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RESEARCH ARTICLE

OCCURRENCE OF ORGANOCHLORINE PESTICIDE RESIDUES IN BUFFALO MILK OF DHANBAD CITY, JHARKHAND, INDIA

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ABSTRACT

Despite the fact that the consumption of pesticides in India is still very low, about 0.5 kg/ha of pesticides, there has been a widespread contamination of food commodities with pesticide residues, basically due to non-judicious use of pesticides. The widespread application of pesticides in agriculture, industry and infrastructure, public health can result in the accumulation of pesticides in the environment. Therefore, a survey was conducted to analyze the levels of organochlorine pesticide residues in buffalo milk from different locations of Dhanbad city, Jharkhand, India. Milk samples were collected seasonally, and pesticide residues were assessed using a gas chromatograph (GC) with an electron capture detector (ECD). The results indicate that the milk samples were contaminated with aldrin, isomers of hexalchlorocyclohexane (HCH; alpha, beta, and gamma), dichlorodiphenyltrichloroethane (DDT) and its metabolites (DDE and p, p'-dichlorodiphenyldichloroethane [DDD]), and isomers of endosulfan (alpha, beta and endosulfan sulfate). Seasonal variations of these pesticide residue levels were also observed in all the milk samples. Samples collected during winter season were found to contain higher residue levels as compared to other seasons.

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INTRODUCTION

Milk claims a unique place in human diet worldwide. With all its valuable constituents like fat, protein (casein and lactalbumin) and lactose, in a balanced proportion, milk has special importance on nutritional standpoint at different stages of human life (infants, adults, old age). The contamination of milk by pesticides is considered as one of the main dangerous aspects in the last few years. Dhanbad is the most populous city of Jharkhand having a total population of 1,195,298 (Census, India 2011). Pesticides constitute an important component in agricultural and commercial development and protection of public health in Dhanbad. Universally important organochlorine pesticides (OCPs) are para, para, dichlorodiphenyltrichloroethane (p,p'DDT), hexachlorocyclohexane (HCH), chlordane, aldrin, dieldrin, endrin and endosulfan. Due to the highly lipophilic nature of organochlorine pesticides, they easily concentrate in fatty food like milk leading to bioconcentration and biomagnifications through food chain, leading to many adverse acute and chronic health effects.

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Huge portion of the population starting from infants to youth to aged people have milk as a daily constituent of their diet. Per capita availability/day of milk in Dhanbad was 152g in 2014 (Directorate of Economics and Statistics, Govt of India).

Several studies have been carried out on pesticide contamination of bovine milk from different parts of the world (Abou Donia *et al*., 2010; Lehotay and Mastovska, 2005; Bosnir *et al*., 2010; Ashnagar *et al*., 2009). OCP residues like aldrin, different isomers of DDT and HCH, endosulfan etc were also reported in bovine milk samples from various regions in India (Kumar and Nath, 1996; Sharma *et al*., 1999; Agnihotri *et al*., 1999; John *et al*., 2001; Pandit and Sahu, 2002; Battu *et al*., 2004; Kumar *et al*., 2006; Nag and Raikwar, 2008; Aslam *et al*., 2013). Despite the fact that the consumption of pesticides in India is still very low, about 0.5 kg/ha of pesticides against 6.60 and 12.0 kg/ha in Korea and Japan, respectively, there has been a widespread contamination of food commodities with pesticide residues, basically due to non-judicious use of pesticides. In India, 51% of foods commodities are contaminated with pesticide residues and out of these, 20% have pesticides residues above the maximum residue level values on a worldwide basis (De *et al*., 2014).

The purpose of the present study was to detect OCP residues in buffalo milk and to determine the concentration of the detected OCPs in buffalo milk samples collected from different locations of Dhanbad city, Jharkhand, India during different seasons of the year. By determining the pesticide residue contents in the buffalo milk, an estimation of Dhanbad region environmental contamination was made for better scientific and public health knowledge.

