Bio-monitoring of Persistent Organochlorines in Human Milk and Blood Samples from Sub-Himalayan Region of India

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Abstract In the present study, concentrations of organochlorine pesticide residues viz. Dichlorodiphenyltrichloroethane and its metabolites (DDTs) and Hexachlorocyclohexane isomers (HCHs) in human breast milk and human blood samples, collected from several high altitude regions of Garhwal Himalaya in Uttarakhand, India viz. Devprayag, Chamoli, Uttarkashi, Joshimath, Bhatwari and Gangnani (altitude ranging from 472 to 1,982 m above sea level) were determined. Mean concentrations of HCH and DDT in human milk samples ranged from 4.53 to 34.32 mg/kg and 6.09 to 12.98 mg/kg, respectively. While the human blood showed mean values ranging from 6.64 to 281.7 µg/L and 12.37 to 104.10 µg/L for HCH and DDT, respectively. The study showed much higher concentrations of organochlorine residue contamination in the Garhwal region as compared to other parts of India. Risk assessments for infants were also calculated and were found within WHO limits.

Keywords DDT · HCH · Organochlorines · Persistent · Residues

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Organochlorine pesticides are a wide group of chemicals, many of which persist in the environment for a long time (Ntow et al. 2008). Long range transport in the atmosphere is the most important global transport path for persistent organic pollutants (POPs) (Wania and Mackay 1996). In warm regions, POPs tend to evaporate into the atmosphere and are transported long distance in the atmosphere before ending up on cold surfaces in the Polar Regions. Similarly to Polar Regions, low temperature in mountainous regions can also allow higher mountains to act as cold condensers for POPs in the atmosphere and play important role in the global transport of POPs (Blais et al. 1998). Unfortunately human blood and breast milk are not devoid of compounds that may adversely affect a child's health. These include persistent organochlorine compounds found in human breast milk all over the world (Jensen and Slorach 1991; Banerjee et al. 1997). Therefore the burden of these pesticides in the mother's body is indicative of the route of these pesticides to the infant's up to a significant level. Many investigations in different countries have reported contamination of breast milk with dichlorodiphenyltrichloroethane (DDT) and it matobolites and other organochlorine pesticides (Yu and Zhu 2001). Higher levels of DDT and its metabolites in human blood samples from India were reported as compared to other parts of the world (Sharma and Bhatnagar 1996; Bhatnagar 2001). Levels of DDT and hexachlorocyclohexane isomers (HCH) have been reported in soil, water and whole blood samples from bioenvironmental and insecticide sprayed areas of malaria control in India (Dua et al. 1996). Recently Dua et al. (2001) found that the mean DDT and HCH contamination in human blood samples from population residing in the hilly district of Nainital, India was higher than the contaminations by the same in nearby plains.

The present research was a systematic study of monitoring the OCs residues (DDT metabolites and HCH isomers) in human breast milk and human blood samples collected from higher altitudes of Garhwal region to compare the results with those reported from other countries to assess the spread and magnitude of contamination of theses toxic chemicals.

Materials and Methods

All samples were collected from the districts of Garhwal Region of Uttarakhand state situated in Sub-Himalayan region of North-India. The districts of Devprayag, Chamoli, Uttarkashi, Bhatwari, Joshimath, Gangnani, situated at an altitude of about 472, 1,069, 1,156, 1,677, 1,890, and 1,982 m above mean sea level respectively, were selected for the sample collection (Fig. 1).

A total of 240 Blood samples were collected following the method as reported by Dua et al. (1996). In short, 100 μL blood was collected with the help of heparinized marked capillary glass tube of 100 μL capacity (Drumand Scientific Co. USA) on a filter paper by pricking the finger tip with the disposable pricking needle (Lancet, Monoject Scientific, Div. Sherwood Medical, UK). The paper was dried and stored for further analysis. The dried blood spots on the Whatman no.1 paper were cut into small pieces and immersed into 5 mL of n-hexane: acetone (1:1 v/v) for 20 min in a conical stoppered test tube and mixed thoroughly for 10 min with vortex mixer. Solvent phase was separated and the filter paper was re-extracted twice with 5 mL n-hexane: acetone mixture. The extracted solvent was pooled and concentrated to 2 mL in vortex evaporator. Extraction recoveries of the HCH and DDT from spiked samples were more than 80 %.

