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RESEARCH ARTICLE

Quantitative Structure activity relationship and risk analysis of some pesticides in the cattle milk

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ABSTRACT

Milk of cattle was collected from various localities of Faisalabad, Pakistan. Pesticides concentration was determined by HPLC using solid phase microextraction. The residue analysis revealed that about 40% milk samples were contaminated with pesticides. The mean±SE levels (ppm) of cyhalothrin, endosulfan, chlorpyrifos and cypermethrin were 0.38±0.02, 0.26±0.02, 0.072±0.01 and 0.085±0.02, respectively. Quantitative structure activity relationship (QSAR) models were used to predict the residues of unknown pesticides in the milk of cattle using their known physicochemical properties such as molecular weight (MW), melting point (MP), and log octanol to water partition coefficient (Ko/w) as well as the milk characteristics such as pH, % fat, and specific gravity (SG) in this species. The analysis revealed good correlation coefficients (R2 = 0.91) for cattle QSAR model. The coefficient for Ko/w for the studied pesticides was higher in cattle milk. Risk analysis was conducted based upon the determined pesticide residues and their provisional tolerable daily intakes. The daily intake levels of pesticide residues including cyhalothrin, chlorpyrifos and cypermethrin in present study were 3, 11, 2.5 times higher, respectively in cattle milk. This intake of pesticide contaminated milk might pose health hazards to humans in this locality.

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INTRODUCTION

Quantitative Structure Activity relationship refers to the mathematical expressions which interrelate the physicochemical or structural properties of a series of compounds with their biological activities (Cronin, 2004; Kawakami *et al*., 2011) which may be useful for estimating activities of any compound and predicting structures of high activity. The idea behind this methodology is that biological activity is a function of chemical structures that can be described by molecular or physicochemical variables, e.g. molecular weight, hydrophobicity and steric properties etc. (Hansch and Leo, 1995; Muhammad *et al*., 2009).

Pesticide residues in different food commodities mediate different sort of toxicities but their use is unavoidable because they play a crucial role in sophisticated agricultural technologies. Moreover, the use of different pesticides has become mandatory to meet the growing demand of enhanced food productivity for rapidly increasing population of the world. Most of the plant origin foods are grown using pesticides which have increased the agricultural productivity to a great extent (John *et al*., 2001). However, these economic benefits are directly associated to the health hazards in human beings and risk of environmental damage. Many pesticides and their residues have been reported as contributory factors in several diseases such as heart diseases, cancers, Alzeheimer's disease and Parkinsonism (Khaniki, 2007; Tsiplakou *et al*., 2010). Pesticide residues in feed and fodders may transfer into cattle through food chain and assimilate into the body systems of the animal (Prassad and Chhabra, 2001).

After ingestion, lipophilic pesticides get absorbed from the intestine into the general circulation. Highly lipid soluble pesticides tend to concentrate in tissues with higher lipid contents including adipose tissue, brain, liver, kidneys and in milk (Tsiplakou *et al*., 2010).

Cattle milk and dairy products are commonly used commodities in almost all the societies of the world and have a pivotal role in human nutrition. The occurrence of pesticides residues in the milk is a matter of public health concern, so, it is very important to ensure the quality of milk with respect to pesticide residues. In this regard, most of the developed countries have established their maximum residue levels (MRLs) of pesticides in milk and other dairy products.

Some pesticides are biodegradable while others persist in the soils for longer times (Tariq *et al*., 2006). Pesticide residues have been detected in the vegetables in Karachi (Perveen *et al*., 2005), in fruits and vegetables in Islamabad (Tahir *et al*., 2001) and in various tissues of fish in the local lakes (Saqib *et al*., 2005). In order to avoid the toxic health hazards, it is necessary to determine the levels of pesticides in edible tissues such as milk of common food animals such as cattle that are reared on pesticides spraying areas.

Keeping in view, this study was designed to determine the pesticides (cyhalothrin, endosulfan, chlorpyrifos and cypermethrin) residues in the milk of cattle and develop QSAR models of pesticides residues in milk using the milk characteristics and physico-chemical properties of pesticides. These QSAR models will be helpful in computing the pesticide residues in the cattle milk especially in developing countries like Pakistan where experimental or observed values are not available or difficult to determine due to lack of instrumentation and funding constraints.

MATERIALS AND METHODS

Milk sampling: The milk samples were collected randomly from different villages situated within a radius of 25-35 km on two different localities (east and west) of Faisalabad city, Pakistan. A total of 200 samples were collected with a frequency of 10 samples/locality/month and stored in 25mL clean sterilized plastic bottles. All the samples were kept at -4°C until further analysis.

Sample preparation/Analytical procedure: The concentrations of four different pesticide species (cyhalothrin, endosulfan, chlorpyrifos and cypermethrin) were determined in the milk samples of cattle by following the methodology of Cardeal and Clauda (2006) with slight modifications. Briefly, milk samples were prepared for analysis by mixing with a buffer solution (acetic acid and sodium acetate pH (4.5) with a 1:9 ratio (v/v) that resulted in the precipitation of milk proteins. All the samples were centrifuged and from each sample a 16 mL aliquot of the upper layer was transferred to a 20 mL head space vial. A manual solid phase micro extraction (SPME) holder along with 100 µm thickness dimethyl siloxane (PDMS) fiber assembly was purchased from Supelco (Bellefonte, PA, USA). The fiber was immersed directly in to glass vial containing the sample for half an hour with continuous stirring. The fiber was retracted and immersed into methanol for elution of residues.

