INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com ISSN : 0976-4550

Volume-6, Issue-3, July-Sept-2015 Coden IJABFP-CAS-USA Copyrights@2015

Rceived: 20th Jan-2015 *Revised: 4th June-2015* *Accepted: 8th June-2015*

Research article

GC-MS DETERMINATION OF ORGANOCHLORINE PESTICIDES IN BOVINE MILK FROM TIRUCHIRAPPALLI, SOUTH INDIA- A PRELIMINARY ASSESSMENT

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ABSTRACT: The study was aimed to determine the concentrations of organochlorine pesticides (OCPs) in bovine milk (both branded and unbranded) collected from in and around Tiruchirappalli city, Southern India. The results of the present investigation indicated that these milk specimens were found to be contaminated with 7 different pesticides. Mirex (higher incidence), Heptachlor, o,p'-DDD, o,p'- DDE, Aldrin, cis and trans-chlordane were detected in the milk samples. The residues were quantitatively determined using Gas Chromatograph-Mass Spectrometer-Quadrupole on electron ionization (EI) mode. The study showed that unbranded milk samples contained higher concentrations of residues than branded. The derived average daily intake of aldrin and DDT has exceeded WHO's Acceptable Daily Intake.

Key Words: Bovine milk, OCPs, Mirex, Aldrin, GC-MS, India

INTRODUCTION

Contamination due to hazardous compounds has become an unexceptional incident. The widespread occurrence of hazardous chemical such as organochlorine pesticides (OCPs), polyaromatic hydrocarbons (PAHs), dioxins, furans and heavy metals in the environment is a matter of public health concern. In general, organochlorines are highly stable in nature, persist for long time and tend to accumulate in the environment, because of low solubility in water and high stability in sunlight. Likely, DDT and endosulfan has low solubility in water, high lipid solubility and stability in fatty tissue. Milk is an important source of nutrition for the infant, young and elderly. Milk is also rated very highly amongst vegetarians (over 50% of the 800 million population) and is often taken during illness and convalescence (Gebremichael *et al.,* 2013). The consumers get exposed by these fat rich dairy products to significant levels of contamination (Bentabol and Jodral 1995). Therefore, milk can be used as an evaluation index of environment contamination by these insecticides. The contaminated feed and grass/hay, and inhaled air are sources/modes of insecticides residues by milk producing animals (Kumar *et al.,* 2005). OCPs are primarily stored in fat-rich tissues because of lipophilic nature and subsequently translocated and excreted through milk fat. Investigation on the level of organochlorine pesticides in milk and dairy products has been reported in several parts of India. But studies on pesticides in milk in the southern part of India, especially in Tamil Nadu is rare. So, the present study aims at acquiring a preliminary/baseline data on the dietary exposure of organochlorine pesticide through the intake of bovine milk and to assess safety for human consumption.

MATERIALS AND METHODS

Samples

The milk samples (branded as well as unbranded) were collected randomly from Tiruchirappalli city (urban site with a population of about 1 million) and nearby villages (Suriyur, Mandaiyur, Kattur, Gundur) in Tamil Nadu state, India. Out of the 24 samples, 12 samples of branded milk were bought from commercial outlets and unbranded (12) milk samples were obtained directly from the farmers and milk vendors. All samples were collected in a pre-cleaned, oven dried and n-hexane rinsed glass stoppered bottles. The samples were stored in a refrigerator at 4º C until extraction.

Extraction and cleanup

The methodology of Kumar *et al.* (2005) was followed. A Known volume of milk (25 ml) was taken in 250 ml separatory funnel and 60 ml n-hexane: acetone (1:1 v/v) was added, and then shaken well for 15 minutes and left for layers separation. The supernatant organic layer was collected in a conical flask. The milk colloidal mass was re-extracted twice with 30 ml n-hexane each time. All the extracts were combined in a conical flask. For the clean up of the extract, 25 ml sulphuric acid (98% purity, density 1.835 g/ml) was added drop wise into the extract in a separating funnel. Then the lower acid layer was discarded and the extract was subsequently washed with hexane-washed water. Then the extract was passed through anhydrous sodium sulfate for dehydration and evaporated using rotary evaporator (Buchi, Switzerland) and subsequently purged with N_2 gas until 1ml, and final extract was stored at 4º C or less temperature in an amber glass vial until GC-MS analysis.

