International Journal of Fauna and Biological Studies

Available online at www.faunajournal.com

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Uzma Mehboob
Department of Zoology, Federal Urdu University of Arts, Science and Technology, Gulshan – e-Iqbal Campus, Karachi, 75300, Pakistan.

Muhammad Arshad Azmi
Department of Zoology, University of Karachi, Karachi-75270, Pakistan.

Shazia Nisar
Department of Zoology, University of Karachi, Karachi-75270, Pakistan.

Abid Raza
Department of Environmental Science, Federal Urdu University of Arts, Science and Technology, Gulshan – e- Iqbal Campus, Karachi, 75300, Pakistan.

Sadaf Tabussam
Department of Zoology, Federal Urdu University of Arts, Science and Technology, Gulshan – e-Iqbal Campus, Karachi, 75300, Pakistan.

Nadia Fatima
Department of Zoology, Federal Urdu University of Arts, Science and Technology, Gulshan – e-Iqbal Campus, Karachi, 75300, Pakistan.

Mussart Memon
Department of Zoology, University of Karachi, Karachi-75270, Pakistan.

Syed Naeem ul Hasan Naqvi
Baqai Medical University Karachi 51, Toll Plaza Super Highway, Gadap Road, P.O. Box: 2407, Karachi – 74600, Pakistan.

Correspondence:
Uzma Mehboob
Department of Zoology Science, Federal Urdu University of Arts, Science and Technology, Gulshan – e- Iqbal Campus, Karachi, 75300, Pakistan.

Childhood pesticide poisoning via maternal milk in Karachi, Pakistan

Uzma Mehboob, Muhammad Arshad Azmi, Shazia Nisar, Abid Raza, Sadaf Tabussam, Nadia Fatima, Mussart Memon, Syed Naeem ul Hasan Naqvi

Abstract
The aim of the current study is to determine whether level of pesticide in breast milk in the general populations of Karachi city are correlated with demographic parameters, life style factors and occupational exposure. Human milk specimens (n=200) were collected from nine different divisions between late 2003 to early 2013. These divisions were categorized in two groups i.e., highly polluted area (R_1) and less polluted area (R_2).the detection and quantification of different pesticide was performed by HPLC using UV detector. Extraction was performed by n-Hexane and methanol and concentrations of persistent Pyrethroids, OCs and Ops were determined. Pesticide levels in R_1 were consistently higher than R_2 and a significant difference ($p<0.005$) was observed. Permethrin (15%), Cypermethrin (20%), Deltamethrin (11%), Malathion (8%), DDE (7%) and DDT (4%) were recorded in the milk samples of R1. The mean ±S.E of Permethrin, Cypermethrin, Deltamethrin, Malathion, DDE and DDT were (21.50 ±1.18), (12.58±1.25), (9.67±3.03), (15.44±0.83), (4.35±1.10), (1.28±0.39) in R1 respectively, whereas in R2 only three pesticides were detected on very low concentration: Permethrin; (5.823±0.696), Cypermethrin; (1.938±0.56), Deltamethrin; (1.315±0.214). Maximum residue limit was also higher than those recommended by WHO/FAO. The presence of these pesticides has negative impact on newborns health.

