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Specific Pattern of Persistent Organochlorine Residues in Human Breast Milk from South India

Shinsuke Tanabe,*,† Futoshi Gondaira,† Annamalai Subramanian,‡ Aramandla Ramesh,† Dhandapani Mohan,‡ Palaniappan Kumaran,‡ Vanniyoor K. Venugopalan,‡ and Ryo Tatsukawa†

Department of Environment Conservation, Ehime University, Tarumi 3-5-7, Matsuyama 790, Japan, and Centre of Advanced Study in Marine Biology of Annamalai University, Parangipettai 608 502, Tamil Nadu, India

Human breast milk samples collected from four locations in Tamil Nadu state, South India, were analyzed for understanding the levels of persistent organochlorines such as 1,2,3,4,5,6-hexachlorocyclohexane (HCH (BHC)) isomers, 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT) compounds, and polychlorinated biphenyls (PCBs). On the basis of the overall concentrations of these compounds, $\sum$HCH (sum of α, β, γ, and δ isomers) levels were higher than the other two. Unlike that of developed countries, the concentration of HCH isomers in Indian breast milk was more in vegetarians than nonvegetarians. Interestingly, high levels of HCH isomers indicated a shift toward their preferential accumulation in the body relative to DDT compounds, which had higher levels than HCH isomers in earlier years. In view of the increasing usage of technical HCH for agriculture and vector control, the body burden of HCH isomers may still go up in Indians. We also detected PCBs in Indian breast milk, but at low levels.

There has been a steady increase over the past 3 decades in the use of man-made chemicals in developing countries, most of which are located in the tropical zone (Mowbray, 1986). Some of these hazardous chemicals such as HCH (BHC), DDT, and PCB remain in the environment for a long time and have an impact on global environmental contamination due to their persistent nature and harmful biological effects.

The use of these persistent organochlorines in tropical areas has particular implications with regard to the environmental quality and human health due to the specific climatic conditions (such as high temperature and heavy rainfall) and socioeconomic status of people inhabiting these areas when compared with that of temperate countries. For example, our previous studies (Ramesh et al., 1989) showed that tropical areas play a vital role in the global contamination of persistent organochlorines through the atmosphere. Such specific behavior of these toxicants is also expected in other regimes of the tropic environment, including the biota.

The present investigation regarding organochlorines in human breast milk forms a part of our study aimed at elucidating the distribution, behavior, and fate of HCH isomers, DDT compounds, and PCBs in abiotic and biotic regimes in India. India has a representative tropical area where HCH and DDT are still being used and account for >50% of the insecticides currently used, amounting to 47 000 tons for HCH and 19 750 tons for DDT annually (Ray et al., 1985). Indian breast milk could be used as a sort of indicator for understanding the biological specificity in the accumulation of organochlorines in the tropical environment as well as assessing the extent of environmental pollution caused by these chemicals.

MATERIALS AND METHODS

Samples. A total of 25 milk samples were collected during August to December 1988 from rural, urban, and cosmopolitan areas in Tamil Nadu state, South India. The background information on the sample collection sites is as follows:

Madras. This is one of the largest cosmopolitan and industrial cities of India, located on the southeast coast.

Chidambaram. This is a semiurban area situated 250 km south of Madras. Breast milk was collected from donors residing in the nearby rural areas. Eighty percent of the population in the surrounding villages are farmers. Paddy is the main crop grown in this area.

Nattarasankottai. This is a rural area 250 km south of Chidambaram. Most of the inhabitants of this area are also involved in rice farming.

Chinnoor Parangipettai. This is a fishing village. Fishing and marketing of fish are the major occupations of people. This remote hamlet is located 30 km north of Chidambaram.

Formal consent was obtained from all donors prior to sampling. Donors were given a questionnaire listing age, height, weight, nature of delivery, parturitional details, dietary habits, occupation (including husband's), previous illness, involvement in farm/industry work, etc. Some of these characteristics are given in Table I.

Milk samples (4–60 g each) were collected from the donors of 3 days to 17 months postpartum. They were manually expressed by donors into solvent-washed screw-capped glass bottles of 100-mL capacity. Samples were immediately preserved in 10% formalin.

Chemical Analysis. The AOAC method (1984) was employed to extract fat. The procedure of Tanabe et al. (1984) was followed for the analyses of organochlorines.

