and 0.3 mg/kg. Peak area was calculated and calibration curve was plotted for concentration vs. response area or peak area. The calibration curves Figure 1 were best fitted to a linear curve. The correlation coefficients (R) were 0.9947. The quantification was performed from the mean of two calibration curves surrounding the samples.

Precision-repeatability

Precision of the analyte was measured from the standard deviation as described by Horwitz (1982). Precision of the method was estimated from the results of each analyte by an external standardization method with a standard deviation of less than 10%. Four replicates of milk and feed/fodder samples were spiked with four different levels of cypermethrin ranged from 0.001 to 0.1 mg per kg with standard deviation of ± 2 to 6.

Repeatability in this validation was measured from the mean of 4-replicate of each sample (milk and diet). Repeatability was calculated as given ISO 5725-2. The relative standard deviation of the repeatability must be less than or equal to the standard deviation proposed by Horwitz (1982).

Accuracy-recovery

The accuracy was determined by the recovery of standard samples at four concentration levels. The average relative recovery was between 70-120%. Quantification limits (LOQ) are calculated from the results at the lowest accepted standard level, as 6 times the standard deviation (absolute recover) (Table 1).

Health risk assessment (including estimated acceptable daily intake, bioconcentration factor and cancer potency factor)

Health risk assessment

A detail survey of around 100 household living within the vicinity of dairy farms was carried out. The households were divided into three age groups one age group is children aged from 2-10 years, adults from 20-40 years and elderly 50 to 70 years. Their body weight and milk consumption per day was recorded in a survey form and presented in Table 3. The average daily dose of chloropyrifos and cypermethrin was calculated using a formula as described by Pundit and Sahu (2002).

Acceptable daily intake

Daily intake of pesticide residues was estimated using a formula as described by FAO (1997) and given below:

$$EADI = Ca \times F \times I$$

Where, EADI, is the Estimated acceptable daily intake (µg pesticide/kg bw/day) (Pandit and Sahu, 2002); Ca, is the average residue content of pesticide in milk; F, is the fat content in milk and I, is the intake of milk in ml per kg bw (body weight) per day.

Bio-concentration factor (BCF)

BCF of pesticides and their metabolites in cattle's milk was calculated by dividing the concentration of pesticides and their metabolites in tissue (fat, milk, muscle) with concentration of same pesticides and their metabolites in diet as described by Snodgrass (2001). Concentration of pesticides will be in mg per kg. When BCF is 1 or less than 1 than there will be no accumulation (Table 5).

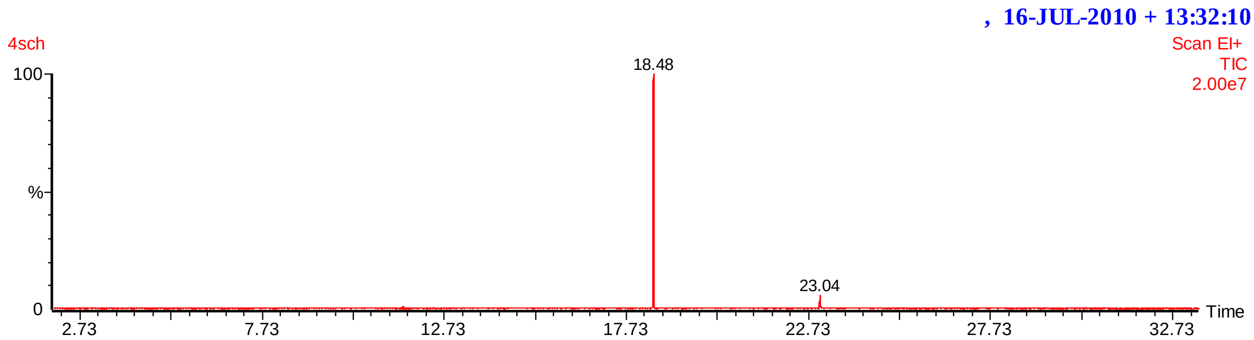

Figure 2. Chloropyriphos analytical standards Retention time: 18.47, 18.48 min for m/z 97 m/z of chloropyriphos 97, 125, 258.

