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Persistent Organochlorine Pesticide Residues in Milk and Butter in Agra City, India: A Case Study

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Organochlorine pesticides (OCPs) have been used extensively in tropical countries in malaria control programme and against livestock ectoparasites and agricultural pests (Fytianos et al., 1985; Curtis 1994). OCPs are used in India to combat the propagation of vectors transmitting diseases, like malaria. DDT and HCH have been used in the form of dusting powder; emulsion sprays to control ectoparasites in livestock and for preventing agricultural products loss due to pest (Spiric and Raicevic 1994; Hosie et al. 2000; John et al. 2001). Due to their high fat solubility, bioaccumulation and persistence, these pesticides can be found in the environment (Juhler et al. 1999). Milk producing animals such as cow and buffalos accumulate the residues of these insecticides when they eat contaminated grass, hay and inhale contaminated air (Deka et al. 2004; Vasanthi et al. 2003; Nerin et al.1996; Rudel 1997; Harner et al. 1999). Owing to their lipophilic properties, OCPs are initially stored in fat rich tissues and subsequently are translocated and excreted through milk. They get accumulated in fat rich dairy products such as butter and cheese etc, as such, and consumers of milk and dairy products are exposed to these residues (Kanan et al 1992; Bentabol and Jodral 1995; Waliszewaski et al. 1997). These facts have caused a great concern since milk and its products play a major part in human nutrition. Thus consumption of contaminated milk and its products may involve health risk situations.

In fact, milk is recommended as complete food item. A large percentage of the Indian population is vegetarian, and consumes milk and its products. India is an agriculture-based country and among the largest users of these OCPs in the world, but now some of these OCPs have been banned in India but their residues are still being reported in milk and milk products by different workers of the country (Jonh et al. 2001; Saxena and Siddiqui. 1982; Kathpal et al. 1992; Kumar and Nath. 1996; Nigam and Siddiqui; 2001). A study was undertaken to monitor the trace level of OCPs in milk and butter samples from Agra city, India, to assess the consumer risk.

MATERIALS AND METHODS

The selected area for the present study was Agra, located in Uttar Pradesh, India. This city has a great importance due to world heritage monument Taj Mahal.

Milk samples (100 mL) were collected from 65 dairies. Similarly butter samples (100 g) were collected from 46 dairies. All the samples were collected in cleaned plastic bottles and stored at 4^0C. Analysis is done within 24 hours. 25 mL Milk was taken

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for the analysis, 60mL n-hexane: acetone (1:1v/v) added then shaken well and left for two minutes to separate the layers. The supernatant organic solvent was decanted off and collected in a conical flask. The milk colloidal mass was re-extracted twice with 30mL n-hexane each time. All the extracts were combined in a conical flask. For the clean up of the co- extractives, 25mL sulphuric acid (98% purity, density 1.835 g/mL) was added drop wise in the extracts. After then the acid layer was discarded and the extract was subsequently neutralized by washing with distilled water. The neutralized extracts were passed through anhydrous sodium sulfate for dehydration and finally evaporated under rotary vacuum evaporator till dryness. The final volume was made up in 1mL with n-hexane and 2µL aliquot was injected into GC-ECD. All the solvents used in the extraction and cleanup procedure were special analytical grade for pesticide residues (E.Merk India Ltd.). Sodium sulfate used in the study were procured from E.Merk India Ltd.

10 g Sample of butter was thoroughly homogenized with 20 g of activated sodium sulphate. The sample was transferred to a 30 x 1.5 cm i.d chromatographic column. Butterfat was extracted with 100mL acetonitrile: n-hexane (1:1v/v) by passing through the above column. The eluent extract was collected in a conical flask and for the clean up of the co-extractives, procedure was followed as described for milk. The detailed methodology was given by Naik et al (2000) and Pandit et al (2003). To check repeatability and accuracy of the method, a recovery study was performed. Three replicates for each pesticide concentration levels were taken for the study and the fortifications levels were 0.1, 0.5, 1.0 mg/L in milk and butter (mg/Kg) samples, which is presented in Table 1.

Table 1. Recovery of OCPs in milk and butter.

Pesticide	Average percent recovery at different fortification levels							
	Milk (mg/L)				Butter (mg/Kg)			
	0.1	0.5	1.0	Mean	0.1	0.5	1.0	Mean
α-HCH	86.5	87.8	92.4	88.9±3.1	85.5	88.9	91.2	88.5±2.8
β-HCH	90.6	92.3	92.8	91.9±1.1	89.7	91.8	93.8	91.7±2.0
γ-HCH	85.6	90.4	91.4	89.1±3.1	90.2	91.4	93.6	91.7±1.7
pp'DDT	91.2	89.3	90.5	90.3±0.9	90.8	93.5	95.5	93.2±2.3
op'DDT	90.2	90.1	91.2	90.5±0.6	89.0	92.3	94.3	91.8±2.6
pp'DDE	87.9	90.5	91.5	89.9±1.8	90.5	91.6	92.1	91.4±0.8

