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PERSISTENT ORGANOCHLORINE RESIDUES IN HUMAN BREAST MILK FROM VIETNAM: CONTAMINATION, ACCUMULATION KINETICS AND RISK ASSESSMENT

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ABSTRACT

Despite the ban of persistent organochlorines (OCs) in most of the developed nations, their usage has remained until very recently in many Asian developing countries for agricultural purposes and vector-borne disease eradication programs, including Vietnam. In this study, we collected human breast milk samples from the two biggest cities in Vietnam: Hanoi city (n = 42) and Hochiminh (n = 44) and determined the concentrations of persistent OCs such as PCBs, DDT and its metabolites (DDTs), hexachlorocyclohexane (HCHs), hexachlorobenzene (HCB), chlordane compounds (CHLs) and tris-4-chlorophenyl-methane (TCPMe). The contamination pattern of OCs was in the order of DDTs > PCBs > HCHs > TCPMe > CHLs > HCB. Compilation of available data indicated that DDT residue levels in human breast milk from Vietnam were among the highest values reported for Asian developing countries as well as for developed nations. This result suggests recent usage of DDTs in both north and south of Vietnam. In general, concentrations of OC compounds in primiparas were higher than those in multiparas, indicating that number of childbirth is an important factor influencing the OC burden in humans. Risk assessment studies indicated that current DDT levels approached the cautioning levels associated with adverse effects in children. Estimated infant daily intake of DDTs for some individuals exceeded the WHO guideline, which may raise concerns for children health.

Keywords

DDTs; PCBs; TCPMe; Human breast milk; Vietnam; infant health.

INTRODUCTION

Widespread contamination and toxic effects of persistent organic pollutants in humans and wildlife have been of great concern and received considerable attention during the past four decades. Despite the ban on persistent organochlorines (OCs) in most of the developed nations since the early 1970s, their usage continued until very recently in many developing countries for agricultural and public health purposes. As these compounds are highly lipophilic and persistent, human chronic exposure via food chain has led to the accumulation of both parent compounds and their metabolites in lipid rich tissues such as adipose tissues and human breast milk. Among human tissues, breast milk is a convenient sampling matrix for measuring residue concentrations of persistent OCs. The samples are easy to collect and highly suitable for estimating body burdens of persistent OCs, and thus may provide useful information about

their accumulation kinetics in humans. In addition, The OC residue concentrations in human breast milk are a key factor for evaluating the toxic potential of contaminants in infants.

Recently, hormone-like activities of some persistent OCs such as PCBs, DDTs, and HCHs have been suggested (Cheek *et al.*, 1999; Colborn *et al.*, 1993; Kelce, 1995; Vos *et al.*, 2000). These facts have raised public concern towards the adverse effects of such OCs to human health, especially for infants due to their susceptibility to environmental impacts. Walkowiak *et al.* (2001) observed association between postnatal exposure to PCBs and mental/motor development of children from 30 months onward. In the Netherlands, Vreugdenhil *et al.* (2002) reported a considerable association between prenatal exposure to PCBs and changing play-behavior of children. In the United States, Longnecker *et al.* (2001) reported a strong association between *p,p'*-DDE levels in mother's serum and the likelihood of premature birth and baby's weight at birth. These findings highlighted the role of persistent OCs, particularly PCBs and DDTs in the neuropsychological development in children.

Despite the fact that great efforts have been done worldwide to phase out the usage of OC insecticides such as DDT, HCHs and CHLs, some recent inputs of DDTs have been recorded in many developing countries like Mexico, India, Thailand and Vietnam (Pandit *et al.*, 2002; Stuetz *et al.*, 2001; Minh *et al.*, 2002; Nhan *et al.*, 1998). In Vietnam, recent input of DDTs to environment has been suggested in both northern and southern parts of the country. For example, relatively high levels of DDTs have been found in various environmental compartments such as surface waters, sediment (Hung *et al.*, 2002; Phuong *et al.*, 1998) as well as fish, mollusks and birds (Nhan *et al.*, 1998; Minh *et al.*, 2002). These elevated concentrations of DDTs could be a result of recent application of DDT for malaria control and, to a lesser extent, for sanitary purposes (Nhan *et al.*, 2001). However, comprehensive studies examining the contamination of persistent OCs in Vietnamese human as a result of bioaccumulation through food chains have not been made in recent years. Therefore, appropriate perspectives of the authorities and public to the possible adverse impacts of DDTs as well as other OCs on human health are still obscure.