HPLC analysis of pesticide residues: The eluted methanol was injected in to High performance liquid chromatography (HPLC) system (Shimadzu) equipped with UV-Vis detector for analyses of pesticides. Thermohypersil-C_{18} column was used for analysis and a wavelength of 235 nm was used to get absorption spectra. A mixture of acetonitrile and methanol in a ratio (11:9 v/v %) was used as mobile phase and injection volume taken for each of the samples and standards for analysis was 20µl.

Statistical analysis: The quantitative data on the pesticide concentrations in milk samples was subjected to one way analysis of variance (ANOVA) and results were expressed as mean ±SE. Multiple regression analysis was conducted on experimentally determined pesticide concentrations in the milk and data of physical parameters of both milk and pesticides using least sum of squares on Microsoft Excel version 2007. A QSAR model was suggested to calculate the concentrations of unknown pesticides in the milk using their known physico-chemical properties. Furthermore, QSAR models were suggested to predict the concentrations of these pesticides using the milk characteristics of cattle in the study area. Risk analysis was computed based upon the determined pesticide residues in the cattle milk and provisional tolerable daily intakes of these pesticides as established by the regulatory authorities.

RESULTS

Milk samples of cow collected from different localities in the east and west of Faisalabad were analyzed for four different pesticides (cyhalothrin, endosulfan, chlorpyrifos, and cypermethrin). The residue analysis revealed that about 40% milk samples was found contaminated with pesticides. Results of quantitative HPLC analysis of milk samples for pesticide residues are presented in the Fig. 1. This figure shows that mean±SE levels (ppm) of cyhalothrin, endosulfan, chlorpyrifos and cypermethrin are 0.38±0.02, 0.26±0.02, 0.072±0.01 and 0.085±0.02, respectively. The levels of all the pesticide species under study were found to be exceeding MRLs as recommended by USFDA (Table 1) except endosulfan that could not surpass the MRLs.

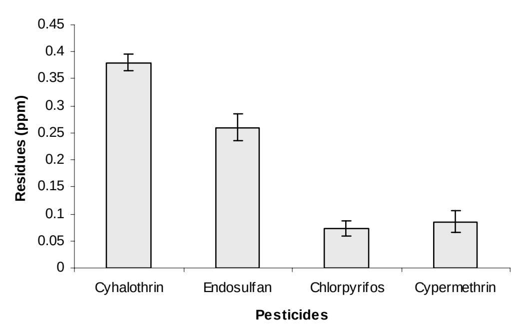

Fig. 1: Mean ± SE values (ppm) of pesticides in the cattle milk collected from Faisalabad-Pakistan

From all the pesticides under study, the occurrence of cypermethrin residues exceeding MLRs was the highest (20% from all the milk samples examined) followed by cyhalothrin (19%) and chlorpyrifos (17%); whereas the endosulfan was under the MLRs in all the milk samples examined. To study the relationship of residue levels of these pesticides to their physico-chemical properties such as MW, MP and log octanol to water partition coefficient (Ko/w) (Table 1) as well as to the characteristics of milk including pH, %fat and specific gravity (Table 2), multiple regression analysis with significance level of 0.05 was conducted. The analysis revealed an excellent correlation coefficient (R^2=0.91) for QSAR model (Equation 1). This higher R^2 value of QSAR model reflects a good prediction power of this model.

QSAR model:

$$\text{Log Kr} = C + \alpha\text{MW} + \beta\text{MP} + \gamma\text{Ko/w} \qquad \ldots\ldots\ldots\ldots(1)$$

Where, intercept C = 0.00, MW coefficient α = 0.00094, MP coefficient β = -0.0019, and Ko/w coefficient γ = -0.0408.

The coefficients of equation show that residue levels in milk are directly proportional to molecular weight while inversely proportional to melting point and Ko/w of pesticides. Multiple regression equation and coefficients for pesticide residues in cattle milk with respect to milk characteristics are presented in Table 3. The correlation coefficients (R^2) for cyhalothrin, endosulfan, chlorpyrifos and cypermethrin residues in QSAR models were 0.95, 0.96, 0.99 and 0.78, respectively. Results showed a positive correlation of all the pesticide residues under study with respect to the %fat (indicated by β) except endosulfan. On the other hand, cyhalothrin and chlorpyrifos residues showed a negative correlation with respect to pH and specific gravity in QSAR; whereas, endosulfan and cypermethrin revealed a positive correlation with respect to these two physico-chemical characteristics of milk.