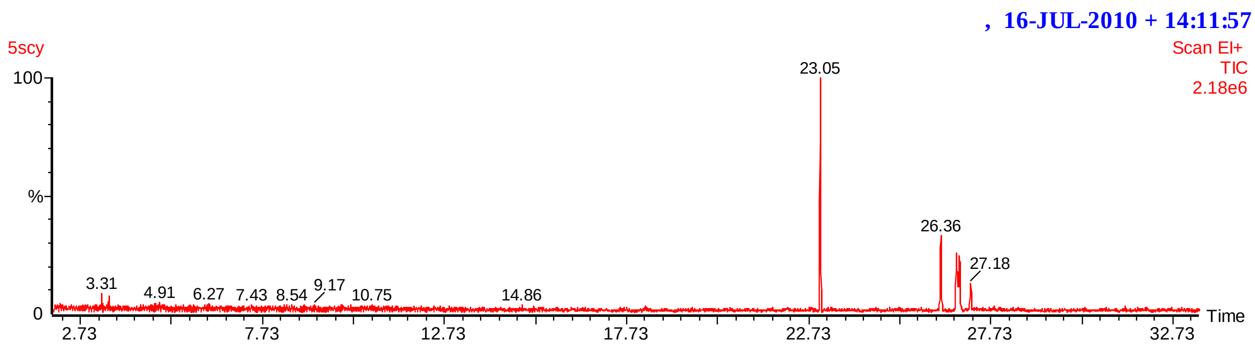

Figure 3. Cypermethrin analytical standard. Retention time: 26.34, 26.36, 26.81, 26.86, 27.16, 27.21, 27.22, 27.24, min m/z of cypermethrin 163,165, 91, 181, 209.

Cancer potency factor

ADD= C×F×I

Where, ADD is the average daily dose; C is the concentration of pesticide in milk; F is the fat content in milk in g; I is the daily intake of milk g per kg body weight per day; Risk is the ADD×ED×1/70×CPF×10^{-6}; ADD is the average daily dose; ED is the Exposure duration or age of the children or elderly and CPF is the Cancer potency factor in mg per kg bw per day according to US EPA classification (1997)

RESULTS

Total ion chromatogram (TIC) of pesticides standards is shown in Figures 2, 3 and 4. The peaks of the standards were identified from m/z ratio of the pesticides as appeared on GC-MS chromatogram. Chlorpyriphos (3,5,6 trichloro 2 pyridinyl phophorothioate) belongs to organophosphate group of insecticides, m/z of chloropyriphos is 97 the retention time of chlorpyriphos on GCMS chromatogram is 18.48 min (Figure 2). Figure 3 shows the TIC of cypermethrin, (RS)-cyano (3 phenoxyphenyl) methyl(1RS)- *cis-trans*-3-(2,2-dichloroethenyl)-2,2 dimethyl-s-s-cyclopropane carboxylate, belongs to pyrethroid group. The m/z's of cypermethrin is 163 whereas the retention time on GCMS chromatogram is 26.36 min. Figure 4 shows the TIC of profenophos (o-(4-bromo-2-chlorophenol)-o-ethyl-s-n-propyl phosphorothiote) belongs to organophosphate group. The m/z's of profenophos is 63 whereas the retention time on GCMS chromatogram is 20.53 min.

Total ion chromatogram of cotton khal, sugarcane khal and mixed diet is shown in Figures 5, 6 and 7. The average content of cypermethrin, chlorpyriphos and profenophos in the diet of dairy cattle farmed fed in various diets is presented in Table 1.

The result shows that the main difference in the amounts of cypermethrin, chloropyrifos and profenophos was that the low content of profenophos was extracted from all feeds followed by chlorpyriphos and cypermethrin.

It was noted that approximately same amounts of cypermethrin was found in all types of dairy cattle feed whereas significantly greater (P<0.05) content of chlorophyrifos was noted in cotton khal and low content was extracted from wheat bran and intermediate amount from all other feeds. Profenophos was low in wheat bran and wheat grain than other four feeds.