MATERIALS AND METHODS

Collection of milk samples

Five hundred forty buffalo raw milk samples were collected randomly from Dhanbad city, Jharkhand, India (Dhanbad is popularly known as the coal capital of India), during summer, rainy, and winter seasons of the year 2014-2015. Milk samples (fat-7.8%) were collected from the local dairy units. Immediately after collection the milk samples were transferred into the ice-box and then kept in deep-freezers (below 4^0C) till the extraction of the samples. All the samples were extracted within a period of 24-36 hours. All the milk samples were extracted and analyzed in the laboratory of the Department of Environmental Science and Engineering of Indian School of Mines (ISM-IIT), Dhanbad, Jharkhand.

Chemicals and Pesticide Standards

All the chemicals used in the study were of analytical reagent (AR) grade and high-grade purity especially supplied for HPLC or GLC work. The chemicals used during extraction, clean-up and estimation of the pesticide residues were acetone, n-Hexane, concentrated H_2S0_4, anhydrous Na_2S0_4. The multiple standard pesticide (GLC grade) was purchased from Sigma Aldrich.

Extraction and Cleanup of samples

A method described by De Faubert Maunder *et al*. (1964), with certain modification by Dhaliwal and Kalra (1977) as described below were used for extraction of samples (Figure 1). This particular method was chosen on the basis of its maximum efficiency of extraction of pesticide residues.

20 ml of thoroughly mixed milk sample was taken in 100 ml stoppered separating funnel. Then 40 ml each of n-hexane and acetone (GC grade) was added in the sample. The separating funnel was stoppered and thoroughly shaken for two minutes. It was allowed to stand for twenty minutes or till there was a clear separation of phases obtained. The upper n-hexane layer was drawn out with the help of a vacuum pipette and dried by passing through 5g of anhydrous sodium sulphate (Na_2S0_4) taken in a funnel. The lower aqueous acetone phase was re-extracted twice with 40 ml n-hexane. All the three batches of n-hexane layers were combined and dried over anhydrous Na_2S0_4. Again the combined n-hexane extract was concentrated to about 1 mg (1 ml) on a rotary vacuum evaporator (with n-hexane). The concentrated residue was dissolved in 40 ml n-hexane. Pesticide residues in n-hexane layer were cleaned from fat and co-extractives by acid digestion method as mentioned by Veirov *et al.* (1977), Kapoor *et al.* (1980), Kapoor and Kalra (1988, 1989). The clean up was done using a separator funnel and neutral litmus paper with concentrated H_2S0_4 (40 ml) anhydrous Na_2S0_4, (5-10 gms).

Pesticide Residue Analysis

Gas chromatography (GC) analysis was performed using Chemito series 2865, micro-processor controlled Gas Chromatograph equipped with Ni (63) Electron capture detector (ECD).