Keywords: Human Milk, Pesticide Residue, Pyrethroids, OCs, Ops

1. Introduction
Pesticides are omnipresent pollutants of our surroundings and have been found in atmosphere, water, top soil and animal tissues and human being world widely. Pesticides accumulate mostly via skin, eyes, Inhalation or ingestion. Water base pesticides and triglyceride base pesticides are diffused in the human body through abrasions, skin and sores. The respiratory tract is also absorbed pesticides vapors and 5µm droplets of aerosol. Inhaled particles and larger droplets are ingested by the digestive tracts. Fat soluble pesticides absorbed in the intestine after ingestion, but highly Fat soluble pesticides accumulate in tissues with higher content of triglycerides including milk, liver, adipose tissue, brain, and kidney [1]. The prominent route of exposure to Pesticides in adult humans was diet [1, 2, 3] The food web, indoor air Inhalation and dermal uptake is the main route of entry of PBDEs into the human body [4]. Most of the plant are gown using pesticides w/c have increased agricultural productivity are used as plant origin food [5]. The residues of commonly used pesticides viz Methyl parathion, Dieldrin, Cyfluthrin, Monocrotophos, Methamidophos and Cypermethrin were studied in three varieties of mango collected from grower fields in Multan Division [6]. Pesticide contamination was studied in fruits and vegetables in Islamabad [7] in the vegetables in Karachi [8]. The major contributors of Pesticide in the human body were meat, processed foods, and dairy products after weaning till reproductive age [9, 10]. Similarly, it was reported that chlorinated pesticides; HCB and PCBs were reported in different fish species from Karachi coast [20] and in various fish tissues in the local lakes [11]. In Pakistan, the drinking water may also be polluted by direct contamination of water systems or through use of similar containers to mix pesticides and to transport drinking water [12]. Insecticide residues in ground water specimens was reported from Mardan Division [13]. The inhabitants were exposed to comparatively high levels of OC pollutants probably through bovine milk [14] OPs, Pyrethroids, Carbamate residue were detected in cow, goat. Camel and sheep milk in local areas of Lahore [15]. Similarly, Endosulfan, Chlorpyrifos, Cyhalothrin, and Cypermethrin were reported in goat and cattle milk from Faisalabad [16, 17].

Adverse effects on child health were also noticed by some researchers due to the extensive use and exposure of different pesticides. The health risk such as reduced brain mass, decreased synthesis of brain DNA and respiratory syndrome in offspring during postpartum due to the exposure of organophosphate pesticides was reported [18]. Similarly it was reported that the organophosphorus pesticides such as Malathion was high neurotoxin in nature [19]. In Pakistan, the exposure of multiple pesticides for instance; Monocrotophos, DDE, Diazinon, Deltamethrin, Cypermethrin and DDT for delayed period. It disturb different organ system`s normal function and probably created characteristics clinical effects like burning sensation in urine and hepatitis dyspnea in humans [20]. Similarly, the correlation of some adverse health effects such as birth defects, fetal death, endocrine and neurologic syndromes with different pesticides e.g., DDT and Pyrethroids residues in the maternal milk was also reported [21, 22]. The analytical techniques were used for the detection of contaminants in the blood, maternal milk, fat, urine and biological samples [23]. In the present study, for the identification, separation, and quantification of ten commonly used pesticides, a technique used HPLC with UV detection. The compounds studied were match, Cypermethrin, Deltamethrin, Malathion and Permethrin, DDE, Polytrin-C, Diazinon, DDT and Monocrotophos. These females are living in highly polluted regions. Polluted regions are nesting ground for germs and insects. These people used immediately killing pesticides especially for the indoor sprays. Pesticides are also accumulating via food chain. The hypothesis was that it should be proved by the current data that their practice is very harmful for them and their families especially; infants.

2. Material and Method
2.1 Selection of site
Sampling sites were selected according to demographic factors like poor waste disposal overuse of harmful substances, inadequate services, and environmental pollution, excessive use of indoor residual spray (IRS). Human breast milk samples were collected from nine different divisions of Karachi city. These divisions were categorized in two groups. 100 samples were collected from each group.
These categories were setup on the basis of highly polluted area (R_1) and less polluted areas (R_2). Liaquatabad, Essa nagri colony, Hazara colony, Moosa colony &, Rehri Goth are located in R_1. Korangi, Hazara Colony, Shah Faisal Colony and Machhar Colony are located in R_2

2.2 Sample collection and preservation
The milk samples were collected in sterilized glass vials and kept in ice box immediately after collection. After collection, samples were preserved in freezer till analysis. Questionnaire on dietary aspect was also designed.

2.3 HPLC analysis of pesticide residue
For detection and isolation of pesticide from the milk sample HPLC equipped with electron capture detector (ECD) was performed.

2.4 Extraction of fat
The pesticide is lipophilic in properties and accumulates with lipids, therefore for the extraction of pesticides, fat must be extracted, for such purpose following procedure were adopted.