Total ion chromatogram of cypermenthrin, chloropyrifos and profenophos in milk samples are shown in Figures 8

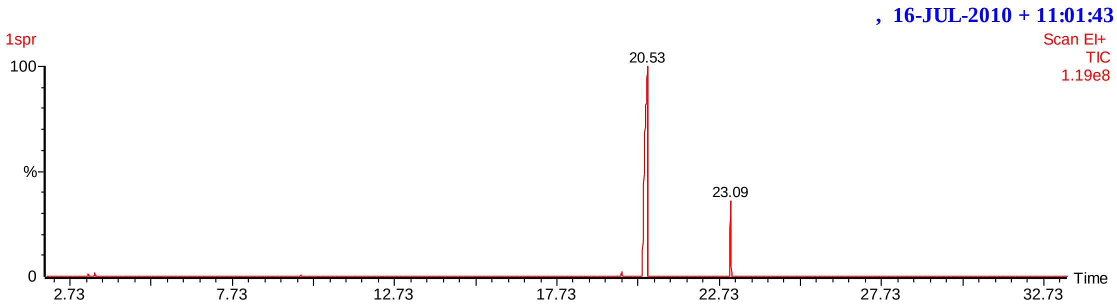

Figure 4. Profenophos analytical standard. Retention time: 20.44, 20.52, 20.53, min m/z of profenophos 63, 142, 144, 206, 208, 210.

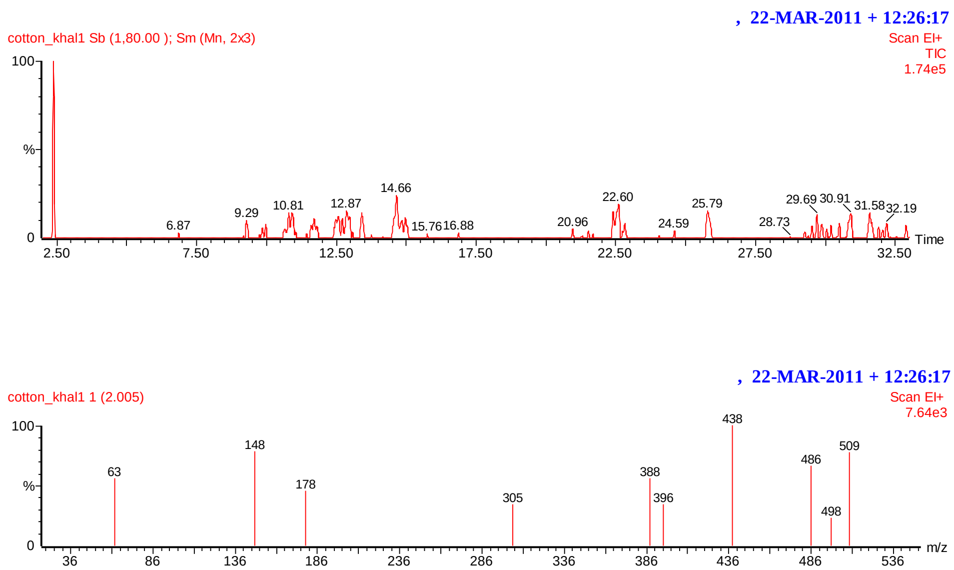

Figure 5. Total ion chromatogram and spectrum of cotton khal. Only profenofos was determined m/z 63.

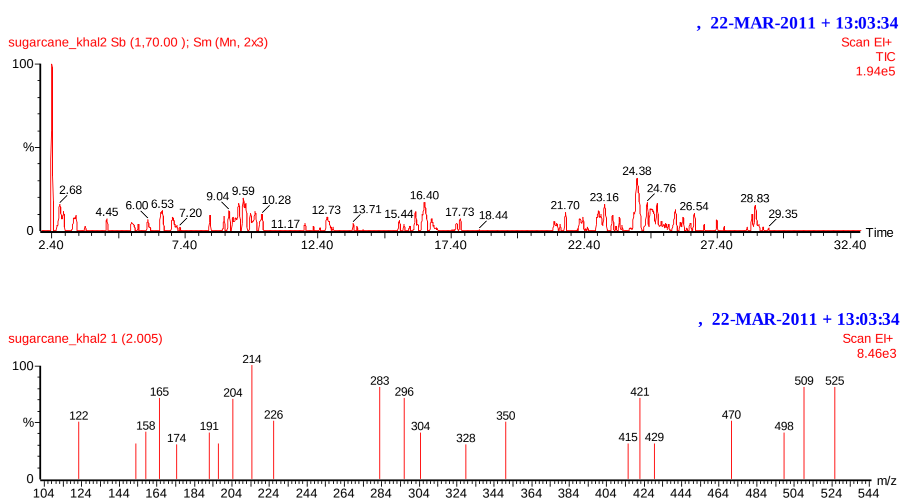

Figure 6. Total ion chromatogram and spectrum of sugarcane khal. In sugarcane khal 165 cypermethrin and 191 chloropyrifos was found