A total of 80 Milk samples were collected from lactating mothers in individual glass tubes containing saturated $K_2Cr_2O_7$ solution in 1 % amyl alcohol. Mothers were requested to express manually about 5–10 mL of milk in the glass tube and samples were stored in deep freezer till the analysis. The 5–10 mL milk was mixed with 10 mL methanol and 0.05 g sodium oxalate. 5 mL ethyl ether was added to above solution and contents were mixed vigorously for 1 min and then 5 mL petroleum ether was added, and again shaken for 1 min. The solution was centrifuged at 1,500 rpm for 5 min to separate the solvent layers. Petroleum ether extract was transferred to separating funnel containing 650 mL distilled water and 10 mL saturated solution of sodium chloride and shaken for 2 min. The petroleum ether portion was separated, and the aqueous residue was re-extracted twice with 10 mL portions of ethanol:petroleum ether (1:1 v/v). The combined extracts were than washed with 100 mL water cautiously and the water layer was discarded. The final petroleum ether extract was passed through an anhydrous sodium sulfate column. The organic solvent was then dried in a vacuum evaporator to obtain fat. The fat was weighted to determine the fat content of milk. Weighted fat content was dissolved in 5 mL petroleum ether in a beaker and transferred to a separating funnel containing 30 mL of acetonitrile. The solvent was shacked vigorously for 1 min and the layers were allowed to separate (∼5 min). Acetonitrile layer was transferred to another separating funnel containing 50 mL petroleum ether and shacked for 1 min it, and was again extracted twice with 15 mL petroleum ether. It was then passed into another separating funnel containing 500 mL distilled water, 30 mL of saturated solution of sodium chloride and 100 mL petroleum ether. The sample was re-extracted with three additional 30 mL portion of acetonitrile as above. All four extracts were combined into another separating funnel and mixed vigorously for 30–40 s. The aqueous layer was passed into another separating funnel. 100 mL petroleum ether was added to aqueous layer and shaken for 15 min. The aqueous layer was discarded. The combined petroleum ether extract was washed with 200 mL

Fig. 1 The sampling site of Garhwal region of Uttarakhand
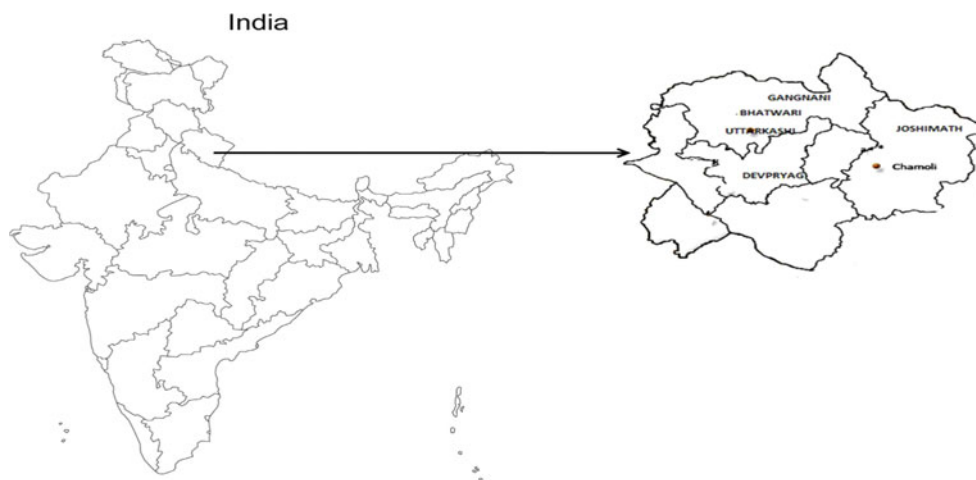
portion of distilled water. The petroleum ether extract was then passed through an anhydrous sodium sulphate column and was evaporated in a vacuum evaporator to 2 mL.

The samples thus extracted from different matrices were processed to remove co-extracting impurities by column chromatography. The chromatographic glass column was packed with 10 cm of 5 % deactivated alumina and 1.5 cm of anhydrous sodium sulphate. The bottom of the column was packed with glass wool. The packed column was tapped gently to ensure tight packing and was washed with 50 mL n-hexane. The concentrated extract of samples (blood and milk) was loaded onto column. The column was eluted with 100 mL n-hexane: benzene (1:1 v/v) in a 250 mL flat bottom flask. The elute was concentrated to a volume of 1–2 mL of and transferred to a conical test tube. The flask was rinsed with 5 mL n-hexane and also transferred to the same tube. This insecticide extract was evaporated to dryness on a vacuum evaporator and stored till further analysis.

