

Short communication

Evaluation of Persistent Organochlorine Pollutants in Mother's Milk from Kalaburagi District, Karnataka, India

V. PRASHANT^{1*}, K. VIJAYAKUMAR¹ AND SANKET, M²

¹Department of PG studies and Research in Zoology, Gulbarga University, Kalaburagi, India

²PG Pharmacology, Department of pharmacology, MR Medical College, Kalaburagi, India

E-mail: pvc.132@gmail.com

*Corresponding author

ABSTRACT

In present study, traces of organochlorine pollutants and related compounds namely DDE p, p' and DDE o, p' were analysed in mother's breast milk living in rural areas of Kalaburagi district. Traces of organochlorine pollutants were found in almost 80% of the samples analysed from rural areas, indicating that residents in these areas have been exposed to the contaminants. Kalaburagi is having fertile land, a variety of seasonal crops are cultivated in the selected study area, farmers practice large amounts of pesticides to increase the crop productivity and control the pests. Exposure of humans to these hazardous chemicals occurs directly in fields as women work as agricultural labour and indirectly due to intake of contaminated food. The organochlorine pollutants are lipophilic in nature and their presence in mother's milk has been documented in different parts of the country. Breast milk samples were collected from mothers who were willing to donate the samples after signing the informed consent form. The levels of total OCPs in milk ranged from 5 to 9 ng g⁻¹. The results are within permissible limits and harmless.

Key words: DDE; Breastfeeding; Kalaburagi; Pesticides.

INTRODUCTION

Mother's milk is very essential to newborns for overall nourishment, immunological and boosts complete growth. Specifically related with low threat of obstacles which affects the acute premature baby being feeding complications and necrotizing enterocolitis (Cacho et al. 2016). Indeed, when premature babies lack its own mother's milk and hence pasteurized donor milk is usually preferred substitute (Parker et al. 2013, Kantorowska et al. 2016). Humans are exposed to Persistent Organochlorine pollutants through food chain and environmental conditions in their whole life-span. OCPs are manmade chemical compounds that break down at a very slow pace, amass in the human body which are toxic in nature. OCPs get accumulated in blood and fatty tissues and are also found in breastfeeding women. In present investigation study, the traces of Organochlorine pollutants in lactating mother's milk generally are below the permissible limits provided. There is no threat for babies when exposed to these substances via mother's milk. The

outcome of the present research work therefore does not give reason to stop breastfeeding, based on the present survey of OCPs in lactating mother's milk. These results also show that concentrations of OCPs in lactating mother's milk have reduced during the last decades. This is because many persistent OCPs have been banned by the government of India, also Stockholm convention that restricts the practice of OCPs. As OCPs generally break down at a very slow rate, even now also OCPs persists in the environment. At an average it requires five to ten years for the traces of OCPs to get reduced by half in mother's milk. POPs are released by industries, through combustion procedure in the industrial processes, they can be even found in insecticides, pesticides and herbicides which are practiced in agriculture commonly. For the present study, we have collected human milk samples from January 2021 to April 2022, samples were analyzed in 5 batches soon after the sample collection was done. Since from 1969 WHO has recorded OCPs in feeding mother's milk. Due to the developments in the accumulation of OCPs in lactating mother's milk maybe compared

between countries and monitored.

MATERIALS AND METHODS

Study area

In Karnataka's north-eastern region, 'Kalaburagi' (Earlier Gulbarga) means stony land in Kannada. The most prominent language used in Kalaburagi is Kannada. Kalaburagi is recognized for the ancient structures made by the Bahamani rulers. Furthermore, it is also a commercial hub for Karnataka and also Hyderabad. In addition to that an educational capability, the centre also ready as a regional marketplace and facility hub for the region. It's location in a poor area has enabled the city to become a focal point for many development initiatives, which have attracted residents from surrounding districts. Out of 30 districts in Karnataka, Kalaburagi's administrative centre is located here. Kalaburagi is situated in Deccan Plateau located at 17°33" North and 76°83" East and area of 10,951 Km² (Fig. 1). Study was conducted in Basaveshwar hospital and Sangameshwar hospital, which is part of Mahadevappa Rampure Medical College.