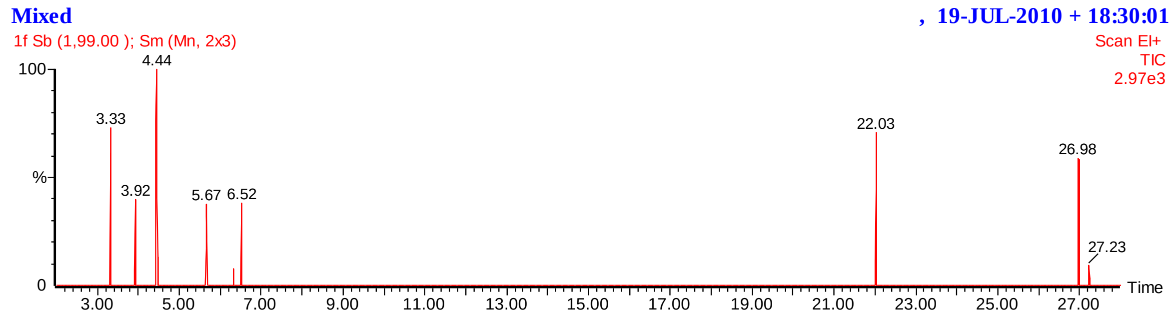

Figure 7. Total ion chromatogram and spectrum of wheat bran and mixed. Mixed shows 63 profenofos, 165 cypermethrin, 191 chloropyrifos.

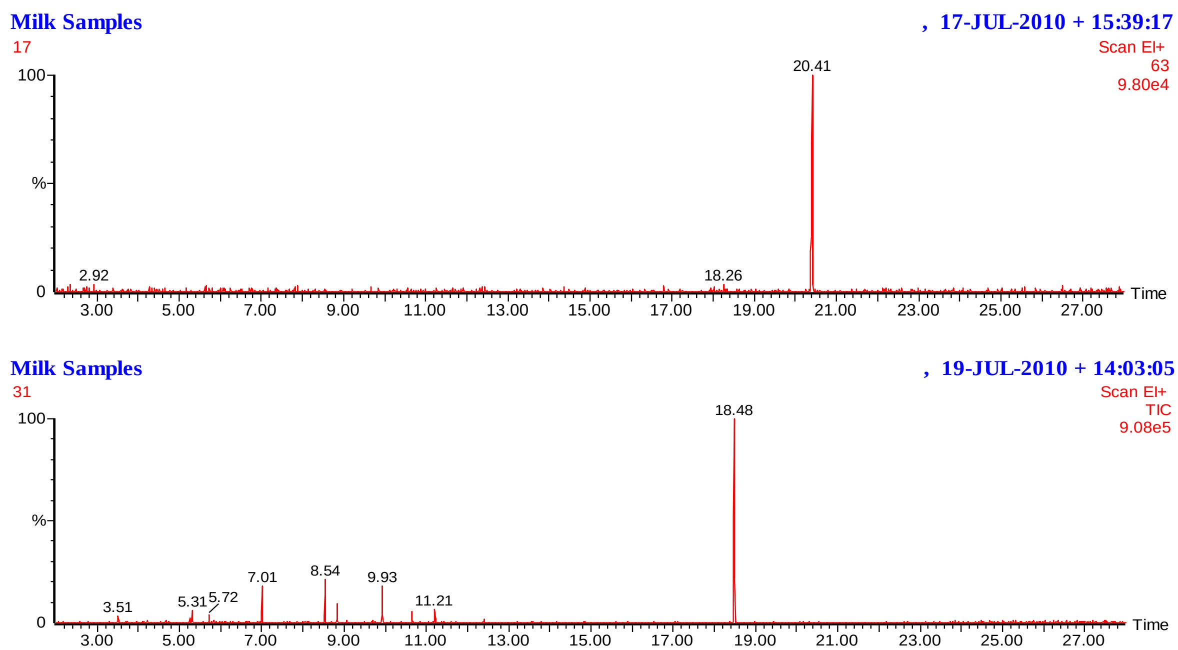

Figure 8. Peaks of cypermethrine and profenofos in milk samples.

