Evaluation of Health Risk in Infants by Organochlorine Pesticides (OCPs) Through Breast Milk

BHUVANESHWARI RANGARAJAN, S. RAJAGURU,
SHANMUGAM GOVINDARAJ, RAMASWAMY BABU RAJENDRAN

Department of Environmental Biotechnology, School of Environmental Sciences, Bharathidasan University, Tiruchirappalli- 620024, Tamil Nadu, India,
Corresponding Author: ecobhuvi2007@gamil.com

Abstract

The aim of our study was to obtain the data on the exposure of breast fed infants to organochlorine pesticides (OCPs) in South India. The breast milk samples were collected from lactating women of agricultural occupation from Virudhunagar District and were analysed for the presence of OCPs viz., Hexachlorocyclohexane (HCHs), Hexachlorobenzene (HCB), Aldrin, Dieldrin, Endrin, cis & trans-Chlordanes (CHLs), Dichlorodiphenyltrichloroethane (DDT) & its metabolites and Mirex using Gas Chromatograph- Mass Spectrometer (GC-MS). The analysis of breast milk showed the presence of almost all the chlorinated pesticide residues. The concentrations of HCHs and CHLs were found to be exceeding the tolerable daily intake value for infants' proposed by Health Canada.

Keywords: OCPs; breast milk, GC-MS; Tolerable Daily Intake

Introduction

Oflate, levels of organic contaminants have increased exponentially in the environment. This has intensified the concern on its toxicity to both human beings and wildlife. Organochlorine pesticides are ubiquitous contaminants in aquatic environments as a result of uncontrolled spillage, stream transport, surface runoff and atmospheric deposition. They have been accumulated through food chain and reported in human adipose tissue, blood and breast milk (Kanja et al., 1992; Sanghi et al., 2003; Poon et al., 2005). The female breast is most vulnerable to chemical absorption during periods of significant development, including the prenatal period, adolescence, pregnancy and lactation (Davis et al., 1997). Breast milk has several advantages as a sampling matrix: It is simple and noninvasive, with samples collected by the mother. Breast milk is considered to be the important source of all nutrients and antibodies to the infants. Unfortunately, breast milk is not free of contaminants and its ingestion represents an important exposure pathway to organochlorine pesticides and other environmental and pharmaceutical chemicals to children (Sauer et al., 1994; Patandin et al., 1999; Berlin et al., 2002). It monitors body burdens in reproductive age women and it estimates *in utero* and nursing-infant exposures, all important to community health. Time-trend data from breast milk monitoring serve as a warning system that identifies increasing body burdens and human exposures to chemicals (Hooper and She, 2003).

Lipophilic xenobiotic compounds are habitually eliminated in the fatty fraction of milk. In women, there is a strong correlation between the concentrations of organochlorine pesticides in their adipose tissue at the end of the pregnancy and those in the fatty fraction of their milk (Dorea, 1997). The mean fat content of human milk is 3.5%, it is more than enough for milk to be considered as a vehicle for

toxins during breast-feeding The reports regarding the presence of pesticides in the breast milk reported in South India are less. So, the present study aims on estimating the levels of organochlorine pesticides in breast milk samples from Tamil Nadu, South India.

Materials and Methods

Sample Collection

Milk samples were collected from lactating women (n=13) of agricultural occupation from Virudhunagar District, Tamil Nadu, India. In order to prevent external contamination, sterile containers were used and the breast and hands were washed with water before manual expression of the milk into the containers. After sample collection the samples were transferred to laboratory in cold condition and deep frozen at -20^0 C until the residue extraction and analysis.

Chemicals and Solvents

Solvents (hexane, acetone) were purchased from Qualigens Fine Chemicals Pvt Ltd, India and were of HPLC grade. Anhydrous Na_2SO_4 was purchased from Himedia Laboratory Pvt. Ltd., India. Organochlorine pesticides standards were procured from Accu Standard, USA.

Sample Preparation

Samples were extracted according to the methods report of Poon et al. (2005) and Ntow et al. (2008) with minor modification. Briefly, 5ml of milk was taken in a conical flask with 10g anhydrous sodium sulfate and ground with mortar and pestle for 5min. Then extracted with 50 ml of acetone and n-hexane (1:1) by shaking at 35°C (ORBITEK, Chennai, India) for 12h. The extraction was repeated and the combined extract was concentrated to 5ml using a rotavapor at 40°C. Then the extract was transferred to preconditioned (18ml of n-hexane) Florisil SPE cartridge (Phenomenex, USA) and eluted with 20ml of n-hexane. The eluent was condensed to 1 ml using rotavapour and subsequently by passing N_2 gas and collected in 1 ml amber glass vial and stored at 4°C until GC-MS analysis.