Samples were extracted with mixed solvents of ethyl alcohol (20 mL), diethyl ether (25 mL), and hexane (100 mL). The extracts were transferred to 100 mL of hexane by shaking in a 300-mL separatory funnel containing hexane-washed water and 100 mL of hexane. The funnel was allowed to stand undisturbed for 30 min, and then the hexane layer was collected and concentrated to 10 mL in a Kuderna-Danish (KD) concentrator. Two milliliters of the KD concentrate was used for extraction of fat. The remaining 8 mL of the concentrate was passed through 20 g of Florosil packed in a glass column. Organochlorines adsorbed on Florosil were isolated by eluting with 120 mL of acetonitrile containing 30 mL of water and then transferred to hexane in a separatory funnel containing 600 mL of water and 100 mL of hexane. After partitioning, the hexane

† Ehime University.
‡ Annamalai University.

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Table I. Sampling Details and Relevant Information on Breast Milk Collected from South India

sample no.	sampling location	collection date	mother's age, years	no. of previous children	age of nursing child, days	freq of eating (per week) fish	freq of eating (per week) meat
CM1	Chidambaram	Aug 7, 1988	20	0	120	1–2	*a*
CM2	Chidambaram	Aug 7, 1988	27	3	10	4	2
CM3	Chidambaram	Aug 7, 1988	24	2	10	2	1
CM4	Chidambaram	Aug 7, 1988	22	0	30	0	0
CM5	Chidambaram	Aug 7, 1988	25	2	420	1–2	0.5
CM6	Chidambaram	Aug 7, 1988	23	1	3	1–2	0.5
CM7	Chidambaram	Aug 9, 1988	19	0	4	2–3	4
CM8	Chidambaram	Aug 9, 1988	25	2	3	0.5	1
CM9	Chidambaram	Aug 9, 1988	24	0	26	5	5
CM10	Chidambaram	Aug 14, 1988	21	1	5	0	0
CM11	Chidambaram	Aug 14, 1988	28	1	5	1	1
CP1	Chinnoor Parangipettai	Sept 1, 1988	22	2	150	7	1–2
CP2	Chinnoor Parangipettai	Sept 1, 1988	28	2	90	4	1
CP3	Chinnoor Parangipettai	Sept 1, 1988	33	4	150	7	0.25
CP4	Chinnoor Parangipettai	Sept 1, 1988	24	0	330	3	0
CP5	Chinnoor Parangipettai	Sept 1, 1988	*a*	2	510	5–6	0.25
M1	Madras	Sept 6, 1988	23	1	240	1	1
M2	Madras	Sept 6, 1988	22	1	270	2	1
M3	Madras	Sept 6, 1988	28	2	90	2	2
M4	Madras	Sept 6, 1988	29	1	180	2	2
M5	Madras	Sept 6, 1988	28	2	210	1	1
M6	Madras	Sept 6, 1988	24	1	360	1	1
N1	Nattarasankottai	Dec 28, 1988	24	1	150	0	0
N2	Nattarasankottai	Dec 28, 1988	21	0	150	0	0
N3	Nattarasankottai	Dec 31, 1988	26	2	60	0	0

[a] No data available.

layer was separated and concentrated to 6 mL. The concentrate was fractionated with 1.5 g of silica gel (Wako gel S-1) packed in a glass column. The first fraction eluted with 240 mL of hexane contained the PCBs and *p,p'*-DDE; the second fraction eluted with 40 mL of dichloromethane and 160 mL of hexane contained the HCH isomers, *p,p'*-DDD, and *p,p'*-DDT. Each fraction was concentrated, cleaned with 5% fuming sulfuric acid, washed with water, and then injected into a gas chromatograph.

Quantification of HCH isomers and DDT compounds (*p,p'*-DDD and *p,p'*-DDT) was performed by injecting the aliquots of final extracts into a gas chromatograph (Shimadzu Model 9A) equipped with a ^{63}Ni electron capture detector (GC-ECD) and moving needle type injection system (splitless and solvent cut mode). The column consisted of a fused-silica capillary (0.25-mm i.d. × 25-m length) with chemically bonded OV-1701. The temperature was isothermally maintained at 220 °C. Injector and detector temperatures were kept at 260 °C. Nitrogen was used as both carrier and make-up gas.

PCBs and *p,p'*-DDE were also quantified by a GC-ECD (Shimadzu Model 7A) equipped with a fused-silica capillary column (0.23-mm i.d. × 30-m length) coated with silicone OV-101. Column oven temperature was programmed from 170 to 230 °C at a rate of 1.5 °C/min for PCB analyses. Both injector temperature and detector temperature were kept at 260 °C. For detecting DDE, the column temperature was isothermally kept at 230 °C. Total PCB concentration in the sample was calculated by adding the concentrations of the individually resolved peaks of different PCB isomers and congeners.