Instrumentation & QA/QC

The samples were analysed in GCMS (Gas Chromatograph- Mass Spectrophotometer) (QP 2010 Shimadzu Corp, Japan) equipped with capillary column DB-1 (30m long, ID 0.32mm) and 5% methyl phenyl silicone. The limit of detection (LOD) of OCPs was determined three times of the standard deviation of the blank. Before analysis, standards were run to check for the column performance, peak height and resolution. From stock solution of organochlorine pesticides standard containing cocktail of 17 pesticides hexachlorocyclohexane (α, β & γ- HCH), Cyclodiene (aldrin, dieldrin and endrin), heptachlor, hexachlorobenzene (HCB), trans-Chlordane, cis-Chlordane, mirex and diphenyl aliphatic (p,p'-DDE, o,p'-DDE, o,p'-DDD, p,p'-DDD, o,p'-DDT, p,p'-DDT), 200 ppb (200 ng/ml) of working standard was prepared. For the standard calibration, eight different concentrations from 5ng/ml to 200ng/ml were prepared. All standards show a linear range from 5 ppb to 200 ppb. The coefficient (R2) values ranged from 0.9746 to 0.9985 for 8 concentrations levels. The limit of detection (LOD) as 3S varied from 0.69 to18.23 ng/ml (3S) for OCPs.

Average Daily Intake (ADI) of OCPs

The ADI is the estimate of dietary intake of substance or chemicals introduced into food inadvertently through contamination resulting from processing. The exposure factor (EF) equals 1 representing a daily exposure to the contaminant.

$$\text{ADI } (\mu g/kg) = \frac{\text{Contaminant Concentration} \times \text{Intake rate of milk} \times \text{Exposure Factor}}{\text{Body Weight}}$$

RESULTS AND DISCUSSION

The mean, range and incidence percentage of organochlorine pesticides residues in branded and unbranded milk (collected from urban and rural areas) were shown in Table 1. The analysis revealed the predominance of seven compounds Viz., Mirex, Aldrin, o,p'- DDE, o,p'- DDD, trans-Chlordane, cis-Chlordane and Heptachlor. Mirex was detected in all the samples with mean concentrations in both branded and unbranded milk samples as 15.92 and 37.08 µg/L; the incidence percentage is found to be 100 and 92%, respectively (Table 1). The presence of mirex in milk and milk products is not reported elsewhere in India so far and this is the first report of mirex in the milk samples. Mirex was not utilized for agricultural purposes, but used against wasps, bugs and termites in cattle sheds and also to eradicate ants (Tanabe and Subramanium 2006). The possible source for mirex may be the contaminated cattle feed and/or the animals might have accumulated through inhaled air. The average daily intake of mirex in branded and unbranded for adult and child is quantified as 0.07 & 0.48 and 0.16 & 1.112 µg/Kg, respectively (Table 2&3) based on per day consumption of milk by Indian adult and child with an average weight of 60 and 10 kg is 250 and 300g, respectively (Gopalan and Rao 1980).

The concentration of aldrin was found to be highest next to mirex. The branded milk samples had less concentration (9.689 µg/L) than unbranded milk samples (25.72 µg/L). The mean concentration of aldrin observed in the branded milk samples in this study is elevated than the concentration observed in pasteurized milk (2 µg/L) reported in Spain (Martinez *et al.,* 1997). The mean concentration of aldrin in unbranded milk samples (25.72 µg/L) is 7 times greater than the raw bovine milk samples (3.6 µg/L) reported from Haryana, India (Sharma *et al.,* 2007). A study conducted on OCP residues by John *et al.* (2001) illustrated the mean concentration of aldrin (457 µg/L) in toned milk at different seasons during 1993 - 1996 from Jaipur city, India which was exceptionally higher than the present study. Malhat and Haggag (2012) also reported presence of aldrin in the cow milk samples from Egypt with concentration varying from >0.01 to 3.80 µg/L, with a mean concentration value of 0.382±0.749 µg/L. The elevated concentration in unbranded milk attributed to higher incidence of aldrin in that particular location.

The practical limit of aldrin in milk is 150 µg/L (WHO 1973). In the present investigation lower levels of aldrin observed in both branded and unbranded samples than practical limit of WHO. For a child with an average weight of 10 kg which consumes 300g of milk per day, the average daily intake is calculated to be 0.3 & 0.7 µg/Kg in branded and unbranded which exceeds the maximum acceptable daily intake (ADI) of aldrin which is 0.1 µg/Kg (WHO 1973), whereas the average daily intake for adult (0.07 µg/Kg) is below the ADI (Table 2&3).

The incidence of o,p'- DDE in branded and unbranded milk was observed to be 50 and 75%, respectively. In the present investigation only metabolites of DDT, DDE and DDD were detected which shows that there is no recent input/source of DDT to bovine or DDT would have been degraded into its metabolites by the animals. The mean o,p'- DDE in unbranded milk samples was observed to be 3.826 µg/L (Table 1) which was slightly higher or comparable with the value obtained by Sharma *et al.* (2007) with the mean value of 1.5 µg/L in raw bovine milk from Haryana, India. The sum of o,p'- DDE and DDD in branded and unbranded milk are 0.868 and 3.826 µg/L, respectively. The total DDT (DDE + DDD) observed in branded milk sample was higher than reported by Martinez *et al.* (1997) in the pasteurized milk sample in Spain which was 0.07 µg/L. John *et al.* (2001) reported a mean DDT concentration 595 µg/L in buffalo milk collected during different seasons from Jaipur city which was many fold higher than the present observation. The concentration of total DDT in cow's milk from Uttar Pradesh was reported to be 116 µg/L (Maurya *et al.* 2013). DDT has exceeded maximum acceptable daily intake which is 0.005 mg/kg, respectively (WHO 1973) for child in branded milk sample (0.023 µg/Kg) and for both adult and child in unbranded milk sample i.e., 0.16 and 0.115 µg/Kg respectively.