2.5 Soxhlation method
The extraction carried out in a well known soxhlet apparatus appears to be one of the best procedures for extraction of residues from finally milk samples. For the extraction of fat from samples of milk Holden and Marsden (1969) method was used [24].

2.6 Sorption
The process of sorption was carried out in chromatographic column of alumina [24, 25, 26].

2.7 Analysis of pesticides by High Performance Liquid Chromatography
The detection by this technique is simple, rapid and sensitive. HPLC have been used for the separation of pesticides, by using a packed column, (Zorbax TM NH_2) a polar bounded phase with particle size of about 7 μm in diameter. The columns are packed to uniform bed density by using a high pressure slurry loading technique. This column was used with fractionated n-hexane with the flow rate of 2.5 milliliter per min in a mobile phase. A UV detector was used with a wave length of 205 nm, pressure 200kg/cm$_2$ and absorbance 0.08 with chart speed 10mm/min, for the detection of pesticide.

2.8 Mobile Phase
10 ul of each sample was injected and peaks were obtained. The comparison was made on the basis of inhibition or activation of pesticides among standard and sample.

2.9 Statistical Analysis
The quantitative date on the pesticide concentration in milk samples were subjected to Analysis of Variance Technique (ANOVA) and generalized linear Model (1998) by using Minitab 15.

3. Result
Pesticide residues in human milk samples were studied from local women resident in south eastern region (R_1) and north western region (R_2) of Karachi city were tested during the period of 2006-2012. Results showed the difference were found and presented in **Table-1.** Maximum residue limit of Cypermethrin, deltamethrin DDT and DDE was higher than the recommended dose of WHO/FAO. **Table-1** [27, 28].
The result indicated the remarkable difference b/w the two regions. Malathion, DDT, DDE was not detected in R_2 on the other hand it was detected in R_1 **Table-2 and 3.**
The mean concentration of Permethrin was higher than other detected pesticides (21.50μg/10μl) in R_1 and in R2 (5.823). Cypermethrin was detected in twenty samples of R_1 (mean value => 12.58) on the other hand it was detected in ten samples of R_2 (mean value => 1.938). Deltamethrin result showed mean value = 9.67 & n=11 in R_1 while in R_2 it`s mean value = 1.315 & n=15. All samples of this region showed lower concentration of Deltamethrin. Malathion was detected in eight samples of R1 and their mean value was 15.44. DDE was found in seven samples (mean value => 1.277) On contrast of it DDT was detected in four samples (mean value => 4.53) in R_1 **Table-2 and 3.**

Table 1: Maximum Pesticide Residue Level (µg/10µl) in Milk Sample of Both Regions.

MRL	Permethrin	Cypermethrin	Deltamethrin	Malathion	DDT	DDE
MRL R_1	35.86	22.42	26.80	17.2	6.25	3.06
MRL R_2	8.82	4.31	2.71	ND	ND	ND
MRL, WHO/FAO	-	5	5	-	2	2

ND; Not Detected, MRL; Maximum Residue Limit

Table 2: Descriptive Statistical Data of Pesticide Residue in Milk Sample of R_1

| Pesticide	N	%	Mean	S.D	S.E	Range
Permethrin	15	23%	21.50	4.58	1.18	18.86-23.93
Cypermethrin	20	30%	12.58	5.60	1.25	9.86-15.11
Deltamethrin	11	16%	9.67	10.05	3.03	2.92-16.43
Malathion	8	12%	15.44	2.350	0.831	13.47-17.40
DDT	4	6%	4.35	2.20	1.10	0.86-7.85
DDE	7	10%	1.277	1.020	0.386	0.33-2.22

Table 3: Descriptive Statistical Data of Pesticide Residue in Milk Sample of R_2

| Pesticide	N	%	Mean	S.D	S.E	Range
Permethrin	8	24	5.823	1.968	0.696	4.177-7.468
Cypermethrin	10	30	1.938	1.772	0.560	0.671-3.2005
Deltamethrin	15	45	1.315	0.829	0.214	0.856-1.775