Table 1: Physico-chemical properties and maximum Residue Limits (MRL) for pesticides established by USFDA (FARAD)

Pesticides	Molecular weight (MW)	Melting point (°C)	Ko/w	Maximum Residual Limits (ppm)
Cyhalothrin	450	10	6.90	0.2
Endosulfan	407	106	3.58	0.5
Chlorpyrifos	351	41	5.00	0.01
Cypermethrin	416	70	6.30	0.05

Table 2: Characteristics of cattle milk collected from different sites of Faisalabad-Pakistan

Sample collection sites	Milk pH	Fat (%)	Specific gravity
1	6.82	4.8	1.05
2	6.57	4.2	1.03
3	6.77	5.6	1.002
4	6.73	5.2	1.001
5	6.55	5.5	1.04

Table 3: Multiple regression equation and coefficients for pesticide residues determined in the cattle milk samples with respect to the milk characteristics

$$\text{Log Kr} = C + \alpha\,(\text{pH}) + \beta\,(\%\text{fat}) + \gamma\,(\text{specific gravity}) \qquad \ldots\ldots\ldots\ldots(2)$$

Pesticide	R^2	C	α	B	γ
Cyhalothrin	0.95	0.447	-0.039	0.046	-0.039
Endosulfan	0.96	-2.214	0.070	-0.016	2.053
Chlorpyrifos	0.99	0.949	-0.007	0.009	-0.863
Cypermethrin	0.78	-0.571	0.006	0.024	0.492

DISCUSSION

The major source of contamination of dairy products by different hazardous pesticides is the presence of their residues in animal feedstuffs. Other factors that may also contribute to this sort of contamination include the application of pesticides on farm animals, environmental contamination and accidental spills (Goodarzi *et al*., 2010). Milk contamination with the pesticides residues can be avoided/controlled by preventing the contamination of feedstuffs.

In this monitory study, residues of different pesticides were determined by HPLC analysis which showed high levels of cyhalothrin, chlorpyrifos and cypermethrin residues in milk samples exceeding MRLs. The result of our findings were comparable to those determined by Wong and Lee (1997) who analyzed the milk samples collected from local market of Hong-Kong for organo-chlorine pesticides and reported that 42 out of 252 (16%) samples exceeded the level prescribed by codex committee on pesticides.

The percentage of milk samples contaminated with pesticide residues in present study was lower as compared to those reported by Parveen *et al.* (2005) who analyzed the 206 samples of different vegetables from Karachi, out of which 63% samples were found to be contaminated while 46% samples exceeded the MRL. At the same time, percentage (40%) of milk samples contaminated with pesticide residues was also lower than different food commodities (60%) as reported by Agnihotri (1999). Higher percentage of contaminated food commodity (fruits and vegetables) as compared to milk might be due to the direct exposure of these food items to pesticide. Regarding QSAR model, multiple regression equation (1) suggested that molecular weight, melting point and Ko/w were main determinant of pesticide residues in cattle milk. The equation (1) indicated a direct dependence of residue levels in milk upon molecular weight and inverse dependence upon melting point and Ko/w.

The residues of pesticides under study except endosulfan showed a positive correlation of all the pesticide residues under study with respect to the %fat (Table 3). This finding is in accordance to the findings of Fytianos *et al*. (2001) and Abou Donia *et al*. (2010) who reported the accumulation of a high level of pesticide residues in high fat milk as compared to low fat milk. Cyhalothrin and chlorpyrifos residues showed a negative correlation with respect to pH and specific gravity in QSAR model; whereas, endosulfan and cypermethrin revealed a positive correlation with respect to these parameters.

Yang *et al*. (2004) and Sheng *et al*. (2005) showed that different pesticides show higher solubility at lower pH (acidic) level and this is consistent with the findings obtained for cyhalothrin and chlorpyrifos; whereas, results of endosulfan and cypermethrin are not consistent with the findings of Sheng and his coworkers; and showed higher solubility in milk samples with higher pH values (Table 3). The variable pattern of pesticide residue dependence upon milk characteristics signifies the need to construct the QSAR model for residue prediction in the cattle milk.

The daily acceptable intakes (DAIs) of cyhalothrin, endosulfan, chlorpyrifos and cypermethrin are 0.2, 0.5, 0.01 and 0.05 ppm, respectively (Table 1) (WHO, 1997). According to Food and Drug Administration, the daily recommended milk requirement for a healthy adult individual is 1.5 kg (Riviere, 1999). Based upon which daily intake levels of pesticide residues including cyhalothrin, chlorpyrifos and cypermethrin in present study are 3, 11, 2.5 times higher, respectively in cow milk. Since endosulfan did not surpass the MRL limits so risk assessment for this pesticide was not calculated. These higher values imply that consumers of the region are potentially at high risk by these pesticides and particularly are of major health concern for the infants with poor metabolic activities (Mishra and Sharma, 2011).

Conclusion: Findings of the present study revealed that the 40% milk samples of cow were contaminated with pesticides and 17-20% milk samples of cattle surpassed the MRL levels for different pesticides. The suggested QSAR models can be successfully employed to assess the pesticide residue burden in this locality. Nevertheless, it is indispensable to monitor the presence of these compounds for their relevant implication in health care system and assess their levels in other zones of the country to shape a major representative panorama. Moreover, these findings suggest creating awareness in dairy farmers and general public regarding the avoidance of pesticide residues in milk.

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