Instrumentation

Analysis was performed using a gas chromatograph (GC-2010) interfaced with a quadrapole mass spectrometer (QP-2010) (Shimadzu Corporation, Japan). The GC-MS was operated with an interface temperature of 270°C and an ion source temperature of 230°C. The mass spectrometer was tuned using perfluorotributylamine (PFTBA). Chromatographic separation was achieved with DB-1 fused silica capillary column (30m x 0.32mm I.D; film thickness- 0.25µm; J & W Scientific, Folsom, CA, USA). Helium with a purity of 99.99% was used as the carrier gas at a flow rate of 2.25ml/min. The gas chromatograph was equipped with splitless injector port and operated at 250°C. The samples were injected in the splitless mode at a column temperature of 70°C. The oven temperature was programmed as follows: initial temperature 70°C for 1min, from 70 to 160°C at a rate of 20°C/min and from 160°C to 190C at a rate of 2^0C/min and finally increased to 320^0C at a rate of 5^0C/min and held for 5min. The mass spectrometer was operated in the positive ion electron impact (EI) mode using an ionization energy of 70eV and an emission current of 60µA. Full scan data were obtained with a mass range of m/z 35- 500. Scanning interval and SIM sampling rate were kept at 0.5 and 0.2sec, respectively. 1µl of the extract was injected using AOC autosampler for anlaysis.

Estimated Daily Intake (EDI) of OCPs by Infants from Breast Milk

The EDI 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.

$$\text{EDI (μg/kg/day)} = \frac{\text{Contaminant Concentration} \times \text{Intake rate of milk by Infant} \times \text{Exposure Factor}}{\text{Body Weight}}$$

Ethics

All the samples were obtained after getting the approval from the institutional ethical committee of the Bharathidasan University, Tiruchirappalli. Volunteers were informed before sampling and their data are kept as confidential. A questionnaire was obtained from the volunteers which contains a range of questions related to their residence (rural or urban), occupation, age, sex etc. Breast and uterus tissues were obtained from the archived samples.

Results and Discussions

All the samples contained measurable quantities of the organochlorine pesticide residues. The mean levels of organochlorine pesticide residues, standard deviation (SD) and frequency of detection in human milk samples were shown in Table 1. The mean concentration of Endrin was found to be higher among all other residues detected in the breast milk samples and lowest being γ-HCH. α-HCH was detected in almost all the samples with the detection frequency of 92.3% . We considered HCHs as the sum of the three isomers (α,β & γ) and DDTs as the sum of the six Compounds: o,p'-DDE, p,p'-DDE, o,p'-DDD, p,p'-DDD, o,p'-DDT & p,p'-DDT, Cyclodienes as the sum of aldrin, dieldrin & endrin and CHLs as *trans*-chlordane & *cis*-chlordane. The mean concentrations and standard error of total DDT and its metabolites, HCH isomers, HCB, Cyclodienes, chlordane compounds (CHLs) and Mirex in the samples collected were shown in Fig 2. The mean concentrations of the OCPs decreased as Cyclodienes > Σ-HCHs > Σ-DDTs > CHLs > Mirex > HCB.

Breast milk is used as very good matrix for environmental monitoring of persistent pollutants of lipophilic in nature (Malisch et al., 2003). The analysis of breast milk showed the presence of almost all the chlorinated pesticide residues. Residues such as α-HCH, dieldrin, endrin, *cis*-chlordane, p,p'-DDE and mirex were found predominant in the breast milk samples. Among the pesticide residues, the mean concentration of endrin was found to be high. Endrin is a cyclodiene insecticide used on cotton, maize and rice. The major use (about 80%) of endrin is used as a spray to control insect pests of cotton. Higher level of endrin in this study is quite astonishing since when compared to aldrin and dieldrin the degree of persistence of endrin in organisms is lower which may due to its rapid excretion in bile (FAO and WHO, 1970). Burke et al. (2003) also detected dieldrin in nearly all breast milk samples from the rural areas of Indonesia. The detection of dieldrin in the breast milk indicates direct exposure to dieldrin from the environment or may be due to the exposure of aldrin that might have metabolized to dieldrin in the breast tissue (Burke et al., 2003). The comparison of OCPs in milk samples from various countries with the present study was represented in the Table 2. The concentrations of HCHs, DDTs and HCB were much lower in the present study than the levels reported in breast milk from different parts of the world (Kanja et al., 1992; Kunisie et al. (2004&2006); Minh et al., 2004; Mueller et al., 2008). The concentration of

CHLs is comparable with breast milk analysed from a metropolitan city Chennai in Southern India (Kanja et al., 1992; Subramanian et al., 2007).