Over the last few years, in the frame-work of the Asia-Pacific Mussel Watch Program, our laboratory has conducted comprehensive investigations on the distribution, behavior and fate of persistent OCs in various environmental compartments such as air, water, soils, sediments and biological samples (fish, mussels and birds) and foodstuff from Vietnam (Kannan *et al.*, 1992, 1995; Iwata *et al.*, 1994; Thao *et al.*, 1993; Monirith *et al.*, 2000; Minh *et al.*, 2002). Recently, through the Core University Program supported by the Japan Society for the Promotion of Science (JSPS) in which we are also involved, we collected human breast milk from the two big cities in Vietnam, Hanoi and Hochiminh and determined the concentrations of persistent OCs such as PCBs, DDTs, HCHs, CHLs, HCB and TCPMe to evaluate the status of contamination in Vietnamese population in comparison to other countries in the region. In addition, accumulation kinetics of OCs in Vietnamese human were studied. Potential risk for breast-fed infants due to OC exposure was also evaluated.

MATERIALS AND METHODS

Sample collection

Human breast milk samples were collected from two big cities in Vietnam; Hanoi ($n = 42$) and Hochiminh ($n = 54$) during the years 2000 and 2001. These milk samples were kept in ice immediately after collection, shipped to our laboratory in Japan with dry ice and preserved there at -20°C until analysis. We randomly selected the breast milk donors from two communities in Hochiminh city, Vinh Loc and Dong Thanh; and two communities in Hanoi, Me Tri and Tu Liem. Data on the biological characteristics of the donors and relevant information of sampling sites are given in Table 1.

Table 1: Related information of breast milk donors from Hanoi and Hochiminh city, Vietnam.

Location		Age	Weight (kg)	Height (cm)	Number of children	Number of pregnant	Occupation
Hanoi (2000, <i>n</i> = 42)	Mean	29	50	157	1.6	1.6	Housewife: 58%
	Range	20 - 44	40 - 63	150 - 166	1 - 7	1 - 7	Others: 42%
Hochiminh (2001, <i>n</i> = 44)	Mean	27	49	155	1.6	1.7	Housewife: 61%
	Range	18 - 37	31 - 64	140 - 170	1 - 5	1 - 7	Others: 39%

The biological characteristics show similarity between such cohorts. The informed consents were obtained from all the donors. Questionnaires on dietary aspects were recorded. Years of residence of the donors in their area varied widely from 1 to 37 years indicating that the cohorts actually consist of women from various places and not entirely represent only resident communities. However, the migrations were believed as regular and also within the regions of each city.

Chemical analysis

Chemical analysis of OCs in human milk followed the method described in Minh *et al.* (2004). The procedure comprise extraction using diatomite earth, gel chromatography column for clean up and lipid removal, fractionation using florisil wet column and quantification using gas chromatograph with electron capture detector (GC-ECD)GC-ECD and gas chromatograph equipped with mass selective detector (GC-MSD). The recovery rates of the target chemicals through this analytical method (*n* = 6) were 99.5% ± 3.4 for DDTs; 101% ± 4.9 for PCBs, 99% ± 2.5 for HCHs, 98% ± 2.1 for HCB, 101% ± 2.2 for CHLs, 108% ± 2.9 for TCPMe and 90% ± 5.9 for TCPMOH. Concentrations were not corrected for recovery rate. DDTs represents the sum of *p,p'*-DDT, *p,p'*-DDD and *p,p'*-DDE, and CHLs include *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, and oxychlordane. HCHs include α , $-\beta$ and γ -isomers. In this study, concentrations of α - and γ -HCHs were mostly below the detection limit (0.1 ng/g). Therefore, HCHs represent mainly concentrations of β -HCH. Similarly, TCPMOH was found only in less than thirty percent of the examined samples at levels above the detection limit of 1 ng/g lipid wt. Concentrations of OCs were expressed as ng/g on a lipid wt basis, unless otherwise specified.