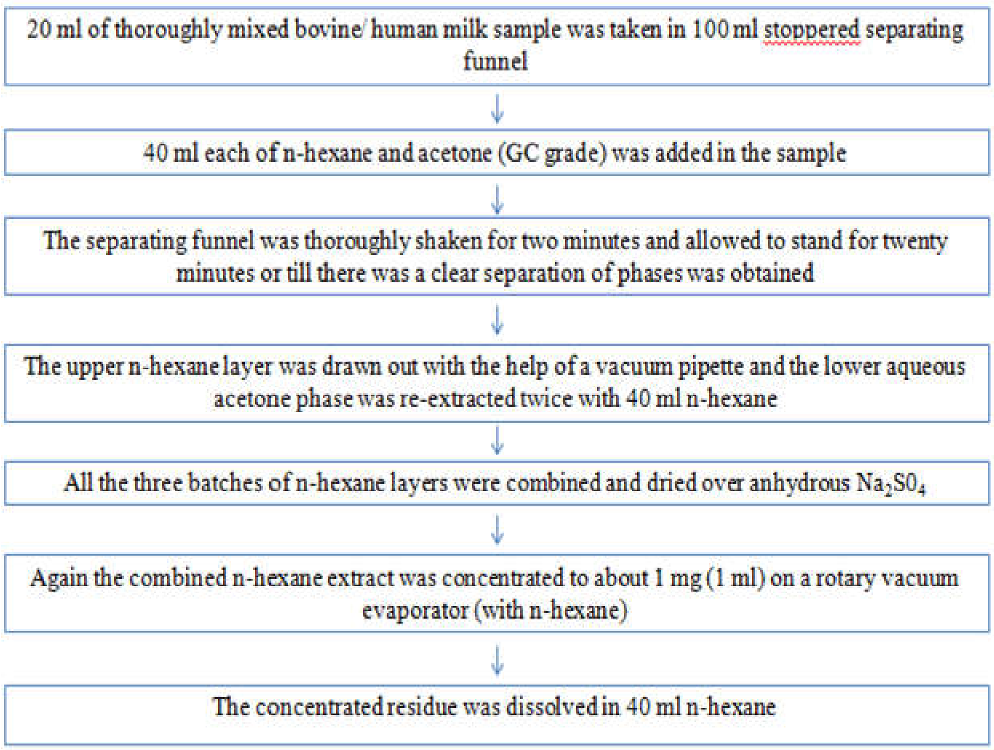

Fig. 1. Flow diagram of extraction of liquid milk samples for multiresidue analysis of pesticides

The GC system was equipped with 2 m glass, 0.25 in internal diameter (I.D.) packed with OV-17/1.95% QF- 1 on Gas Chrom Q (100-120 mesh) capillary column. 10 LAR-1Nitrogen (N_2) was used as the carrier gas. The separation was operated under the following conditions: Oven temperature program: Injection port temperature: 220^0C; column oven temperature: 200^0C (6 min); 215^0C (5 min) and 230^0C (5 min); detector temperature: 280^0C; flow rate: 50 ml / minute.

Gas chromatograph was standardized by giving several injection of standard (working standards prepared) simultaneously. 2 to 8 ml of aliquot of the clean-up extracts was injected so that the injection represented about 5 mg sample equivalent. If required, the extract was diluted to get peak height within the scale. Qualitative and quantitative analysis analyses were done by comparing the retention time and peak area of the sample, respectively, with those of the calibrated reference standards. Recoveries were determined by spiking of milk with pesticide solution of known concentration. The average samples of fortified samples were exceeding 95%. Further confirmation was done by thin layer chromatography (TLC) according to the modified method of Thompson *et al.* (1970).

RESULTS AND DISCUSSION

Milk samples analyzed for OCP residues revealed that all the milk samples were contaminated with pesticides, aldrin, isomers of hexachlorocyclohexane (alpha, beta and gamma HCH), isomers of dichlorodiphenyltrichloroethane (p,p'DDE, p,p'DDD, o,p'DDT and p,p'DDT) and isomers of endosulfan (alpha, beta and endosulfan sulfate). Among the OCP residues, the level of HCH was the highest. Average concentrations of the pesticides detected from raw buffalo milk during different seasons are given in Table 1. The Rf values of pesticides by TLC are given in Table 2. Levels of detected pesticides in milk samples during different seasons are represented in Fig 2 respectively.

Table 1. Organochlorine Pesticide residues in Buffalo milk during different seasons (concentration in mg/l)

Pesticides detected	Summer Season	Rainy Season	Winter Season
Aldrin	0.1106	0.0533	0.2332
α HCH	0.0371	0.0805	0.0161
β HCH	0.0407	0.1339	0.2994
γ HCH	0.0202	0.0530	0.1426
∑ HCH	0.0980	0.2674	0.4581
p,p'-DDE	0.0659	0.0189	0.1543
p,p'-DDD	0.0067	0.0172	0.0450
o,p'-DDT	0.0003	0.0012	0.0044
p,p'-DDT	0.0123	0.0209	0.0435
∑ DDT	0.0852	0.0582	0.2472
α-Endosulfan	0.0037	0.0027	0.0050
β-Endosulfan	0.0030	0.0023	0.0043
Endosulfan Sulfate	0.0004	0.0003	0.0007
∑ Endosulfan	0.0071	0.0053	0.0101