Gas chromatographic (GC) analysis was performed using a Perkin Elmer GC (Clarus-500) with Electron Capture Detector (Ni[63]). The column used for the analysis was 0.25×0.25 mm id. The initial oven temperature was held at 150°C for 1 min and then increased at the rate of 10°C/min up to 275°C and then held at this temperature for 10 min. The injection port temperature was kept at 275°C and the Detector temperature was 300°C. The carrier gas was Nitrogen at a flow rate of 1 mL/min. Nitrogen was also used as a make up gas at 75 mL/min.

The final confirmation of the DDT and HCH residues in Human blood and milk samples were carried out by gas chromatography mass spectrometry (GCMS) analysis. A Varian CP 3800 gas chromatograph equipped with mass detector (Varian Satrun 2000 Corporation, USA) was used for GC–MS analysis. Chromatographic separation was carried out using a DB-5 capillary column supplied by Varian Pvt., Mumbai (30 m $\times$ 0.25 mm id with the film thickness of 0.25 μm). Ultra pure helium at a flow rate of 0.8 mL min^{-1} and in splitting ratio of 1:10 was used as carrier gas. The injector temperature was 200°C while the oven initial temperature was 150°C increased to the final temperature of 275°C at the rate of 5°C min^{-1}. MS detector was operated at 70 eV in EI auto ionization mode. The trap temperature, manifold temperature and transfer line temperature were 170, 40 and 270°C, respectively for MS detector.

Results and Discussion

Human blood is the most accessible body fluid for ascertaining the organochlorine residue levels. A total of 240 human blood samples collected from Devprayag, Chamoli, Uttarkashi, Bhatwari, Joshimath and Gangnani were analyzed for HCH and DDT residues and the results are given in Table 1.

The principal contributor to the total HCH was δ-HCH (31.06 %) followed by γ-HCH (28.6 %), α-HCH (22.49 %) and β-HCH (17.8 %). In case of total DDT, the major contributing contaminant was p,p-DDT contributing about 46 % to the total DDT concentration. The next major component was p,p-DDE (17 %) while the frequency of occurrence of other metabolites were 14.8 % for o,p-DDE, 10.85 % for p,p-DDD and 10.74 % for o,p-DDT. DDT is converted to DDE over time. The p,p-DDE/p,p-DDT ratio was found to be about 0.37 in the present study. This low p,p-DDE/p,p-DDT ratio suggests a more recent use of p,p-DDT. It has been reported that incessant exposure might be due to the large amount of DDT residue disposed in the past (Wong et al. 2002; Subramaniam and Solomon 2006).

A total of 80 milk samples were collected from Devprayag, Chamoli, Uttarkashi and Joshimath. All the samples were analyzed for the residues of DDT metabolites and HCH isomers and their results are shown in Table 2. The mean concentrations of total HCH were highest in Joshimath, 34.32 mg/kg followed by Chamoli, 20.86 mg/kg, Uttarkashi, 5.86 mg/kg and lower in Devprayag, 4.53 mg/kg. All the breast milk samples were found to be contaminated by HCH isomers α-HCH, β-HCH, γ-HCH and δ-HCH. The β-HCH isomer was the predominant contaminant in the breast milk samples and comprised more than 68 % of the total HCH concentration. The β-isomer is the most persistent HCH isomer, and is less readily metabolized (Steinwandter and Schlüter 1978). It is eliminated 5 times more slowly from the body than other HCH isomers (Pfeilsticker 1973). In addition it accumulates 10–30 times more in fatty tissues than Lindane. Also α and γ isomers are known to isomerise into β-isomer in living organisms (Jensen 1983). Thus the ratio between different HCH isomers changes from the start of the food chain till excretion in human milk, resulting in the more persistent β-HCH being the predominant isomer in human milk (Szokolay et al. 1977).

It has been noticed that p,p-DDT was the major metabolite and it contributes more than 36 % of the total DDT contamination followed by p,p-DDE (35.6 %), p,p-DDD (19.98 %), o,p-DDE (5.92 %) and o,p-DDT (2.49 %). Similar results were reported by Klumpp et al. (2002) in a Fujian province showing high proportions of p,p-DDT which, indicated the recent releases of this chemical to the environment. Very high levels of p,p-DDT contamination has been reported in breast milk of population living in China by Slorach and Vaz (1985). Organochlorine pesticide residues are the toxic entities that enter the human body through the food chain and cause serious health problems. Nature has devised several routes to eliminate these toxicants from the human body.