Regarding quality assurance and quality control, our laboratory participated in the Intercomparison Exercise for Persistent Organochlorine Contaminants in Marine Mammal Blubber organized by the National Institute of Standards and Technology (1999, Gaithersburg, MD, USA) and Marine Mammal Health and Stranding Responses Program of the National Oceanic and Atmospheric Administration's National Marine Fisheries Service (Silver Spring, MD, USA). We analyzed Standard Reference Material SRM 1945 for selected PCB congeners and persistent OC insecticides. Our results were in good agreement with the standard reference values. However, this analytical method applied Soxhlet extraction, which is little different with solid phase extraction used for the human breast milk in the present study. The method for human breast milk was qualified by cross-checking analysis using corresponding real samples to compare with earlier result reported by Kunisue *et al.* (2002b) and standard deviations of approximately 15 percent for PCBs and chlorinated pesticides was considered as satisfactory result.

Statistical Analysis

Test for significant difference and correlation ($p < 0.05$) were performed using respectively, Mann-Whitney U test and Spearman test, which are available in StatView version 5 (SAS Inc., 1998).

Residue Levels and Accumulation Pattern

Lipid-normalized concentrations of OCs in human breast milk from Hanoi and Hochiminh city, Vietnam are given in Table 2. We considered the accumulation of OCs in 2 groups: mothers who give first child (primiparas) and mother who have more than one child (multiparas). In general, the residue pattern of OCs in human breast milk in Vietnam followed the order of DDTs > PCBs > HCHs > CHLs \approx HCB \approx TCPMe. Significant differences in OC levels between two cities were found only for HCHs. The pattern of OCs in human breast milk observed in this study is different to that found in Vietnamese foodstuff analyzed 10 years ago (Kannan *et al.*, 1992) or in human breast milk from Hochiminh city (Schechter *et al.*, 1989), which showed higher level of HCHs compared to PCBs. However, the present pattern is in accordance with those recently observed in birds collected from Red river estuary, (Minh *et al.*, 2002), mollusks from Hanoi region (Nhan *et al.*, 2001), and in biotic samples from Red river delta (Nhan *et al.*, 1998).

Table 2. Concentrations of organochlorines in human breast milk (ng/g lipid wt.) in Hanoi and Hochiminh city, Vietnam^a.

	Age (years)	Fat content (%)	PCBs	DDT compounds				CHL compounds				Δ HCH	HCB	TCPMe
				<i>p,p'</i> -DDE	<i>p,p'</i> -DDD	<i>p,p'</i> -DDT	DDTs	oxy	t-nona	c-nona	CHLs			
Hanoi														
Primiparas	27	2.5	76	2200	13	190	2400*	0.88	2.2	1.9	2.5	69	4.2	3.7
Multiparas	31	2.1	72	1500	8.9	150	1700*	0.92	0.86	0.3	1.4	46	3.5	3.9
Overall ^b	29	2.3	74	1900	11	170	2100	0.90	1.53	1.1	2.0	58	3.9	3.8
Range	20-44	0.7-5.7	26-210	420-6300	3-50	34-960	480-6900	0.25-1.6	<0.21-8.1	<0.21-3.2	<0.72-13	11-160	0.62-9.5	1.4-8.3
Hochiminh														
Primiparas	24	3.3	88	2700*	8.3	310	3020*	2.7	4.2	0.8	7.8	14	2.8	7.2
Multiparas	29	2.9	70	1300*	5.7	220	1500*	2.1	3.2	0.8	6.0	13	2.1	7.2
Overall	27	3.1	79	2000	7.0	265	2300	2.4	3.7	0.8	6.9	13.5	2.5	7.2
Range	18-37	1.5-6.9	29-200	340-16000	2.7-18	100-1000	440-17000	<0.25-9.3	1.3-15	<0.21-2.1	1.3-26	4.1-35	1.3-10	1.1-26

oxy: oxychlorane, t-nona: *trans*-nonachlor, c-nona: *cis*-nonachlor

DDTs = *p,p'*-DDE + *p,p'*-DDD + *p,p'*-DDT, CHLs = oxy + t-nona + c-nona

^a Arithmetic means were given; ^b Average concentration from primiparas and multiparas; * p = 0.056; + p < 0.05