Study population and sampling

Participants were recruited from two maternity hospitals in Kalaburagi, one was Basaweshwar Teaching and General Hospital (Sedam road) and the other was Sangameshwar Hospital (Central Bus stop road). These hospitals were selected to study the possible traces of chemicals in lactating mother's milk. The present work was submitted and approved by the Integrity forming Committee of Mahadevappa Rampure Medical College, Kalaburagi (approval no. HKES/MRMCK/ IEC/ 20211107) and also the trial has been registered in



Figure 1. Study Area Kalaburagi

Clinical Trial registry of India (CTRI/2022/02/040556), more than 150 subjects were approached for the trial but only 25 agreed to donate us with the breast milk samples, informed consent form was collected from each participant during the time of enrollment. Batch 1 of rural residents were selected for sample collection which included 5 rural women who contributed in the method development for the present research study, later during the time of screening; donors resided in Kalaburagi district rural population were enrolled. Demographic information was collected by research investigators (Table 1). A questionnaire was provided where they were asked about their information such as, age, place of residence, employment etc. and the events that could

Table 1. General demographic characteristic of sample individuals

Charecteristics	Mean	SD	Median	Minimum	Maximum
Age	25.86	25.8	25.80	25.8	26.1
Number of offspring	2.21	1.181	2	0.99	4
Number of years residing at present address	5.72	2.99	5	2.38	12
Infant weight (kg)	2.60	0.73	2.7	0.48	4.2
Age at sampling	7.14	2.66	7	2	12.4
Mother's weight	56.16	4.336	57	49	64

impact pesticide exposure of any kind. Breast milk samples from 25 women of Kalaburagi district were collected after getting signatures of donors on informed consent forms, women for this study were randomly selected and grouped in 2 categories viz.,: 1) from rural areas 2) urban areas. Samples were collected in chemically cleaned amber vials to protect the samples from UV-light and to keep the samples safe and stable during transportation, samples were transferred under chilled conditions to laboratory and stored at -20°C until analysis.

Extraction of pesticide residues

Kalra and Dhaliwal (1978) slightly modified the technique of Godly et al. (1964) for extraction of pesticide residue which was considerably effective. A well mixed sample of human milk was obtained in a 100 mL stoppered separating funnel. Each of n-hexane and acetone (GC grade) were added to the sample in a quantity of 40 mL each. Thoroughly shaking the separating funnel for two minutes continued, so that distinct separation of phase is achieved after letting it stand for 20 minutes. A vacuum pipette was used to remove the top n-hexane layer, and then the sodium sulphate (Na_2SO_4) soaked funnel was used to dry it. Adding 40 mL of n-hexane, the base layer was extracted twice. The three layers from base were combined and parched above anhydrous Na_2SO_4 . Lastly, the extract was concentrated over a rotating vacuum evaporator to around 1 mg (1 mL). 40 mL n-hexane was utilized to liquefy the residues.

Clean up method

A 250 mL separatory funnel was filled with concentrated nhexane and 40 mL concentrated sulphuric acid (H_2SO_4), sp. gravity 1.84 was added in it drop wise slowly to allow the contact time of the extraction (1 hour). The lower dark reddish brown/ dark yellowish brown/ dark yellowish layer of digested lipids and H_2SO_4 was discarded. The organic solvent layer (upper n-hexane layer) was washed with lukewarm distilled water (6 or more times using 50 mL each time) and ensured that extract was free from acid with the help of neutral litmus paper. The n-hexane extract was dried up by passage over 5-10 gm of anhydrous sodium sulphate (Na_2SO_4) in a funnel. The contents were finally transferred to graduated glass tube up to 5 mL for

estimation. Clean up sample were tagged, named and kept in cold storage up until analysis. Pesticide Residues Estimation Gas chromatographs were standardised by simultaneously injecting multiple injection standards. Standard mixture were injected at all the different concentration levels of standard prepared to obtain 30 to 40% and 60 to 80% full scale deflection (FSD) for various compounds and checked variation due to non-linearity of electron capture detector. We administered aliquots of the clean-up extracts varying in volume from 2 to 8 mL, with an injection equivalent to approximately 5 mg of sample. If necessary the extracts were diluted until we get peak height within the scale, for identification of organochlorine pesticides.