and 9. Analysis of GC-MS chromatograms of dairy cattle milk samples from various farms differs from the diet given to the cattle which showed that approximately similar amounts of cypermethrin and chloropyriphos appeared on the chromatograms. The average content of cypermethrin, chlorophyriphos and profenophos in the dairy milk of dairy cattle fed on different diet is presented in Tables 2 and 3.

The main differences between the diets in the pattern of appearance of pesticides were that, low amounts of all of them were found in cattle milk fed on sugarcane khal than other diets. Greater concentrations of cypermethrin and profenophos were noted in the cattle milk fed on mixed diet whereas chloropyrifos appeared to be high in the cattle milk fed on cotton khal+wheat bran and mixed diets.

When the concentration of cyperme-thrine, chloropyrifos and profenofos in the milk samples of all cattle's irrespective of the diets given to them was compared and it was noted that more or less same amount of cypermethrine and chloropyrifos were obtained from all milk samples whereas the concentrations of profenofos was low in all milk samples. No differences in the total milk production among cattle's were noted regardless of the diet.

Health risk assessment of various age groups among children and adults

For non-cancer health risk, the value of RfDs for chloro-pyrifos and cypermethrine is 3×10^{-3} and 1×10^{-2} mg per kg

Table 2. Comparison between average pesticides level in different types of diets.

Type of feed	Pyrethroid (mg/L)	Organo phosphate(mg/L)	
	Cypermethrine	Chlorophyriphos	Profenophos
Cotton Khal	0.026	0.00269[c]	7.6×10^{-4}
Sugar Cane Khal	0.025	0.0012[b]	7.9×10^{-4}
Mixed feed	0.034	0.0016[b]	9.8×10^{-4}
Wheat bran	0.021	0.0009[a]	6.6×10^{-4}
Wheat grain	0.022	0.0011[b]	6.2×10^{-5}
Maize	0.022	0.0013[b]	5.9×10^{-5}

*Means (n=3) showed by different letters within columns are significantly different at the 5% level of probability.

Table 3. Average content (m*g* L^{-1}) of pyrethroid, organo-phosphate, chloronicotinyl and avermectine groups of pesticides in the dairy milk of rural and urban farms.

Farm number	Diet	Cypermethrin	Chloropyriphos	Profenofos
1	Cotton khal + mixed feed	0.221[c]	0.228[c]	0.00152[b]
2	Wheat bran	0.208[c]	0.029[a]	0.0006[a]
3	Cotton+sugarcane khal+wheat bran+mixed feed+maize bran	0.112[b]	0.018[a]	0.00144[b]
4	Maize bran	0.321[d]	0.081[b]	0.00212[c]
5	Sugarcane khal	0.02[a]	0.107[b]	0.00156[b]

*Means (n=3) showed by different letters within columns are significantly different at the 5% level of probability.

Table 4. The average daily intake of milk within the vicinity of dairy farms (urban and rural) of Peshawar district.

Age group	Body weight (kg)	Daily milk intake (ml)	Daily intake of milk in ml per kg body weight per day	EADI µg pesticide kg^{-1} bw day^{-1}
2-10 years-children	10 to 30 (25)	500	20	91
20-40 years-adults	60 to 90 (75)	1000	13	59
50 to 70 years- elderly	45 to 60 (55)	350	6	27

Table 5. Transfer factor of cypermethrine, chloropyrifos and profenofos from cattle feed into milk.

Type of feed	Cypermethrine	Chloropyrifos	Profenofos
Cotton khal			9.50
Sugar cane khal	7.04	77.16	
Mixed feed	5.17	57.87	7.38

per day (US-EPA, 1997). However, cancer potency factor (CPF) for cypermethrin is less than 1×10^{-5}. The results of this study shows that CPF for children less than 10 years have cypermethrin less than 4×10^{-6} and for elderly 0.0077×10^{-6} (Table 4). Daily acceptable intake of pesticides in different age groups remained less than 0.1 mg per kg of body wt. This suggests that human's are not exposed to the pesticide contamination from milk or any other sources.