The concentrations of PCBs are rather uniform in both the cities in accordance with those observed in foodstuffs from different parts of Vietnam (Kannan *et al.*, 1992). The global comparison of PCB residues in human breast milk is given in Table 3. We cited data from recent studies which used high resolution gas chromatography for quantification of individual PCB congeners. Although the cited data may differ between laboratories, it is possible to draw some relevant comparison to understand the magnitude of contamination. In comparison to other developing countries like Cambodia, India and the Philippines, residue levels of PCBs in human breast milk from Vietnam are slightly higher. However, these PCBs levels are still below those reported for developed countries (Table 3). Recent global inventory of PCBs production and consumption has indicated that common applications of PCBs (i.e. for industrial purposes) in Vietnam during the past years were not higher than those in China, Hong Kong, India and the Philippines (Breivik *et al.*, 2002). Hence, the higher PCB residues observed in human breast milk from Vietnam suggest additional sources of PCBs besides industrial sources like transformers, capacitors, etc. A likely source of PCBs in Vietnam could be the release from different kinds of military weapons used extensively during the Vietnam War as suggested earlier (Thao *et al.*, 1993).

Mean concentrations of DDTs in human breast milk were 2100 ng/g and 2300 ng/g in Hanoi and Hochiminh city, respectively and the levels in primiparas group were higher than those in multiparas group (Table 2). This difference indicates that excretion via milk during lactation is an important factor reducing DDT burden in nursing mothers. Further examination of the DDTs composition revealed that *p,p'*-DDE is the predominant compound accounting for 85 to 90 % of the total DDT concentrations. Interestingly, in both cities, the proportion of *p,p'*-DDT was higher in multiparas than those in primiparas. We observed that time interval between the first and the second child of a mother in both the cohorts is usually short around 3 years.

Therefore, the higher proportion of *p,p'*-DDT accumulated in multiparas group could be interpreted as an evidence for continuous intake of DDTs.

Table 3. Comparison of organochlorine residues in human breast milk from various countries (ng/g lipid wt.)

Country	Year of Sampling	n	PCBs	DDTs	CHLs	HCHs	HCB	Reference
<i>Industrialized countries</i>								
Australia	1995	60	500	1200	-	350	-	Quinsey, et al., 1995
Czech Republic	1996	17	1160	1050	-	70	-	Schoula, et al., 1996
Germany	1995-1997	246	550	240	-	40	80	Schade, et al., 1998
Turkey	1995-1996	104	-	2100	-	457	50	Cok, et al., 1997
Russia	1996-1997	140	380	1040	37	280	91	Polder, et al., 2003
Sweden	1997	40	324	143	-	-	12	Noren, et al., 2000
Ukraine	1993-1994	197	594	2700	38	730	168	Gladen, et al., 1999
UK	1997-1998	168	-	470	-	103	43	Harris, 1999
U.S. (Massachusetts)	1993	122	320	-	-	-	-	Korrick et al., 1998
Japan	1998	49	200	290	85	210	14	Konishi, et al., 2001
<i>Developing countries</i>								
Cambodia	2000	28	42	1600	1.8	5.5	1.7	Kunisue, et al., 2002b
China (Guangzhou)	2000	54	33	3550	-	1110	-	Wong, et al., 2002
China (Hongkong)	1999	132	42	2870	-	950	-	Wong, et al., 2002
India	2000	8	30	420	0.9	650	1.0	Kunisue, et al., 2002b
Philippines	2000	10	72	190	15	4.7	-	Kunisue, et al., 2002b
Vietnam (North)	2000	42	74	2100	2.0	58	3.9	Present study
Vietnam (South)	2001	54	79	2300	6.9	14	2.5	Present study
Mexico	1998	60	-	4100	-	60	30	Waliszewski, et al., 2001