Result revealed the presence of higher values of Cypermethrin, Permethrin & Deltamethrin in R_1 as compared to this lower value of these pesticides was detected in R_2. Box plot no (i),(ii),(iii) showing a remarkable difference between the quantities of pesticides detected from both region and significant difference (P = 0.000) using samples T-Test Technique on Minitab. The FAO/WHO ADI of DDE (0.002 mg/g) was lower than these values (p<0.05).
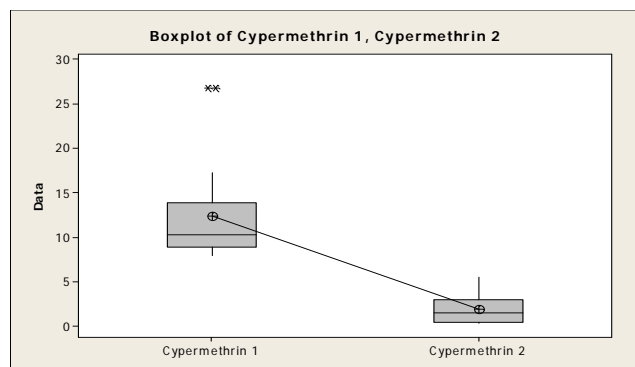
Box plot (i): showing values Cypermethrin 1 in R1 and Cypermethrin 2 in R2 using sample T-Test Technique
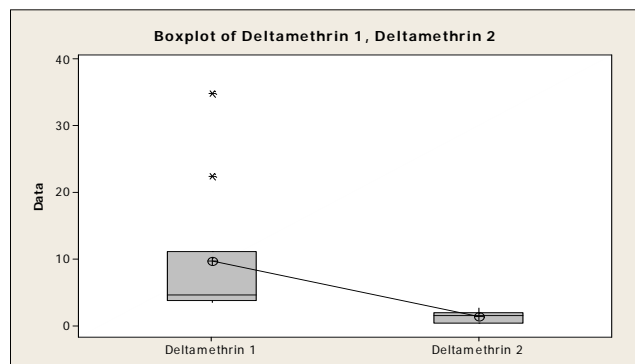
Box plot (ii) showing values deltamethrin 1 in R1 and Deltamethrin 2 in R2 using sample T-Test Technique
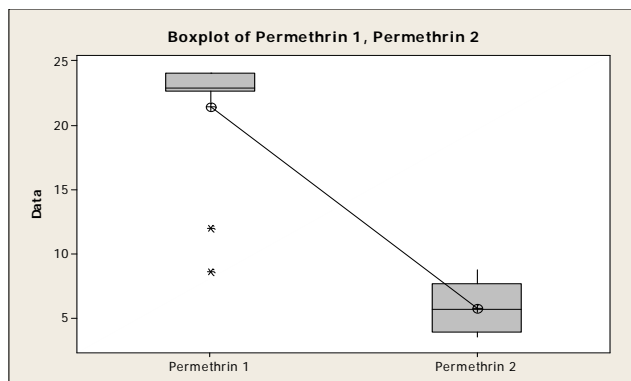
Box plot (iii): showing values Permethrin 1 in R1 and Permethrin 2 in R2 using sample T-Test Technique

4. Discussion

Liaquatabad, Essa nagri colony, Hazra colony and Moosa colony areas are located in R_1, traditionally considered as site of small cottage industries with improper waste disposals and sewage system, it also lack of collection and process a small proportion of sewage and garbage due to this condition it is the nesting ground for insects. It is famous for its fish market. There is also lack of cleanliness was recorded in the houses of the residents of these areas. Maternal milk samples for the measurement were analyzed for isomers of Hexachlorocyclohexane, methyl-parathion, chlorpyrifos, endosulfan, and Malathion from Madhya Pradesh, Bhopal [29]. The maternal milk was contaminated by Total Pyrethroids, deltamethrin, Cypermethrin, Cyfluthrin and Permethrin in 3 urban regions of South Africa, (KwaZulu-Natal) [30]. Similarly In the present case, Permethrin, deltamethrin, Cypermethrin, Malathion, DDT and DDE was detected in 65% samples of R1 mothers and in 33% samples of R2 mothers.