The level of OCP residues in buffalo milk throughout the year was found to be 0.1323, 0.2745, 0.1302, and 0.0075 mg/l for aldrin, HCH, DDT and endosulfan respectively. The bulk of milk consumed in Indian households is for tea and coffee making. Buffalo milk is preferred for this purpose because of its greater whitening quality, as compared to cow milk. The milk man, extraneous dirt, environment or un- clean water may be the reason of milk contamination. Though, the application of many OCPs in agriculture have been banned in India, in the past several OCPs were extensively used in the cultivation of paddy, wheat, maize, gram, oilseeds, many fruits and vegetables. The feed of the cattle supplied in the city are from the villages around the city. The pesticides used in the past to protect agricultural crops from pest damage in the farms of these villages are highly persistent in nature due to which their residues remain in the soil, water, air etc and enters the leafy vegetables, straws of wheat, rice, maize, grasses etc and through the cattle feed these residues enter into the animal body.

Table 2. Recovery factor (Rf) values of OCPs by TLC

Pesticides	Rf values
Aldrin	0.81
α HCH	0.52
β HCH	0.10
γ HCH	0.39
p,p'-DDE	0.36
p,p'-DDD	0.89
o,p'-DDT	0.59
p,p'-DDT	0.63
α-Endosulfan	0.67
β-Endosulfan	0.45
Endosulfan Sulfate	0.36

Hay and oat straw bedding supplies significant amount of aldrin to dairy cattle, even though these had been grown on farms, with no history of aldrin spraying (Wedberg *et al.*, 1978). Air movement of soil particles containing pesticide may be the reason for roughage contamination of these farms. Though not for agricultural purpose, but HCH and DDT are being sprayed by the health department for treating malaria, filariasis, dengue, cholera, louse borne typhus, termites control and the indoor spraying on the walls, floors and roofs might contaminate stored feed and thereby may contribute partly towards the ingestion of HCH and DDT. These pesticides might have also reached milk due to use of OCPs directly on the animals against disease vectors, use in stables for the treatment against flies, in milk processing factories for hygienic treatments against insects. Extensive coals mining in and around the city enhance the problem of pesticide contamination.

Once these OCPs enter the animal body, due to their lipophilic nature, they are not easily removed or degraded, gets accumulated in fat-rich tissues and subsequently translocated and excreted through milk fat.

Seasonal variations and the pesticide residue levels revealed that samples collected during winter season were found to be more contaminated, followed by summer and rainy seasons. Comparative concentration of detected pesticide shows that higher residues of HCH (and its isomers) were present in milk samples during all the seasons, as compared to other pesticides. Total HCH detected in buffalo milk during winter, summer, and rainy season were 0.0980, 0.2674, and 0.4581 mg/l, respectively. Average aldrins detected during winter, summer, and rainy season were 0.1106, 0.0533, and 0.2332 mg/l, respectively. Average DDT detected during winter, summer, and rainy season were 0.0852, 0.0582, and 0.2472 mg/l, respectively.