The detection limit and the percentage recovery of the analyzed organochlorine compounds were 1 ng/g and ~95–98%, respectively.

RESULTS

The concentrations of HCH isomers (α-, β-, γ-, and δ-HCH), DDT compounds (*p,p'*-DDD, *p,p'*-DDE, and *p,p'*-DDT), and PCBs are given in Table II.

HCH Isomers. The mean concentrations of $\sum$HCH (sum of α, β, γ, and δ isomers) in the human breast milk from Nattarasankottai, Chidambaram, Madras, and Chinnoor Parangipettai were 10 000, 8800, 2900, and 2400 ng/g on a fat weight basis, respectively, and the overall mean concentration for all the samples obtained was 6200 ng/g. Samples from rural agricultural areas such as Nattarasankottai and Chidambaram revealed higher levels of $\sum$HCH than samples from the urban area (Madras) and the fishing village (Chinnoor Parangipettai). Interestingly, in the case of Nattarasankottai samples N1 and N2 showed higher levels of organochlorines than sample N3 even though their sample sizes were smaller. Among HCH isomers, the percentage of β-HCH was found to be the highest. This is in agreement with the results of Yakushiji et al. (1979a), who reported that β-HCH constituted >90% of $\sum$HCH in breast milk collected from Japan.

DDT Compounds. The mean concentration of $\sum$DDT (sum of *p,p'*-DDD, *p,p'*-DDE, and *p,p'*-DDT) was 1200 ng/g. Area wide, the mean $\sum$DDT concentrations of Nattarasankottai, Chinnoor Parangipettai, Chidambaram, and Madras were 2300, 2000, 1000, and 760 ng/g on a fat weight basis, respectively (Table II). In most of the samples (14 out of 25 samples) the *p,p'*-DDD concentration was <1 ng/g. The *p,p'*-DDE concentration was highest among the DDT compounds, which was similar to that of previous works in India (Siddiqui and Saxena, 1985) and in other countries (Karakaya et al., 1987; Skaare et al., 1988).

PCBs. The mean PCB concentration of all the areas was 120 ng/g on a fat weight basis. The individual mean concentrations are as follows (Table II): Chidambaram, 180 ng/g; Madras, 110 ng/g; Chinnoor Parangipettai, 72 ng/g; Nattarasankottai, 21 ng/g. The values observed are rather uniform; however, samples CM3, CM9, CM10, CP3, and M1 showed apparently higher levels.

DISCUSSION

A comparison of $\sum$HCH concentration among the four locations revealed that dietary habits and exposure of people to pesticides define their levels in breast milk. The areas of Chidambaram and Nattarasankottai, where higher levels of HCH isomers were observed in breast milk (Table II), have vast stretches of agricultural lands. Personal inquiries of the breast milk donors revealed that most of them own paddy fields and some of them used

Table II. Concentrations of HCHs, DDTs, and PCBs (ng/g of Fat) in Human Milk from South India[a]

sample no.	fat content, %	HCHs					DDTs				PCBs
		α-HCH	β-HCH	γ-HCH	δ-HCH	ΣHCH	p,p'-DDE	p,p'-DDD	p,p'-DDT	ΣDDT	
CM1	0.48	2100	18000	310	320	21000	770	<1.0	260	1000	27
CM2	1.9	270	4700	19	29	5000	370	9.5	100	540	21
CM3	3.3	290	6700	28	17	7000	840	<1.0	160	1000	660
CM4	2.2	380	4500	40	30	5000	1300	17	240	1600	23
CM5	0.26	1300	6500	150	130	8100	340	<1.0	160	500	26
CM6	0.80	310	1100	130	100	1600	32	<1.0	71	100	30
CM7	1.5	410	7200	49	56	7700	880	<1.0	210	1100	72
CM8	2.4	490	3800	51	82	4400	180	<1.0	69	250	30
CM9	3.1	150	3200	6.3	9.3	3400	1700	<1.0	210	1900	270
CM10	1.5	1600	24000	52	54	26000	2100	<1.0	270	2400	770
CM11	3.4	360	6900	21	21	7300	750	<1.0	96	850	52
mean		700	7900	78	77	8800	840	2.4	170	1000	180
		(8)	(90)	(1)	(1)	(100)	(83)	(0.2)	(17)	(100)	
CP1	15	100	940	12	15	1100	68	<1.0	46	110	7.0
CP2	4.1	160	1800	21	20	2000	960	<1.0	320	1300	19
CP3	4.4	110	950	23	29	1100	240	<1.0	100	340	190
CP4	4.1	270	3700	51	17	4000	4700	30	1400	6100	47
CP5	1.3	140	2200	0.0	13	2400	300	<1.0	110	410	32
mean		170	2200	24	20	2400	1600	6.0	400	2000	72
		(7)	(91)	(1)	(1)	(100)	(80)	(0.3)	(20)	(100)	
M1	3.3	410	3300	76	26	3800	79	14	140	230	460
M2	4.3	150	2800	4.4	96	3100	1300	16	<1.0	1300	26
M3	1.7	240	1600	17	27	1900	590	10	230	830	20
M4	2.0	190	1800	14	8.2	2000	650	13	64	730	37
M5	2.0	140	2400	16	20	2600	290	14	160	460	21
M6	0.95	170	3500	25	11	3700	790	18	240	1000	86
mean		220	2600	25	31	2900	620	14	140	760	110
		(8)	(90)	(1)	(1)	(100)	(81)	(1)	(18)	(100)	
N1	1.8	1100	16000	46	25	17000	3500	43	390	3900	29
N2	2.7	490	11000	82	29	12000	2500	33	440	3000	31
N3	5.1	340	1500	53	51	1900	95	<1.0	48	140	3.3
		640	9500	60	35	10000	2000	25	290	2300	21
		(6)	(93)	(0.6)	(0.4)	(100)	(87)	(1)	(12)	(100)	