All the pesticide standards were obtained from Riedel – de Haen (Hannover Germany). The Limit of detection (LOD) for OCPs was 0.002 mg/Kg and Limit of quantification (LOQ) was 0.005 mg/Kg.Gas chromatographic analysis was performed using a Chemito Model (8510) with Ni^{63} ECD. Chromatographic determination of OCPs was carried out in a 50m x 0.2mm x 0.33µm fused silica capillary Column Ultra-2 (5%-phenyl and 95% dimethyl- polysiloxane). The operating conditions were as follows. Injection port temperature 260^{0}C; Column temperature 180^{0}C initial with 1 min hold; 10^{0}C/min to 280^{0}C and detector temperature was 290^{0}C. The carrier gas was IOLAR I grade nitrogen (99.9%). The flow rate of carrier gas was 1 mL/min.

RESULTS AND DISCUSSION

Fortified milk and butter samples were analysed in triplicate and average results are

reported to check the reproducibility and accuracy of method in Table 1. The results of analysed milk and butter samples are presented in Table 2.

Table 2. Range and mean concentration of OCPs residues in milk and butter.

Organochlorine pesticide	Amount of residue in milk (mg/L) and in butter (mg/Kg)	
	Milk n = 65	Butter n = 46
α-HCH	R: (0.016 - 0.020) X= 0.018 (± 0.011)*	R: (0.020 - 0.029) X= 0.024 (± 0.010)*
β–HCH	R: (0.032 - 0.073) X= 0.048 (± 0.013)*	R: (0.044 - 0.078) X= 0.052 (± 0.011)*
γ–HCH	R: (0.038 - 0.089) X= 0.048 (± 0.013)*	R: (0.046 - 0.061) X= 0.056 (± 0.012)*
Total HCH R (Residues)	X= 0.114 (±0.002)*	X= 0.132 (±0.0002)*
pp'DDT	R: (0.021 - 0.069) X= 0.040 (± 0.0123)*	R: (0.023 - 0.070) X= 0.043 (± 0.013)*
op'DDT	R: (0.005 - 0.011) X= (0.010) (± 0.012)*	R: (0.006 - 0.025) X= 0.02 (± 0.013)*
pp'DDE	R:(0.036 - 0.083) X= 0.055 (± 0.0130)*	R: (0.040 - 0.078) X= 0.057 (± 0.012)*
Total DDT R (Residues)	X= 0.105 (± 0.001)*	X= 0.120 (± 0.002)*

R: Range, n = Number of samples, X= Mean value for pesticide, * figures in parenthesis give SD values

The recovery study shows that mean average percentage recovery were 88.9±3.1 to 91.9±1.15 and 88.5±2.8 to 93.2±2.3 in milk and butter respectively. The standard deviation was less than 9 indicating the reproducibility and accuracy of the method. The organochlorine pesticide residue levels are expressed on the fat level basis. HCH isomers (α,β and γ) were detected in all the samples of milk and butter analysed. In the milk sample γ and β isomers of HCH contribute the same mean range, which is 0.048 mg/L while α isomer contribute less. In the butter sample the mean value of α, β and γ HCH were 0.024, 0.052 and 0.056 mg/Kg, respectively that shows the butter to be slightly more contaminated with HCH isomer than milk. The total HCH mean value in butter was 0.132 mg/Kg and total HCH mean value in milk was 0.114mg/L, which is less than butter. The maximum residue limit (MRL) values for β and γ isomers are 0.02 and 0.01mg/Kg, respectively as prescribed by FAO (1993). The HCH isomers level present in this study is higher than their MRL values prescribed by FAO. Among DDT's isomers pp'DDE was the major constituent with mean value of (0.055 mg/Kg) followed by pp'DDT (0.040 mg/Kg) and op' DDT (0.01 mg/Kg) in the milk. The tolerance limit of DDT in milk is 1.25 mg/Kg prescribed by FAO/WHO (1993). The total mean value of DDT in the milk is 0.105 ± 0.0187 mg/Kg, which is lower than the maximum residue limit mentioned by FAO/WHO. In the butter sample

pp'-DDE was the major constituent with mean range 0.057 mg/Kg ± 0.012 followed by pp'DDT (0.043±0.013) and op'DDT (0.02±0.013 mg/Kg). The total mean value of DDT in butter is 0.120 mg/Kg ± 0.0115, which is higher than the total mean value of the milk. In milk and butter,γ-HCH is the major constituent among HCH isomers. Butter is contaminated with DDT and HCH more than milk; this may be due to the high percentage of fat in butter.

This study shows the presence of the OCP residues in milk and butter. As presence of DDT and HCH in cattle feed in India has already been reported (Mukerjee and Gopal, 1995; Battu et al, 1996), these cattle feed are therefore, the main source of contamination. Butter is the product of milk so OCPs were transferred from contaminated milk to butter during its preparation. Total DDT concentrations were higher than total concentration of HCH in milk and dairy products. This present monitoring study reveals that organochlorine pesticide being persistent couldn't be eliminated from commodity by simply avoiding their use, as many years are required for them to disappear from the environment.

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