Conc. of OC compound in specimen of Shenyang mothers was significantly lower than Dalian mothers [31]. Similarly, in present case Malathion, DDT and DDE was detected in R_1 mothers samples and not detected in R_2.

Rehri goth is located on the Karachi sea coast in R_1 & has a large community of fisherman. They mostly consumed sea food as a diet. Poor sanitation & inadequate water supplies, solid waste pollution were also recorded in the Questionnaire. Samples of women of this area showed higher concentration of Cypermethrin, Malathion and Deltamethrin & Permethrin. Some samples were contaminated by more than one pesticide and showed high quantity of Cypermethrin as well as deltamethrin thus confirming the funding of earlier research reported chlorinated pesticides (HCB & PCBs) in different fish species from Karachi coast [20] and in local lakes [11]. Different levels of PBDEs & chlorinated compound from two UK regions. They reported organochlorine levels in south east samples were consistently higher & significant difference (p< 0.05) [4] the same finding were also noticed in R_1 during the present study. box plot (i), (ii), (iii). DDT & HCH were slightly higher in historic specimens from Melbourne. Heptachlor & Dieldrin levels were comparatively higher in

Sydney, Adelaicle, New south Wales & rural Queensland. They also indicated that even ban & restriction of usage from last two decades somewhat than it is responsible for continuous small exposure in Australia [32]. Same situation is also seen here; historically higher values were reported in Pakistan, but in present case lower quantity of some pesticide were also recorded in R_2. The highest concentration of DDE was calculated and the value of mean ± S.D; average was 311 ± 174; 0.000279 mg/g where as our result was lower than the finding [32].

Korangi, Hazara Colony, Machhar Colony, Shah Faisal Colony and Malir are located in (R_2) of Karachi with lack of basic facilities. Several large industries are located in this area. Houses have poor ventilation, due to lack of windows and electricity. There is a lack of cleanliness. There is insecure tenure, poor waste disposal and open sewage Lines are the nesting ground of pesticides. People of Malir are mostly framers. Presently the sequence of pesticide residues was Deltamethrin > Cypermethrin > Permethrin > Malathion > DDT > DDE in R_1 where as in R_2 it was Permethrin> Cypermethrin > Deltamethrin. Previously, pesticide contamination was reported in the sequence of DDT>PCB>HCH> comp of chlordane ≈ HCH ≈ Tris -4- Chlorophenyl – methane in human milk samples of Vietnam [33].

Maternal milk was studied for the measurement of isomers of HCH resident in two residents from Shenyang & Beijing, China. They also reported that other Chinese cities & world wide data was lower than the current data of Shenyang & Beijing [34] similarly, present study also showed that the Cypermethrin is high in R_1 as compared to other researcher finding of Pakistan in goat milk [18] and in cattle milk [17].

Milk specimens of R_1 mothers have relatively higher mean values of Permethrin, Cypermethrin and deltamethrin ((21.50), (12.58), (9.67) than R2 (5.823), (1.938), (1.315). most probable reason for this contamination is indoor residual spray, food chain, environmental pollution by pesticides. The result of our finding is comparable to those determined [34]. Presently, the mean value of deltamethrin is 9.67 in R_1 and 1.315 in R_2 was higher as comparable to those reported [35] the mean ± S.E of Cypermethrin was reported 0.092 ± 0.002 in goat milk and 0.085 ± 0.02 in cattle milk in Faisalabad [17,18]. Pakistan where as our result shown higher values of Cypermethrin in both groups (R_1 and R_2).

5. Conclusion
The pesticide residue was investigated in 200 human milk samples from two groups of inhabitants of Karachi. Almost 65% samples were contaminated in R1 and 33% SAMPLES IN R2. The main route of contamination is food chain, malarial and dengue control pesticides. So, It was concluded that the routes of exposure to any pesticides in millions of inhabitants living in malaria control areas is Pyrethroids, OPs, OCs, although lives were saved during malaria prevention but the insecticide residues correlation with potential newborn health threats via maternal milk.

6. Acknowledgement

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