[a] Values in parentheses indicate percentage compositions of HCH isomers and DDT compounds.

to assist their husbands in farming. Hence it seems inevitable that these donors get exposed to insecticides during agricultural operations by treating crops with these chemicals, from drift of airborne insecticides from nearby fields into residential areas, and by handling and cleaning of stored pesticide containers and application instruments. They also have a higher possibility of exposure to HCH isomers through contaminated food. On the contrary, lower levels of HCH isomers were found in samples from Madras and Chinnoor Parangipettai, where all the donors are nonvegetarians, indicating that the intake of HCH isomers through foodstuffs of animal origin is not so significant. It also suggests that dietary intake of HCH through vegetables may be a principal route of exposure in Indians. This view might be supported by the fact that high HCH levels were observed in the breast milk samples from Nattarasankottai, where the donors are strict vegetarians. Indeed, high levels of HCH have been documented in vegetables (Lal et al., 1989), cereals (Noronha et al., 1980), and edible oils and oil seeds (Dikshith et al., 1989) from India. On the other hand, in developed countries such as Japan, most of the HCH intake was from dairy products (Tatsukawa et al., 1972). The dietary exposure of people to HCH as observed in the present study seems to be unique to India, which has a majority of vegetarians.

Unlike HCH isomers, differences in DDT concentrations in Indian breast milk according to area of living and dietary habits were found to be smaller. In India, DDT was used to control agricultural pests and for public health purposes in earlier years. However, in recent years, this situation has remarkably changed. Out of the total annual DDT consumption, 85% is used for mosquito control in India (Singh et al., 1988). Studies conducted by Battu et al. (1989) showed that indoor spraying of DDT for malaria control (at the rate of 2 g/m^2) in rural dwellings of India resulted in a concentration of 1.8–14.6 $\mu g/m^3$ in air after 1 month of application. They also reported contamination of stored food commodities like wheat grain, straw, and flour due to DDT spraying of rural premises for vector control. DDT is also sprayed in urban areas for mosquito control (Kaushik et al., 1987). Thus the major route of DDT exposure to humans is through inhalation and through contamination of food during spraying of homes, making overall exposure of DDT more uniform in the population of different areas.

The levels of insecticide residues in breast milk recorded in the present study were compared with those of other countries (Table III). Interestingly, HCH and DDT levels in Indian as well as Chinese human milk are apparently higher, reflecting that these two countries are the major consumers of persistent insecticides in the world as of now. Unfortunately, this trend is likely to continue in India in the future because an increasing demand of this insecticide is predicted in the coming 5 years at least (Ray et al., 1985).

