ISSN 2250-3153

Trends in ambient loads of DDT and HCH residues in animal's and mother's milk of PaliaKalanKheeri, Uttar Pradesh-India

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Abstract- Monitoring of DDT and HCH residues in the environment of PaliaKalan during 2009 to 2011 revealed low to moderate levels of these insecticides in cow, buffalo, goat& breast milk samples. The levels of the DDT and HCH residues in animal & human milk collected from rural areas having intensive sugarcane, wheat & paddy cultivation in PaliaKalan were analysedall samples contained detectable quantities of DDT, HCH and its metabolites. Total HCH residues were high than those of DDT in all the samples.The total concentration of DDT & HCH was found lower than the previous studies carried out in India.These results indicate that the overall organochlorine-residue levels in PaliaKalan are slightly degrading. The residues were quantitatively analyzed on Gas Chromatograph with ECD &confirmed by Gas Chromatograph-Mass Spectrometer-Quadrupole on electron ionization (EI) mode.The data from this study is added to the scientific data and knowledge on OCPs levels in milk which was not' available forPaliaKalanKheeri Region, India.

Index Terms- DDT, HCH, Milk, Gas chromatography, Mass spectrometry

I. Introduction

India, having agriculture-based economy, is one of largest insecticide consumers in the world. Moreover two-thirds of the pesticides consumed are Class I and II pesticides (WHO) which are highly toxic. No wonder a number of studies from India have reported widespread contamination of various milk, food and water sources to be contaminated with these pesticides. In India, largest pesticide consumption has been in the state of Uttar Pradesh, according to the data of 1995-1996 and 1999-2000, produced by Central Insecticide Board and Registration Committee, India (Srivastava, S et al.2008). Introduced in the 1940s, organochlorine pesticides (OCPs) were widely used in agriculture and pest control until research and public concern regarding the hazards of their use led to government restrictions and bans. Despite restrictions and bans on the use of many organochlorine pesticides in the 1970s and 1980s, they continue to persist in the environment today. OCPs, such as DDT and HCH, have stable chemical properties and less biodegradability. HCH and DDT exhibit broad-spectrum toxicity and residual activities. DDT and HCH are banned in India for agriculture but are still used for controls of vectors in public health.HCH was banned for use in agriculture in 1998 and Lindane was recommended in its place (Mukherjee and Gopal, 2003). These toxicants enter the human body through the food chain and cause serious health problems (John et al.,2001). The presence of OCPs residues in food commodities (Mukherjee and Gopal, 1996), water (Agnihotri et al., 1993), Mother's milk (Nair et al., 1996; Okonkwa et al., 1999; Anoop et al., 2006)., dairy milk (John et al., 2001; Mukherjee and Gopal, 1995; Nicholas., 2011), Human blood (Waliszewski et al., 2000) and in skin (Due et al., 1998) have been reported in earlier studies. Epidemiological studies provide evidence that exposures to organochlorine pesticide can produce adverse health effects (Kalpana, 1999;Jiawei et al., 2008).

Milk is a 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. Milk can be used as an evaluation index of environment contamination by these insecticides. This alarmingly high daily intake value is a cause for concern, since children are highly susceptible to effects from such environmental contaminants. The observed contamination of mother's milk and the possible transfer of the contaminant from mother to child is an obvious risk associated with breast-feeding (Gebremichael S et al., 2013). The main objective of this analysis is to monitor the levels of OCPs in animals and human milk. It is important to establish the level of OCPs in milk especially in rural area where animal & human are more exposed to these insecticides as they eat agricultural product from same area. The presence of organochlorine pesticide residues in agricultural fields in rural parts of India indicates regular use of there compounds (Mohapatra et al., 1995). OCPs residues are highly persistent and are reported to be sold to unsuspecting consumers, they are still needed to be monitored. There is no report on organochlorine pesticide residues in animal and human milk from PaliaKalanKheeri region, we undertaken, a systematic study of monitoring the OCPs residue level in animal & human milk sample collected randomly from rural parts of PaliaKalanKheeri, region.

II. MATERIAL AND METHODS

A. Site

PaliaKalan (28° 27' 0" North, 80° 35' 0" East) is a small peaceful city of Lakhimpur Kheeri district in the Indianstate of Uttar Pradesh-India. Dudhwa National Park, home to Rhinos, Tigers, with countless animal species, birds, insects, reptiles is adjacent to the town. The main occupation is farming (cane, wheat, paddy etc.) and also houses the Bajaj Industries Private Limited Sugar production plant, distillery and 1 ecofriendly plywood production unit. The sugar production plant is second largest sugar production unit in Asia and the plywood production plant is only 2nd plant in the world whichproduces plywood with beggasse.