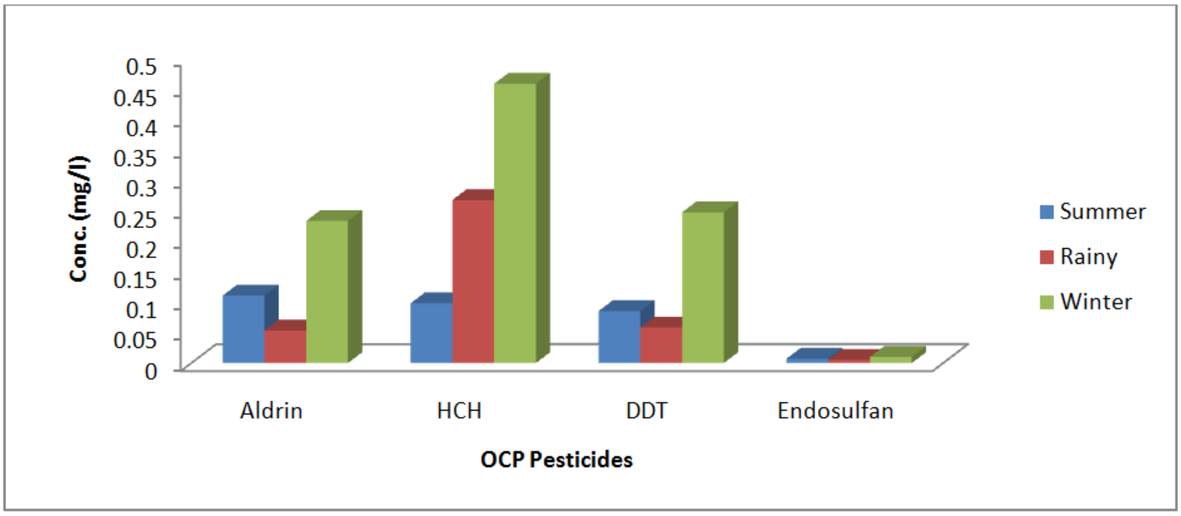

Fig. 2. Organochlorine Pesticide residues in Buffalo milk during different seasons of the year (Conc. in mg/l)

Average endosulfans detected during winter, summer, and rainy season were 0.0071, 0.0053, and 0.0101 mg/l respectively. Residues of HCH show different trend of variations in different seasons as compared to other OCP residues detected.

Occurrence of alarming quantities of OCP residues during winter could be due to greater cultivation of seasonal vegetables and other standing crops. It may be further explained that the influences of heat, wind and rain, which are the characteristics of summer and rainy seasons, are very low during winter seasons, so that the dissipation does not take place very rapidly from the outdoor field conditions where the crops grow on which cattle feed. Diet of buffaloes during winter contains more oil seeds, which is also one of the main sources of OCP accumulation, and through which maximum entry of pesticide residue becomes possible to the cattle body. So the consumption of more pesticide residues during winter season is directly proportional to the residue levels detected during the same season.

During summer, water from the ponds evaporates, leaving the insoluble pesticide in remaining water, and is adhered to the suspended particle. The grass and herbs also dry off, so that the grazing animals rely on the grasses, which rarely grow around the ponds and the other water bodies, or the stored raw materials of wheat, rice, oat, etc., which have been sprayed in the past on those fields where they were grown. The pesticides carried along with the air/wind also result in the contamination of stored foods during summer, whereas during rainy season, the cattle get fresh grasses to eat. The residues, which adhered on the grasses, get drained off to some extent along with the run-off water, reducing the overall intake of pesticides as compared to summer season.

Various OCP residues analyzed indicate that the level of only HCH exceeded the tolerance level prescribed by WHO i.e. 0.1 mg/kg for HCH, whereas levels of aldrin, DDT, endosulfan and its metabolites were below the limit i.e. 0.15, 1.25 and 0.01 mg/kg, respectively. Since DDD and DDE are not used in India, these residues detected in milk samples must have arisen as a result of metabolic conversion and dehydrochlorination of DDT (Dhaliwal and Kalra, 1978).

Due to the ban on DDT, aldrin, endosulfan for agricultural purpose, there might have been a decline in their levels. High concentration of HCH and its isomers reveals the increased improper use of these pesticides both for agricultural purposes as well for control for diseases. HCHs are the main contributors to the total OCs burden in milk, suggesting recent usage of HCHs in Dhanbad city.

Conclusion

The present survey showed the contamination of buffalo milk with OCP residues of aldrin, HCHs, DDTs and endosulfan, as they were persistent in nature due to their slow decomposition rate, long half-life and high stability in the environment. In the case of HCHs, the values detected exceeded the tolerance levels of WHO/FAO. Since the obtained results are alarming, as many of these compounds are carcinogenic, mutagenic, and teratogenic, it becomes essential to check the pesticide contamination problem through the mass media and adopting Integrated Pest Management (IPM) by utilizing alternate methods like mechanical, cultural, biological, and use of botanical pesticides for the control of pests and disease vectors. Seasonal variation of pesticide residues in milk is a common environmental phenomenon and so the state can keep the standard in accordance with the seasonal change instead of having one set standard throughout the year.

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