- data not available

Elevated concentrations of DDTs in various environmental compartments in Vietnam have also been reported in a number of recent investigations. Results of the Asia-Pacific Mussel Watch Program indicated that DDT concentrations in mussels and fish from Vietnamese coastal waters are among the highest values reported for the countries in this region (Kannan *et al.*, 1995; Monirith *et al.*, 2000; Minh *et al.*, 2002). Interestingly, Nhan *et al.* (2001) reported higher levels of DDTs in sediments from populated locations in Hanoi as compared to those from paddy fields in Red river delta. This evidence indicates recent applications of DDTs for other purposes such as sanitary and malaria control rather than for agriculture. The widespread and elevated contamination of DDTs in the environment and food chains may explain the high residue levels found in human breast milk from Vietnam.

To understand the magnitude of DDT contamination in Vietnamese population, residue levels of DDTs in human breast milk in different countries were compiled (Table 3). Interestingly, DDT concentrations in Vietnamese human breast milk were among the highest values reported for the countries surveyed. This observation again suggests the recent use of DDTs in Vietnam and that Vietnam may be a potential source of DDTs in the south Asian region. In general, DDT levels in human breast milk were observed to be higher in developing countries than those in developed nations (Table 3), which could be due to the recent use of this insecticide for malaria control and, to a lesser extent, for sanitary purposes in developing countries. In this context, the role of the south Asian region as an emission source to pristine areas in higher latitudes deserves further monitoring studies. Our recent study on seasonal variations of persistent OCs in migratory birds from Lake Baikal wintering in south eastern Asian region has indicated elevated exposure to DDTs and HCHs in the southern wintering sites, suggesting the south Asian region as a potential source of OC insecticide contamination for higher latitude areas (Kunisue *et al.*, 2002a).

The spatial distribution of HCHs in our study are somewhat in agreement with those reported in earlier studies showing higher HCHs levels in Hanoi compared to Hochiminh (Thao *et al.*, 1993; Iwata *et al.*, 1994; Kannan *et al.*, 1995). There are two possible reasons for this spatial pattern: (i) due to the lower latitudinal position, Hochiminh city has typical tropical climate which perhaps facilitates more volatilization of HCHs; (ii) on the other hand, Hanoi city is located at higher latitude toward the north and close to China, the world's largest producer/user of HCHs (Li *et al.*, 1998). Thus, higher export of HCHs to these areas leading to higher concentrations in environment and biota could be expected. In the global comparison, human breast milk from North Vietnam showed intermediate levels of HCHs, which are lower than those in China, Hong Kong, India and Japan but higher than those in other Asian developing

countries like Cambodia and the Philippines (Table 3). Besides, our recent study on birds demonstrated that HCH concentrations in migratory birds collected from Vietnam were significantly higher than those in resident birds, which could be due to the accumulation in stopover sites in India and South China (Minh *et al.*, 2002).

Specific accumulation according to parity and age

It is known that adult female excrete lipophilic contaminants such as OCs via lactation and thus reducing the body burden of such contaminants. In this study, we observed higher concentrations of OCs in human breast milk of primiparous mothers as compared to multiparas. Figure 1 shows the relationship between parity and DDTs concentrations in human breast milk from Hanoi and Hochiminh cities. The relationship however, is less pronounced for other compounds like PCBs and HCHs (data not shown), probably due to the lower background levels. Indeed, the present result provides another evidence for the influence of lactation on OC burden of nursing women (Albers *et al.*, 1996).

Because of the potential influence of parity on OCs levels, we have examined the correlation between residue levels and mother's age independently for the groups of primiparas and multiparas. In both groups, strong correlations of contaminant levels and age were observed. However, only DDTs levels of multiparas group in Hochiminh city significantly correlated with age ($p < 0.05$; Spearman test). Nevertheless, slopes of the regression lines for DDTs and mother's age (Fig. 2) are quite similar regardless of the differences in groups and areas. This result would probably be interpreted as an evidence of similar intake rates of DDTs in Vietnamese mothers. Besides, it should be noted that other factors such as length of lactation, time of sampling during breast-feeding etc. could also influence the OC levels in women. For instance, Schecter *et al.* (1998) reported that DDE and HCB levels in breast milk of a mother nursing twins might reduce approximately 80 and 90 percent respectively, during thirty-eight month period.