Table 1: OCPs (µg/L) in Branded and Unbranded milk samples from Tiruchirappalli, South India

Pesticide	Branded (n = 12)			Unbranded (n = 12)		
	Mean	Range	(%)	Mean	Range	(%)
Heptachlor	0.048	BDL* - 0.252	25.0	0.339	BDL - 3.51	16.0
Aldrin	9.689	BDL - 68.4	50.0	25.72	BDL - 234.0	67.0
Trans-Chlordane	0.663	BDL - 4.85	50.0	0.367	BDL - 2.94	42.0
cis-Chlordane	0.167	BDL - 1.13	25.0	0.546	BDL - 1.54	67.0
o, p'- DDE	0.093	BDL - 0.9	50.0	3.826	BDL - 45.54	75.0
o,p'- DDD	0.775	BDL - 9.3	8.33	BDL	BDL	0
Mirex	15.92	BDL - 31.83	100	37.08	BDL - 154.4	92.0

* - Below Detection Limit

Table 2: Average daily intake of OCPs (µg/Kg body weight) through bovine milk

Pesticides	**Mean level of OCPs in Branded Samples**	**Estimated Average Daily Intake* (µg/Kg body weight)**	
		Adult	**Child**
Heptachlor	0.048	0	0.001
Aldrin	9.689	0.040	0.3
trans-Chlordane	0.663	0.003	0.020
cis-Chlordane	0.167	0.001	0.005
o,p'- DDE	0.093	0	0.003
o,p'- DDD	0.775	0.003	0.023
Mirex	15.92	0.07	0.48

The mean concentration of heptachlor in raw milk (0.339 µg/L) was higher than branded milk (0.048 µg/L). Heptachlor in branded samples in this study was less than the level reported in the pasteurized milk from Spain (11 µg/kg) reported by Martinez *et al.* (1997). The concentration of Heptachlor was lower than the practical limit in milk and milk products prescribed by WHO i.e., 150 µg/Kg (WHO 1973). The calculated average daily intake for both adult and child in branded and unbranded milk samples (Tabel 2&3) were lower than the acceptable daily intake (0.5 µg/Kg) (WHO 1973).

Table 3: Average daily intake of OCPs (µg/Kg body weight) through bovine milk

Pesticides	Mean level of OCPs in Unbranded Samples	Estimated Average Daily Intake* (µg/Kg body weight)	
		Adult	Child
Heptachlor	0.048	0.001	0.01
Aldrin	9.689	0.107	0.772
trans-Chlordane	0.663	0.002	0.011
cis-Chlordane	0.167	0.002	0.016
o,p'- DDE	0.093	0.016	0.115
Mirex	15.92	0.16	1.112

Branded milk contain transchlordane and cischlordane with mean values of 0.663 and 0.167 µg/L, respectively and unbranded samples contained 0.367 and 0.546 µg/L, respectively (Table 1). The concentration of Chlordane was found to be 101 µg/Kg in the pasteurized milk from Spain (Martinez 1997) but the concentration in this investigation was lower than values reported by them. The calculated ADI of chlordane (cis and trans) for adult as well as child (Table 2&3) was lower than the acceptable daily intake of 1 µg/Kg proposed by WHO (1973) in both branded and unbranded milk samples. Chlordane is mainly used as an insecticide on agricultural crops and for the control of termites (Tanabe and Subramanian 2006). The practical residue limit of chlordane proposed by WHO (1973) is 50 µg/Kg.

CONCLUSION

The study has unambiguously shown the contamination of bovine milk samples with OCP residues. Generally, during milk processing the fat is removed and vegetable fat is substituted, and besides that the milk is also processed at higher temperature (Pasteurization) to decontaminate the milk, these may be the reasons for lower levels observed in branded than in unbranded milk which are directly collected from farmers and sold to the public without any processing. The health department authorities should take steps to ensure the quality of unbranded milk before it is getting supplied to the public.

ACKNOWLEDGEMENT

Authors are thankful to United Nations University, Japan and Shimadzu Corporation, Japan for the GC-MS facility sponsored through the project "Environmental Governance and Monitoring of POPs in the Asian Coastal Hydrosphere". Also thank the university authorities for the facilities provided to carry out this research.

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ISSN : 0976-4550

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