B. Sampling

The healthy women samples were collected from donors between age groups of 25 – 35 years and all had normal child deliveries. Normal healthy Cow, Buffalo & Goat were choosen for collection of samples.50 mL of women milk & 200 mL animal milk were collected in glass bottles by manual suction pump. Milk samples were refrigerated at 4 ^{o}C until analyzed.

C. Extraction of the sample

The milk samples were extracted following procedure of Environmental Protection Agency Protocol (EPA1985) and (Ejobi et al., 1996) after some modification. The procedure involved denaturation, solvent extraction, centrifugation, extraction of organic layer and concentration.Briefly, 5 mL milk samples were mixed with anhydrous sodium sulphate to make a free flowing powder and then transferred into a glass extraction column (150x 20 mm). The dry column was eluted with 100 mL of n-hexane with the first 50 mL allowed to stay in contact with the powder for 10-15 min.The elute was collected in pre-weighed round bottom flask. n-Hexane was evaporated off under a rotary vacuum evaporator. The round bottom flask was then weighed again. The difference in weight is the weight of raw fat extracted. The extracted raw fat was cleaned up in a glass column packed with florosil using eluting solvent of n-hexane &dichloromethane in the ratio of 3:1. The flow rate of elution did not exceed more than 3 mL/min. Then the eluting solvent was passed through anhydrous sodium sulfate and concentrated under rotary vacuum evaporator. Final volume was made up to 1 mL n-hexane and injected 1 μL in gas liquid chromatograph equipped with an electron capture detector (Ni^{63}).

III. INSTRUMENT

A. GC instrument

The residues were quantitatively analyzed on Gas Chromatograph-Shimadzu 2010 (Shimadzu, Kyoto, Japan) equipped with split/splitless auto-injector model AOC-20i. The non-polar stationary phase used was a fused silica capillary column DB-5 (5 % phenyl polysiloxane) of 30 m, 0.25 mm i.d and 0.25 µm film thickness (J&W Agilent Palo Alto, CA, USA). GC Solution software was used for instrument control and data analysis.

B. GC/MS instrument

The residues were further confirmed on Gas Chromatograph-Mass Spectrometer-Quadrupole on electron ionization (EI) mode (Shimadzu 2010, Kyoto, Japan) equipped with with split/splitless auto-injector model AOC-20i. The non-polar stationary phase used was a fused silica capillary column DB-1 (1 % phenyl polysiloxane) of 30 m, 0.25 mm i.d., and 0.25 µm film thickness purchased from J&W Agilent Palo Alto, CA, USA. GCMS Solution software was used for instrument control and data analysis.

IV. ANALYTE RECOVERY AND QUALITY CONTROL

Milk samples (5 mL) were spiked with the Organochlorine insecticides α-HCH, β-HCH, γ-HCH,δ-HCH,op' DDT,pp'DDD and pp' DDE at 0.02, 0.05, 0.1 µg L^{-1} levels. The recovery experiment was performed at the three concentrations and each concentration was analyzed in triplicate. The recovery percentage and standard deviation of organochlorine pesticide are summarized table 3, ranging from 86.2 ± 1.30 to 96.0 ± 1.25 across the three concentrations. The use of milk for recovery studied has earlier has reported by Kanja et al (1983);Ip and Phillips (1986); and Schinas et al. (2000); and Anoop et al. 2006. The limits of detection (LOD) and limit of quantification (LOQ) for OCPs was 0.001 µg/mL and 0.01 µg/mL, respectively. All the solvents & chemical used in the extraction and clean up procedure were special analytical grade for pesticide residues (E.Merk India Ltd.). Pesticide standards were obtained Sigma-Aldrich/ Riedel-de-Haen (Zwijndrecht, The Netherlands).