Besides revealing the status of India in worldwide contamination of human milk, the results of the present study show interesting trends when compared with earlier works on organochlorine insecticide levels in Indians. Our studies indicated that ΣHCH levels are apparently higher than ΣDDT in Indian human milk. However, earlier

Table III. Mean Concentrations of ∑HCH and ∑DDT (ng/g of Fat) in Human Milk from Different Countries

country	year	∑HCH	∑DDT	ref
Japan	1977	2500[a]	1900[d]	Yakushiji et al. (1979b)
United States (Hawaii)	1979–1980	180[b]	2200[d]	Takei et al. (1983)
Canada				
native population	1987	34	840	Davies and Mes (1987)
national survey	1987	220	1000	Davies and Mes (1987)
Great Britan	1979–1980	220[a]	1900	Collins et al. (1982)
West Germany	1979–1981	450	1900	DFG (1984)
Norway	1981–1982	80[a]	970[e]	Skaare et al. (1988)
Denmark	1982	80[a]	1200	Andersen and Orbaek (1984)
Sweden	1979	170[a]	1900	Norén (1983b)
Belgium	1982	200[a]	2070[d]	Slorach and Vaz (1983)
Finland	1984	80	570	Mussalo-Rauhamaa et al. (1988)
Yugoslavia	1981–1982	280[a]	2080[d]	Slorach and Vaz (1983)
Israel	NA[f]	390	2800	Weisenberg et al. (1985)
Turkey	1987	1000	5800	Karakaya et al. (1987)
Greece	1983	15[c]	35	Fytianos et al. (1985)
Mexico	1981	400[a]	4410[d]	Slorach and Vaz (1983)
South Africa	1983	NA	2400[d]	Van Dyk et al. (1987)
Kenya	1983–1985	110[b]	6900	Kanja et al. (1986)
China	1982	6600[a]	6200[d]	Slorach and Vaz (1983)
India	1982	4600[a]	5900[d]	Slorach and Vaz (1983)
India	1988	6200	1200	present study

[a] β-HCH only. [b] β- and γ-HCH. [c] γ-HCH only. [d] p,p′-DDE and p,p′-DDT. [e] p,p′-DDE only. [f] NA: data not available.

Table IV. Mean Concentrations (ng/g of Fat) of PCBs in Human Milk from Different Countries

country	year	concn	ref
Japan	1977	1100	Yakushiji et al. (1979b)
United States			
mainland	1977–1978	1500	Wickizer et al. (1981)
Hawaii	1979–1980	800	Takei et al. (1983)
Canada			
native population	NA[a]	530	Davies and Mes (1987)
national survey	NA	420	Davies and Mes (1987)
Great Britan	1979–1980	500	Collins et al. (1982)
West Germany	1979–1981	1800	DFG (1984)
Norway	1981–1982	1000	Skaare et al. (1988)
Denmark	1982	800	Andersen and Orbaek (1984)
Sweden	1979	1400	Norén (1983b)
Belgium	NA	600	WHO (1988)
Finland	1984–1985	930	Mussalo-Rauhamaa et al. (1988)
Yugoslavia	NA	500	WHO (1988)
Israel	NA	540	Weisenberg et al. (1985)
Thailand	NA	60	WHO (1988)
Vietnam	NA	100	WHO (1988)
India	1988	120	present study

[a] NA: data not available.

workers reported that DDT concentrations were nearly 18 times higher than HCH concentrations in adipose tissues in 1964 (Dale et al., 1965). It was further reported that DDT concentrations were only 1.5 times higher than HCH concentrations in adipose tissues in 1979 (Siddiqui et al., 1981). The concentrations of HCH isomers were higher than those of DDT compounds in maternal blood, placenta, and umbilical cord blood of live-born children during 1979–1980 (Saxena et al., 1983). The increasing consumption of HCH both in agriculture and in vector control might be the main reason for the temporal increase of HCH levels in human tissues and the present high levels in human breast milk from India.

Another interesting finding of this study is the presence of PCBs in Indian breast milk. To our knowledge, this paper is the first of its kind to detect the occurrence of PCBs in Indian breast milk. PCB levels in Indian breast milk are lower than those reported elsewhere (Table IV). However, their occurrence emphasizes the widespread contamination of PCBs even in developing countries.

The PCB concentrations in most of the samples were in the several tens of nanograms per gram range, indicating rather uniform contamination (Table II) from common PCB sources such as transformers, capacitors, and other electrical appliances. However, the concentrations of PCBs in some samples are about an order of magnitude higher than the concentrations in the rest of the samples, suggesting the exposure of these donors to a specific PCB source.

In developed countries fish are the prime sources of PCBs to the human body through diet (Watanabe et al., 1979; Noren, 1983a). However, in the present study, breast milk of fisherwomen (donors from Chinnoor Parangipettai) who consumed fish almost every day did not show high levels of PCBs or of the other organochlorines. This implies that the major source of PCB contamination in India is not present in coastal regions, probably lying in the inland areas. Further investigations with a wide variety of biological and environmental samples are needed to understand the details of the PCB source, pathway, and magnitude of contamination in humans.

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