Bio-concentration factor (BCF)

BCF is less than 1 for any of the pesticides which shows that there is no accumulation of pesticides from diet to milk. When BCF is 1 or less than 1 than there will be no accumulation.

DISCUSSION

Cypermethrin and chloropyrifos (Table 2) in dairy milk samples were greater than the maximum residue limits for such pesticides. Profenofos in milk samples remained within the maximum pesticide residue limits in milk (MRL, 2003). The greater content of cypermethrin and chloropyrifos in milk samples revealed the contention that milk is contaminated. Pesticide contamination in dairy milk has previously been reported in the literature. The result of this study is in-agreement with the findings of Roothwell et al. (2001). They reported that cypermethrin content was 0.015 mg/L in the milk samples of dairy cattle and was found to be greater than the maximum allowable limits of cypermethrin in milk. They concluded that the cypermethrin contamination in milk was because of their elevated levels in cattle diet. Alvarez et al. (2010) reported cypermethrin contamination in cow milk samples obtained from urban farm. They concluded that this is because of cypermethrin accumulation in body fats of cows.

Muir and Baker (1973) reported that the concentrations of organochloro pesticides in cattle milk grazed in orchards were greater than grazed in pastures. They concluded that the difference in the concentrations of pesticides between milk is most probably because of high dose of these pesticides sprayed in the orchards. John et al. (2001) found in Jaipur, India that all milk samples were contaminated with organochlorine pesticides and the levels were more in autumn-winter than in summer. The people in Cartagena, Bogota were exposed to organochlorine compounds through consuming 12.1 mg/g/day in pasteurized milk and 100% of the samples tested were contaminated with the pesticides greater than WHO/FAO limits (Castilla-Pinedo et al., 2010).

The levels of pesticides in this study were higher than those detected by Bordet (2002) which ranged from 26 to 45 ng/gm in milk for organophosphorus pesticides and pyrethroids, and also than those found by Pagliuca et al. (2006) who found that 27.40% of the samples were positive in concentrations less than the maximum residue limit (MRLs) and 6.89% had contamination with chlorpyrifos ranging from 5 to 18 µg/kg. Pesticides enter milk through animal food, water, air and soil. Pesticide residues have been detected in milk frequently in various researches. A study conducted by the USDA Pesticide data programme showed that 96% of the samples contained dicchloro-diflouro ethane (DDE), 99% DPA, 41% dieldrin, 25% synthetic pyrethroids and 9% had 3 hydroxyfurans which is a breakdown product of carbamate insecticides (Thompson and Fishwick, 1970). Sassine et al. (2004) studied cypermethrin contamination in dairy milk samples of Brazil. Dairy cattle were sprayed with 50 g/L of active ingredient of cypermethrin and found 16 fold greater cypermethrin in cattle milk within 24 h of cypermethrin exposure. Nonetheless, cypermethrin was undetectable after 17 days of the exposure. Another study was carried out by Battu et al. (2004) in Ludhiana-India. DDT and Lindane was 3 fold greater in cattle milk samples than MRL, however organophosphorus or synthetic pyrethroid insecticides remained within their maximum residue limits of 0.01 mg/kg in milk samples. Bissacot and Vassilieff (1997) studied the pyrethroid insecticides (flumethrin, deltamethrin, cypermethrin and cyhalothrin) injections given to cow in Brazil.

They found that small amounts of deltamethrin, cypermethrin and cyhalothrin were present in cow's milk and blood samples immediately after injection whereas flumethrin was found after 28 days of injecting pyrethroid insecticides. According to the annual report of USDA (1997), the residues of pyrethroid and organophosphate pesticides are increased in milk samples over a period of a decade. Presence of diiphenylamine residues, DDE and endosulfan in milk was reported since 2004. They concluded that this is more likely due to rapid use of diphenylamine in cattle's drug, in milk processing plants and packaging materials.