V. RESULTS AND DISCUSSION

Total Concentrations of DDTs and HCHs in human breast milk were high than animal milk. This indicates that the residents living in this area have been exposed to relatively high levels of DDTs and HCHs through animal milk, meat, water and agricultural products.o,p'-DDT,p,p'DDD and p,p'-DDD were analyzed and detected 90% of human's milk, 80% of buffalo's& cow's milk and 70 % of goat's milk. α-HCH, β-HCH, γ-HCH and δ-HCH were also analyzed and found 90% of human milk, 80% of buffalo, cow and goat milk. The concentration of DDT and HCH are presented inTable 1 and 2. Dominant pesticide in all samples examined wasHCH in mother's, buffalo's, cow's, and goat's milk in the concentrations of 159, 121, 149 and 108 ng/ml, respectively. Total DDT levels were found to be 158 ng/ml in mother's milk, 116ng/ml in cow's& 92 ng/ml buffalo's &88 ng/ml in goat's milk. These residues show that DDT & HCH used for pest control & agricultural purpose accumulates in human and animal body through the food chain and environment and is excreted through milk. Organochlorine pesticides (OCPs) with their high persistence in the environment accumulate in fatty foods and human adipose tissues. Contamination of human milk by organochlorine and other related compounds has been reported throughout the world (GEMS, 1998). During the recent decade,investigations on persistant pollutant (POPs) pollution in the Asian regionsand found that relatively high residue levels of DDTsand HCHs exist in food stuffs (Kannan et al., 1997),

ISSN 2250-3153

mussels (Monirith et al., 2003) and avian species (Kunisue et al., 2003) from some developing countries and these contaminants are possibly in use for public health purposes even now. Among Asian developing countries, concentrations of DDTs in human breast milk from Vietnam, mainland China, Cambodia, and Malaysia were relatively higher than those from other countries (Kunisue et al., 2004). Human milk, at the top of the food chain represents the major route of elimination of OCPs by lactating women (Rogan et al., 1986; Sim and Neil,1992; IARC, 1991) concluded that there is insufficient evidence in humans but sufficient evidence in experimental animals to classify DDT as a possible carcinogenic to humans. However, body loads of DDT also raise concerns about potential effects on developing infants and children because DDT transfers across the placenta from mother to fetus and exposure continues through breastfeeding after birth (Shen et al.,2007).It is well known that they are very dangerous if ingested as an overdose but there is also biological evidence that chronic low-grade exposure to these chemicals, which are very easily absorbed into the body through the skin and lungs, may have adverse effects on mental health (Zhang et al., 2009). The results obtained from other monitoring studies of organochlorine pesticide in human milk in india and abroad are compared with the results obtained from the present monitoring study in Table 4. Total HCH and total DDT levels in the present study are less than those reported in india. This indicates gradual phasing out of these compounds in India and has resulted in the reduction of their residues in mother's milk.

Table 1
Residues of DDT contaminants obtained in animal & mother's milk

Milk's	No of	Residues in ppm			
type	samples	op'DDT	pp' DDE	PP'DDD	Total
Woman	10	R: (0.051-0.061)	R: (0.043-0.056)	R: (0.048-0.054)	∑=0.158(±0.001)*
		M± 0.056 (±0.007)*	M ±0.050 (±0.009)*	M ±0.052 (±0.008)*	
Cow	10	R: (0.034-0.042)	R: (0.036-0.048)	R: (0.029-0.043)	∑=0.116(±0.002)*
		M± 0.038 (±0.006)*	M ±0.042 (±0.008)*	M ±0.035 (±0.010)*	
Buffalo	10	R: (0.025-0.038)	R: (0.023-0.035)	R: (0.026-0.037)	∑=0.092(±0.001)*
		M ±0.032 (±0.009)*	M ±0.035 (±0.009)*	M ±0.032 (±0.008)*	
Goat	10	R: (0.023-0.031)	R: (0.025-0.035)	R: (0.02-0.031)	∑=0.083(±0.030)*
		M ±0.027 (±0.006)*	M ±0.029 (±0.007)*	M ±0.026 (±0.008)*	

R: range; M= mean; * figures in parenthese gives SD values.

Table 2
Residues HCH contaminants obtained in animal & mother's milk

Milk's	No of	Residues in ppm				
type	samples	α-HCH	β-HCH	γ-HCH	δ-HCH	Total
Woman	10	R: (0.021-0.039)	R: (0.025-0.051)	R: (0.029-0.045)	R: (0.019-0.029)	∑=0.159(±0.007)*
		M± 0.030 (±0.013)*	M ±0.038 (±0.018)*	M ±0.037 (±0.011)*	M ±0.024 (±0.007)*	
Cow	10	R: (0.037-0.058)	R: (0.039-0.063)	R: (0.032-0.068)	R: (0.015-0.025)	∑=0.121(±0.004)*
		M± 0.048 (±0.015)*	M ±0.051 (±0.017)*	M ±0.05 (±0.025)*	M ±0.02 (±0.007)*	
Buffalo	10	R: (0.035-0.045)	R: (0.029-0.038)	R: (0.036-0.058)	R: (0.012-0.022)	∑=0.149(±0.008)*
		M ±0.040 (±0.007)*	M ±0.034 (±0.006)*	M ±0.047 (±0.016)*	M ±0.017 (±0.007)*	
Goat	10	R: (0.032-0.057)	R: (0.023-0.045)	R: (0.021-0.037)	R: (0.012-0.021)	∑=0.108(±0.012)*
		M ±0.045 (±0.018)*	M ±0.034 (±0.016)*	M ±0.029 (±0.011)*	M ±0.017 (±0.006)*	

R: range; M= mean; * figures in parenthese gives SD values.