This lactation period is probably longer compared to those in Vietnamese cohort, which is six months, typically. However, lack of information regarding length of lactation and time of sampling during breast-feeding, etc. in the Vietnamese cohort did not allow us to further examine their influence to the OC levels. Further investigation towards these effects would be necessary in future study. Nevertheless, the available data from this study probably indicates that parity and age play important roles in controlling the OC burden in humans.

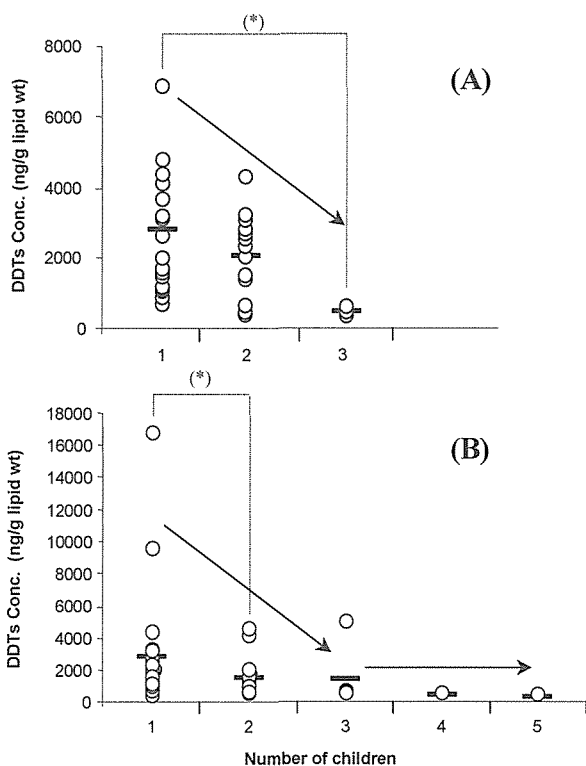


Figure 1. Concentrations of DDTs in maternal milk versus the corresponding number of children (A: Hanoi, B: Hochiminh). The short horizontal bars indicate mean concentration in each group and the stars (*) indicate significant difference ($p < 0.05$).

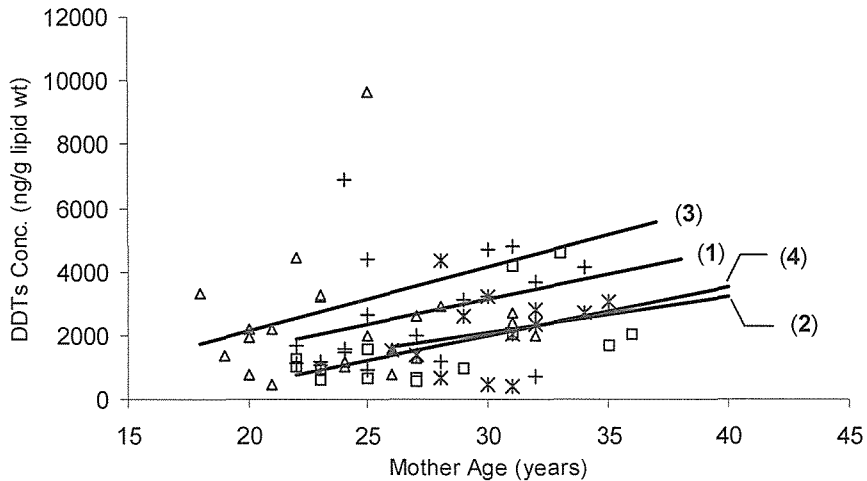


Figure 2. Relationship between DDT concentration and mother's age. The cross mark (+) and line 1 indicate primiparas group in Hanoi; the stars (*) and line 2 the multiparas in Hanoi. Similarly, the triangles (Δ) and line 3 indicate primiparas group in Hochiminh; the squares (\square) and line 4 the multiparas in Hochiminh.