Table 1 Mean concentration in μg/L of HCH isomers and DDT metabolites in the human blood samples of Garhwal region

Sampling site	α-HCH	β-HCH	γ-HCH	δ-HCH	∑HCH	o,p-DDE	p,p-DDE	p,p-DDD	o,p-DDT	p,p-DDT	∑DDT
Devpryag (472 m)[a]	1.15 (0.005–6.7)	1.30 (0.009–5.45)	2.65 (0.037–14.68)	2.62 (0.53–38.14)	6.65 (0.112–39.48)	1.35 (005–2.41)	2.13 (0.013–5.586)	1.86 (0.012–11.41)	0.744 (0.005–1.94)	10.67 (0.089–37.48)	12.37 (1.09–30.49)
Chamoli (1,069 m)[a]	8.81 (0.05–19.20)	9.18 (0.01–37.34)	22.92 (0.20–281.70)	11.54 (0.40–46.70)	47.30 (0.69–480.98)	6.94 (0.013–52.14)	14.33 (0.14–129.80)	22.35 (0.102–396.07)	15.62 (0.033–106.43)	34.22 (2.901–264.80)	81.63 (0.85–457)
Uttarkashi (1,156 m)[a]	12.53 (0.52–84.70)	26.13 (0.54–103.50)	22.73 (0.026–206.32)	49.01 (1.21–289.20)	82.90 (2.56–624.80)	4.46 (0.13–27.18)	10.80 (0.12–35.46)	4.84 (0.052–16.78)	5.40 (0.09–29.18)	51.11 (2.54–433)	71.16 (6.28–437)
Bhatwari (1,677 m)[a]	34.83 (10.60–39.70)	38.61 (20.90–56.24)	67.68 (9.40–65.74)	86.04 (13.20–139.00)	227.20 (61.22–353.80)	7.26 (0.39–11.42)	10.67 (1.39–21.09)	4.61 (1.29–10.96)	4.35 (0.66–11.35)	8.31 (0.25–16.42)	32.90 (24.80–51.25)
Joshimath (1,890 m)[a]	49.29 (0.07–232.50)	69.00 (0.57–521.14)	51.18 (0.33–191.80)	29.05 (0.008–170.00)	201.30 (0.97–651.90)	29.34 (0.017–251.40)	8.95 (0.12–62.14)	6.25 (0.12–64.12)	5.65 (0.039–25.41)	73.01 (2.14–584.20)	104.10 (1.53–720.15)
Gangnani (1,982 m)[a]	49.81 (15.48–158.10)	53.31 (31.48–180.05)	84.01 (10.63–121.54)	94.70 (24.30–102.50)	281.10 (117.50–338.80)	16.41 (0.25–52.73)	28.77 (2.13–105.29)	8.19 (2.69–17.16)	15.89 (10.05–30.41)	28.84 (2.13–64.10)	79.68 (18.06–158.20)

[a] The height from sea level

Values given in parenthesis are the range

n = 40 samples from each site

Table 2 Mean concentration in mg/kg of HCH isomers and DDT metabolites in the human milk samples of Garhwal region

Sampling site	α-HCH	β-HCH	γ-HCH	δ-HCH	∑HCH	o,p-DDE	p,p-DDE	p,p-DDD	o,p-DDT	p,p-DDT	∑DDT
Devpryag (472 m)[a]	0.26 (0.034–0.45)	1.06 (0.20–3.48)	3.73 (0.021–11.20)	0.87 (0.241–2.20)	4.53 (0.68–14.26)	ND	3.63 (0.026–11.45)	0.144 (0.006–0.438)	0.029 (0.008–0.089)	11.03 (0.018–41.13)	12.98 (0.74–41.99)
Chamoli (1,069 m)[a]	0.80 (0.51–1.33)	16.31 (1.41–35.89)	0.561 (0.54–0.65)	5.05 (0.92–9.92)	20.86 (2.53–46.40)	ND	4.84 (0.023–10.00)	1.80 (0.45–5.13)	0.42 (0.063–1.08)	3.70 (2.18–6.21)	10.76 (2.82–22.48)
Uttarkashi (1,156 m)[a]	1.45 (0.021–3.23)	0.77 (0.15–1.726)	0.18 (0.012–4.27)	2.10 (0.021–6.62)	5.86 (0.57–14.68)	0.34 (0.66–1.10)	3.84 (0.59–14.22)	1.74 (0.050–3.60)	0.233 (ND–0.25)	1.02 (0.20–1.94)	6.10 (0.65–19.50)
Joshimath (1,982 m)[a]	1.34 (0.12–3.90)	28.35 (2.31–63.41)	4.08 (0.64–15.78)	1.21 (0.11–2.61)	34.32 (3.99–70.15)	1.03 (ND–1.44)	4.29 (0.23–23.40)	5.63 (0.053–53.81)	0.472 (ND–1.02)	1.06 (0.11–1.97)	11.33 (0.60–54.32)

[a] The height from sea level

Values given in parenthesis are the range

n = 20 from each site

ND not detected

Lactation is one of the most important means of excreting these compounds from the female body (Czaja et al. 1997). On an average basis, when a comparison was made between human blood and breast milk samples, it was observed that human blood samples contained 8.5 times more HCH and 6.34 times more DDT than breast milk samples.