All the three pesticides, that is, cypermethrin, chlorpyrifos and profenofos were detected in all types of diet that is commonly given to cattle. However all these values were within the maximum residue limits of these pesticides in animal diet (MRL, 2003). This shows that diet is not the only source of contamination in milk but there are other sources also like drinking water which usually contains toxic substances from the industrial, agricultural and domestic waste and may enter the food chain of cattle and become accumulated in their fat tissues. Cattle in our country are usually grazed in open fields and drink water from waste water channels. Moreover, a comparatively higher level is acceptable in animal diet but a lower concentration level is taken in very minute quantities on a regular basis, they can lead to harmful effects in the long run.

Nonetheless, the levels of cypermethrin and chloropyriphos of this study in cattle diet were less than the levels of same pesticides as reported by Meglar et al. (2010). They found that "residue levels of seven organophosphorus pesticides (OPPs) widely used in crops used for animal feed were detected between 0.005 and 0.220 mg/kg. Muhammad et al. (2010) found out that 20-25% milk samples surpassed the MRL levels for chlorpyriphos, cypermethrin and certain other pesticides.

Wong and Lee (1997) conducted a survey in Hong Kong and found that 16.6% of the samples contained organochlorine pesticide residues at levels exceeding the Extraneous Maximum Residue Limits of the Codex Committee on Pesticide Residues. Liaska (1968) found out that a well balance US diet contains organochlorines 0.02 mg per kg and organophosphates 0.003 mg per kg.

Most chlorinated insecticides are relatively resistant to processing techniques used for milk and dairy products. Once residues get into milk they are stable and difficult to remove; therefore, the best policy is to prevent their entrance into milk by proper and careful management of the dairy cows. Chloropyrifos dietary exposure in cattle milk reached 0.01 mg/kg when fed on a diet containing 30 mg chloropyrifos per mg per day. According to CCPR report, 0.01 mg/kg is the maximum residue limit of chloropyrifos in cattle fat or milk (Smith, 1968). When dietary intake of chloropyrifos either by human or cattle is more than MRL, that is, 0.03 mg kg^{-1}, it causes inhibition of plasma cholinesterase which causes depression in human, and no inhibition of plasma cholinesterase (ChE) is noted at 0.1 mg/kg of chloropyrifos in diet.

In human, the acceptable daily intake is 0-0.03 mg/kg bw/day oral acute LD_{50}, for rats it is 350 mg/kg and for rabbits, it is 700 mg/kg while a cut reference dose is 1.0 mg/kg bw/day (FAO, 2010). According to FAO (2010) report, profenofos is considered as moderately hazardous. Oral poisoning of profenofos usually occurs accidentally when is placed in an unlabelled container and taken as a drink. Nevertheless, it’s dietary intake when greater than 0.01 mg/kg/day may cause severe health hazards in human and animals. Dermal toxicity occurs when profenofos is in contact with skin accidentally because of leakage or spillage. Profenofos penetrates from the skin into the body. Toxicity symptoms are “excessive salivation, sweating, rhinorrhea and tearing, muscle twitching, weakness, tremor, headache, dizziness, nausea, vomiting, abdominal cramps, diarrhea, respiratory depression, tightness in chest, and cholinesterase inhibition. No teratogenic or mutagenic effects are known in animals (FAO, 2010).” In a research in Multan, Pakistan, 6 spraymen between the age group of 20 to 25 years, were monitored for 4 days for the effects of profenofos on blood cholinesterase levels while spraying on cotton plant. It was found that there was inhibition of blood cholinesterase activity (Loosli, 1952).

Conclusion

The presence of residue of pesticide in milk was a little bit greater than their recommended limits. However, symptoms of adverse health effect in cattle’s were not observed. Similarly, milk production remained unaffected due to pesticide contamination regardless of the diet given to the cattle. The transfer of such pesticides in milk was not consistent with the diet given to dairy cattle as their concentration remained within their maximum residue limits in diet. This revealed the contention that the presence of residue of some of the pesticides in milk is not because of diet and more likely because of drinking or inhalation of contaminated water and dust. However, the presence of such pesticide in milk and their accumulation in cattle fat tissues cannot be ignored and water and dust samples in accordance with milk must be monitored regularly. This is more likely because continuous presence of such pesticides in milk may become a source of severe adverse health problems in cattle’s because of their persistent and non degradable nature within cattle body. No adverse health effects on various age groups of people surveyed were observed during this study. This suggests that although the milk is contaminated, it but is considered to be safe for drinking.

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