Tolerable daily intake

Daily intake of OCs by infants was calculated based on the assumption that the average milk consumption of a 5 kg infant is 700 g/day (Oostdam *et al.*, 1999). The mean values of daily intake of OCs were estimated by using the following equation:

$$DI = \frac{C_{milk} \times 700g \times C_{Lipid}}{5}$$

where DI is daily intake ($\mu\text{g}/\text{kg}$ body wt./day); C_{milk} : concentration of the chemical in milk ($\mu\text{g}/\text{g}$ lipid wt); C_{lipid} : lipid content in milk (%).

The estimated daily intakes are given in Table 4 and individual intakes are shown in Figure 3. It was recognized that although intake of DDTs by most infants is below the guideline proposed by Health Canada (Oostdam *et al.*, 1999) in average, intake by some individuals is close to or exceeds this guideline. This fact may raise greater concerns on infant health because children are highly susceptible to effects from environmental contaminants.

Table 4. Estimated daily intake ($\mu\text{g}/\text{kg}$ body wt./day) of Ocs by infants in Vietnam

	PCBs	DDTs	CHLs	HCHs	HCB
Hanoi	0.25	7.0	0.01	0.17	0.01
range	0.03 - 1.2	0.8 - 27	<0.003 - 0.05	0.05 - 0.43	<0.003 - 0.04
Hochiminh	0.34	11.0 ^a	0.03	0.06	0.01
range	0.11 - 0.94	1.3 - 110	<0.003 - 0.08	0.01 - 0.31	<0.003 - 0.04
TDIs^b	1.0	20	0.05	0.3	0.27

^a Medial value 7.2; ^b cited from Oostdam *et al.*, 1999 (Health Canada guideline)

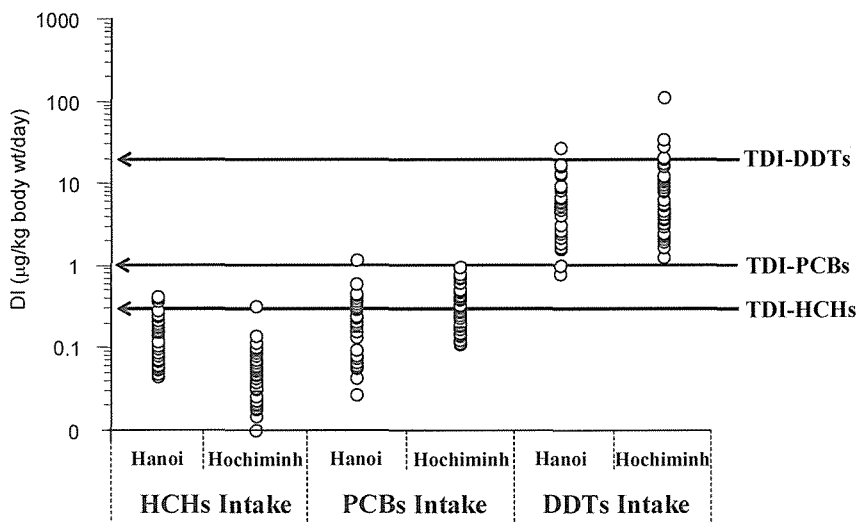


Figure 3. *Estimated daily intake of PCBs and DDTs by infants in Hanoi and Hochiminh city in comparison with the Tolerable Daily Intake (TDI) proposed by Health Canada (Oostdam et al., 1999).*

CONCLUSIONS

To our knowledge, this is the most recent and extensive studies on the contamination by persistent OCs in human breast milk from Vietnam. Our data clearly indicate the recent usage of DDTs in both North and South of Vietnam. Number of childbirth and age of mothers played an important role in influencing the OC burdens in lactating women. The current status of DDT contamination in Vietnamese population is still high with a number of those even being close to the threshold level reported to cause incidences of pre-term birth. Estimated breast-fed children intakes of some chemicals exceeded the guideline proposed by Health Canada. Comprehensive studies on the temporal trends of OCs in Vietnam are therefore necessary to understand the source and evaluate possible long-term impacts of OCs in tropical ecosystem.

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