To understand the magnitude of exposure of HCH and DDT by infants, the average daily intake from the levels of these contaminants in human milk are calculated in this study based on the assumption that an infant ingests 700 mL milk per day and the weight of an infant is 5 kg (Hooper et al. 1997; Minh et al. 2004; Sudaryanto et al. 2006). The levels of the tolerable daily intake (TDI) for HCH and DDT by the WHO are 20 µg/kg body wt/day (Kunisue et al. 2004). Average EDI (Estimated Daily Intake) found in Garhwal region for HCH in Devprayag, Chamoli, Uttarkashi and Joshimath were 0.63 (0.09–2.00), 1.99 (0.35–6.49), 0.82 (0.08–2.06) and 4.96 (0.56–13.46) µg/kg body wt/day respectively. Similarly EDI for DDT were 1.82 (0.10–5.88), 1.36 (0.39–1.60), 0.85 (0.09–2.73) and 1.92 (0.08–13.84) µg/kg body wt/day in Devprayag, Chamoli, Uttarkashi and Joshimath respectively. The acceptable daily intake (ADI) for HCH and DDT in India are 16 and 50 µg/kg body wt/day. Daily intake in Garhwal region was found to be within the recommended limits by WHO.

Concentrations of organochlorines in human breast milk and human blood vary with factors such as altitude and temperature of the particular region. The relationship between concentrations of organochlorines in breast milk and human blood with altitude were examined separately since significant differences in organochlorine concentration levels were observed between them. A highly significant correlation was observed between HCH concentration for breast milk ($r = 0.851$) and human blood ($r = 0.952$) samples with altitude. A moderately significant correlation was obtained between total DDT and height ($r = 0.609$) for blood samples. However, no correlation or very less significant correlation ($r = 0.216$) was found between total DDT and height in breast milk samples (Figs. 2, 3).

This study provides for the first time data regarding levels of organochlorine contamination of human blood and milk samples in Sub-Himalayan region of India. The organochlorine compounds were detected in all human milk and blood samples of Himalayan region indicating that most probably every person is exposed and carries a body burden of multiple pesticides which might be due to a combination of direct and indirect exposure to these pesticides. The presence of these compounds in human blood and milk reveal that they do persist in the body for a good amount of time. It also indicated the presence in the body of the pesticide in its primary form. The organochlorine
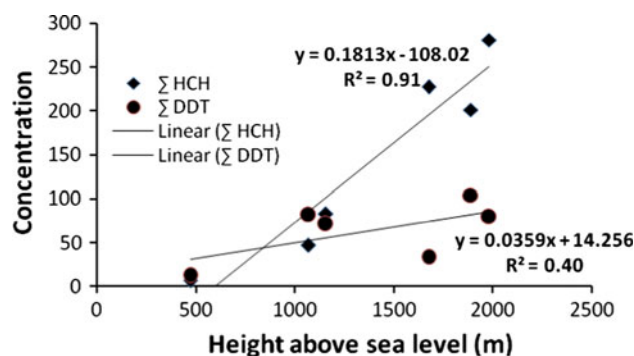

Fig. 2 Correlation between ∑HCH and ∑DDT with Height for Blood samples

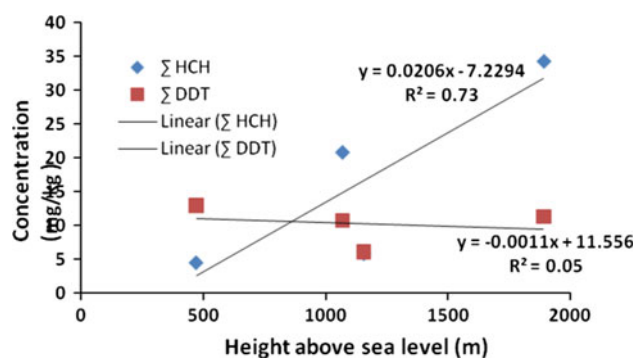**Fig. 3** Correlation between ∑HCH and ∑DDT with Height for Human milk samples

contamination levels in hilly population showed the persistence and presence of these compounds in the hill ecosystem and food chain.

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