Table 3
The effect of pesticide concentration (ppm) on recovery from spiked buffalo milk

Pesticide	**Spiking Level (mg/Kg)**	**Recovery (%)**			**Mean recovery % (± SD)**	**Average Recovery % (± SD)**
		R_1	**R_2**	**R_3**		
α-HCH	0.05	87.2	84.0	83.5	84.88 ± 2.00	86.2 ± 1.30
	0.02	90.9	83.8	82.8	85.75 ± 4.44	
	0.10	85.4	88.5	88.5	87.45 ± 1.78	
β-HCH	0.05	89.0	85.6	89.6	88.04 ± 2.15	87.6 ±1.49
	0.02	85.7	87.9	83.0	85.50 ± 2.45	
	0.10	89.8	93.2	85.5	89.44 ± 3.85	
δ-HCH	0.05	85.8	87.4	86.9	86.69 ± 0.81	88.7 ±1.49
	0.02	95.5	92.4	96.0	94.61± 1.95	
	0.10	82.9	86.6	85.8	85.08 ± 1.94	
γ-HCH	0.05	98.9	92.5	97.9	96.395 ± 3.44	93.6 ± 5.10
	0.02	92.8	95.3	93.7	93.92 ± 1.26	
	0.10	90.0	91.1	90.5	90.53 ± 0.55	
o,p′-DDT	0.05	95.3	89.7	91.9	92.27 ± 2.82	96.4 ±3.87
	0.02	94.7	99.0	98.3	97.31 ± 2.30	
	0.10	100.9	98.9	99.9	99.89 ± 1.0	
p,p′-DDT	0.05	98.8	99.1	95.2	97.68 ± 2.17	92.0 ± 4.93
	0.02	89.9	87.6	89.7	89.06 ± 1.27	
	0.10	88.7	85.2	93.9	89.19 ± 4.37	
p,p′- DDE	0.05	98.0	96.6	97.3	97.29 ± 0.0	96.0 ± 1.25
	0.02	96.4	92.5	95.5	94.78 ± 2.04	
	0.10	96.9	96.0	94.9	95.92 ± 1.00	

R_1, R_2 & R_3 are the replicates

Table 4
Concentration (ng/g) of organochlorine pesticide residues in human milk in various parts of world

Contries	α-HCH	β-HCH	γ-HCH	δ-HCH	DDE	DDD	DDT	DDT	References
Spain	34.2	235	10.5	279.7	604.1	-	12.5	659.8	Hernandez et al., 1993
Poland	17.5	92.5	15	125	610	12.5	47.5	670	Czaja et al.,1997
Turkey	60	380	17	457	2013	-	100	2357	Coke et al., 1997
France	52	287	37	376	2183	-	79	2262	Bordet et al., 1993
Delhi	1125	495	2100	175	1680	5250	4000	26050	Nair et al., 1996
Mumbai	14.82	259.5	17.5	289.75	232	35	288.5	510.5	Sharma et al.,2001
Agra*									
Bakhoti	32	40	51	123	56	65	58	179	
Chiraigaon	34	43	51	128	56	60	54	170	Anoop et al., 2006
Ghodhakhas	37	40	54	131	56	63	55	174	
Minahas	36	39	52	127	56	66	57	179	
PaliaKalan	30	38	37	24	56	50	54	169	Present

*Agra:Bakhoti, Chiraigaon, Ghodhakhas&Minahas

VI. CONCLUSION

We have reviewed the available data/information of organochlorine pesticides contamination in animal & breast milk of PaliaKalanKheeri, U.P-India. The result demostratesthat considerable amount of DDT & HCH residues are transferred from the animal & mother to infant . Milk can be considered as a suitable indicator for monitoring the burden of persistant lipophilic chlorinated insecticides in the environment & human body. In view of our observations suggest that further investigation on animal and human exposure in organochlorinepesticides are needed to elucidate future pollution trends and to assess specially infant health risk.

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