RESULTS AND DISCUSSION

All the samples analysed contained slight amount of OCP's, concentrations of DDE p, p' and DDE o, p' (Table 2). Organochlorine pollutants were detected in breast milk (Fig. 2) the contamination was slightly higher in samples from rural inhabitants than the urban ones. The women in the rural area participate in agricultural activities (Rosa et al. 2011). The other possible reasons for presence of pollutants in human milk may be excessive use of these pesticides for vector control by health department, and also consumption of food grown in agriculture area which had high residue of these chemicals. To evaluate such possibilities we need more research in this field to analyse the important food items available in Kalaburagi. The levels of DDE p, p' and DDE o, p' in mother's milk from Kalaburagi district (Prashant and Vijaykumar, 2022) are much lower than values reported from other parts of India. Compared to the average concentrations of DDE p, p' and DDE o, p' with those reported from Haryana and Punjab it was found the contamination levels are lower in Kalaburagi district.

Table 2. DDE p, p' and DDE o, p' in breast milk

Residues	Whole milk (ng g^{-1})
DDE p, p' Mean \pm SD	0.004 \pm 0.0027
Range	0.008
DDE o, p' Mean \pm SD	0.002 \pm 0.0029
Range	0.007

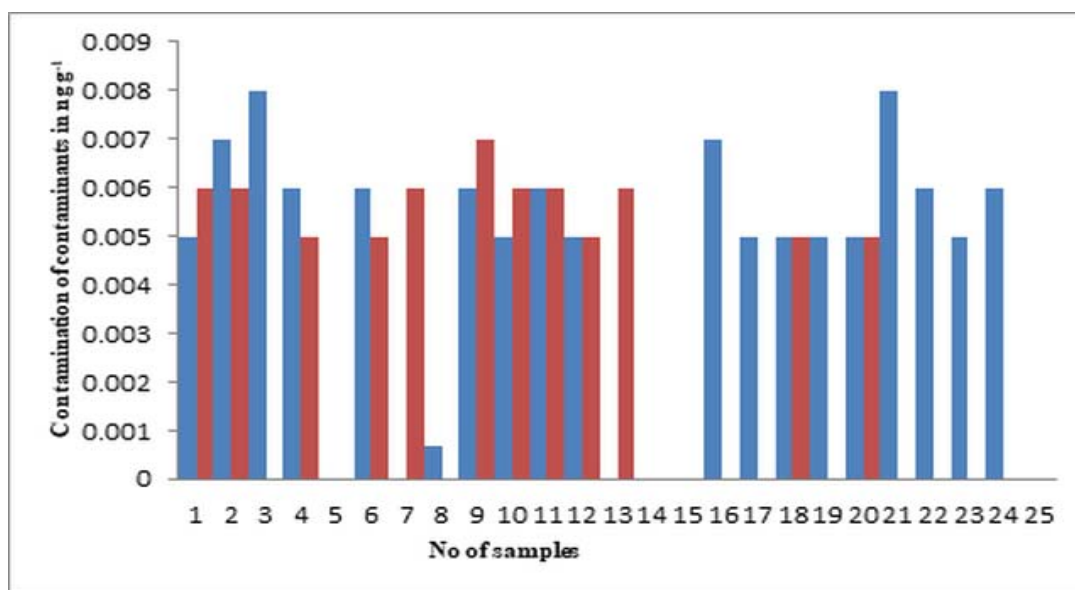


Figure 2. Concentration of contaminant in milk samples from rural area

CONCLUSIONS

Organochlorine contaminants are the most prevalent of OCP's in India. Isomers of HCH and DDT have been principal contaminants in southern region and DDT in various regions of India (western, eastern and northern). Presence of OCP's in present study of Kalaburagi district is lowest among all the previous observations, although no significant correlation was observed in OCP's in mother's milk and age of the mothers. No remarkable difference was found in concentrations of OCP's between primipara and multipara. The study also emphasizes the need for MRL's and ADI's for pesticides for newborn infants in India. The result of the present study also highlights the need for detailed investigation and monitoring of OCP levels in human milk and basic food items as they are the basic pathways to contamination and health risks for human health.

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