Table 1 Levels of Organochlorine pesticides in human breast milk samples from Tamil Nadu, India

Pesticide	Range (ng/ml)	Frequency of Detection (%)
α-HCH	BDL - 16.5	84.6
β-HCH	BDL - 2.05	23
γ-HCH	BDL - 1.34	7.7
p,p'-DDE	BDL - 1.53	69.23
o,p'-DDE	BDL - 1.12	15.4
p,p'-DDD	BDL - 1.18	30.76
o,p'-DDD	BDL - 1.33	30.76
p,p'-DDT	BDL - 3.93	15.4
o,p'-DDT	BDL - 0.94	38.46
Aldrin	BDL - 3.97	30.8
Dieldrin	BDL - 5.29	53.9
Endrin	BDL - 118	53.9
trans-chlordane	BDL - 1.47	38.5
cis-chlordane	BDL - 1.43	61.5
HCB	BDL - 2.01	15.4
Mirex	BDL - 1.42	61.5

Table 2 Comparison of pesticide residues (ng/g) in human breast milk samples

Countries	HCHs	DDTs	HCB	CHLs	Reference
India (Virudhunagar)	7.57*	2.75*	0.30*	1.4*	This study
India (Chennai city)	4500	1200	7.3	4.2	Subramanian et al.(2007)
Australia	380	18	nd	<2	Mueller et al. (2008)
Japan	110	340	80	14	Kunisie et al. (2006)
Malaysia	230	1600	11	23	Sundaryanto et al. (2005)
China	550	870	56	6.7	Kunisie et al. (2004a)
Indonesia	16	1500	1.7	1.7	Kunisie et al. (2004b)
Vietnam	42	2700	3.5	5.2	Minn et al. (2004)

*- ng/ml

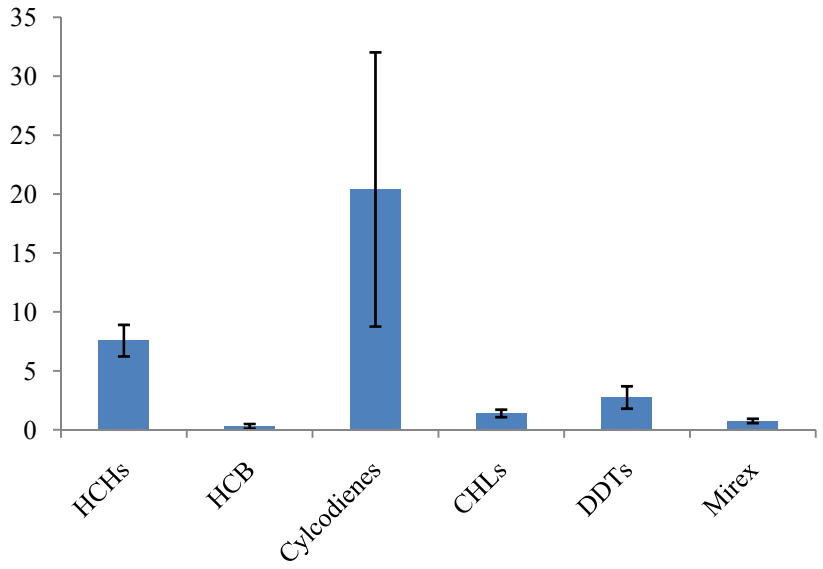

Figure 1 Mean and Standard Error of Organochlorine pesticides levels in human breast milk samples from Tamil Nadu, India

Human milk is considered to be an excellent indicator of exposure since OCPs are lipophilic xenobiotics that accumulate in adipose tissue and breast feeding is the main pathway of elimination through the fatty fraction of milk (Malisch et al., 2003). According to Minh et al. (2004) daily intake of OCs by infants were calculated based on the assumption that the average milk consumption of 5kg infant is 700g/day. In this study daily intake was calculated and compared with the tolerable daily intake. The respective tolerable daily intake proposed by Health Canada (Oostdam et al. 1999) for HCHs, HCB, CHLs and DDTs are 0.3, 0.27, 0.05 and 20 µg/kg body wt/day, respectively. The estimated daily intake of HCHs, HCB, CHLs and DDTs were 1.03, 0.042, 0.196 and 0.284 µg/kg body wt/day. The concentrations of HCHs and CHLs were found exceeding the tolerable daily intake (TDI) of Health Canada. Nevertheless, breast feeding is indeed important for infant's growth. Breast milk is the perfect source of nutrition for infants contains appropriate amount of all nutrients and hormones. It also contains valuable antibodies from the mother that may help the baby to resist infection. So, all the advantages of breast milk and breast feeding made to neglect the disadvantages of lower concentrations of these pesticides residues in the milk.

Conclusion

The results of this study reiterate the importance of monitoring studies on the levels of organochlorine pesticide (OCP) residues in human in particular its transfer from the mother to the infants. The only source of food to the infant's is the mother's milk which contains lots of resistant factors which provide immunological protection to the infant and the emotional bonding it offers between mother and child. It is very unfortunate that even this pristine source to the infants is contaminated with the hazardous chemicals. Breast feeding should not be discouraged instead the usage of pesticides in the agricultural fields can be monitored regularly and awareness on the health risk of exposure to the pesticides can be created.

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