GEOGRAPHICAL DISTRIBUTION, ACCUMULATION KINETICS AND INFANTS HEALTH RISK OF ORGANOCHLORINES IN HUMAN BREAST MILK FROM INDONESIA

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Introduction

Worldwide production and use of organochlorine compounds (OCs) have led to their widespread occurrence in the environment and bioaccumulation in various organisms, including humans. In Indonesia, large usage and production of OCs in the past, particularly OCs pesticides for agricultural and vector-borne disease eradication programs may implicate contaminations of OCs in the environment. Previous studies dealing with mussels as bioindicator reported widespread occurrence of OCs in the coastal environment of this country, and found hot spots of contamination in the waters surroundings Java Island [1)]. Occurrence of OCs were also reported in various environmental compartments including fish [2)], sediment and air [3)]. However, data on levels of OCs in humans are very scarce [4)]. Hence this study has highlighted the accumulation of OCs in human milk from Indonesia, particularly in Java Island where industrial and intensive agriculture are taking place.

In this study, concentrations of classical OCs, such as polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethanes and its metabolites (DDTs), hexachlorocyclohexane isomers (HCHs), chlordane compounds (CHLs), hexachlorobenzene (HCB), and the most recently identified microcontaminants, *tris* (4-chlorophenyl) methanol (TCPMOH) and *tris* (4-chlorophenyl) methane (TCPMe) were determined in human breast milk collected from several locations in Indonesia to elucidate their distribution in relation to their site activities, to assess their possible association with maternal characteristics and to evaluate the possible potential risk of OCs in breast-milk on infant's health.

Materials and Methods

Sample collection: Breast milk samples were collected from four locations having different activities during 2001-2003 (Figure 1), three locations in Java Island, namely Jakarta (major dumping site, urban), Purwakarta (agriculture, rural), Bogor (city, sub-urban) and one location in Sumatra Island, Lampung (coastal area, rural). The donors completed informed consent form and

an exposure assessment questionnaire. The biological characteristics revealed similarity between such cohorts. Half of the donors were primiparae (27/55) and none of the mothers was a smoker.

Analytical methods: PCBs, DDTs, HCHs, CHLs, HCB, TCPMe and TCPMOH were analyzed following the method published elsewhere [5]. Briefly, about 10 g of sample was added to the extraction column (2 cm diameter) packed with pre-cleaned diatomite earth (Merck, Damstadt, Germany) and eluted by diethyl ether. An aliquot of extract was added to a gel permeation chromatography column for lipid removal. The GPC fraction containing OCs was concentrated and passed through an activated Florisil column for fractionation. Quantification of PCBs and OC pesticides was performed using a GC (Hewlett Packard 6890 series) equipped with an ECD. Identification and quantification of TCPMe and TCPMOH were performed using a GC-MS (HP6890 and HP5973) in selective ion monitoring mode [5].

Statistical analysis: Test for significant difference between groups and correlation between parameters ($p<0.05$) were performed using Mann-Whitney *U* test and Spearman rank correlation, respectively, which are available in Stat View program version 5 (SAS Inc., 1998).

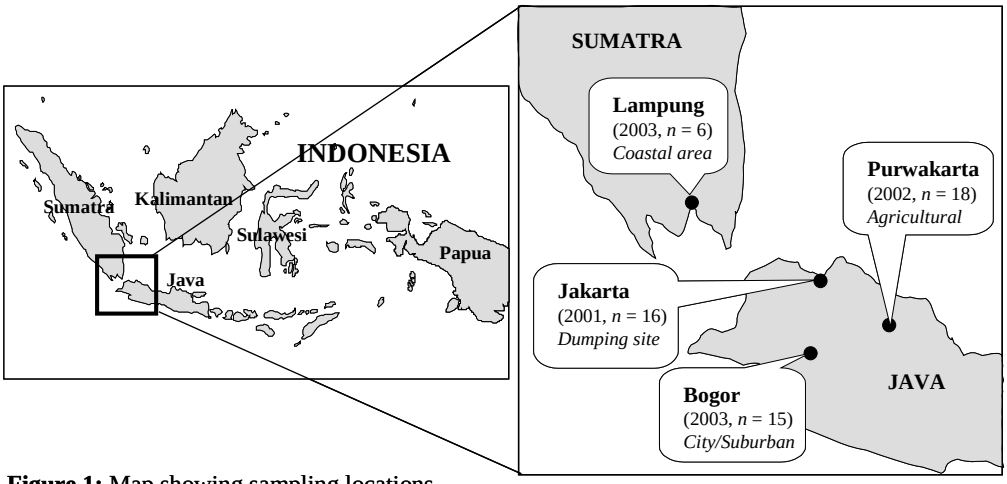

Figure 1: Map showing sampling locations.

Results and Discussion

Contamination status and geographical differences: Except TCPMOH which was present only in few samples (10% of the samples, data not shown), PCBs, DDTs, CHLs, HCHs, HCB and TCPMe were detected in all the human milk examined (Table 1), suggesting that the residents in Indonesia have been exposed to these contaminants. DDTs were the predominantly identified compounds in all the locations up to 15000 ng/g lipid wt. followed by other compounds in the order

of HCHs>PCBs>CHLs>TCPMe>HCB with concentrations 1-3 orders of magnitude less than those of DDTs. This fact highlighted the contamination by DDTs as one of the major problems in Indonesian human. Among locations, DDTs were significantly higher in sub-urban (Bogor) and rural sites (Purwakarta and Lampung) than in urban area (Jakarta). This is probably because of their intensive usage in both agricultural activities and malaria control program in sub-urban and rural sites. Differences in food preference between individuals may also be responsible for variability of DDTs in human breast milk. On the other hand, PCBs, HCHs, CHLs and HCB seemed to indicate little spatial variability which might be due to the low levels of accumulation in Indonesian breast milk corresponding to their background residue levels in the environment. In fact, recent monitoring study using mussels as bioindicator also found low levels of these contaminants in the coastal areas of Indonesia [1]. With regard to TCPMe, the most recently reported persistent organic pollutant in biotic and abiotic samples for which the principal source is still unclear [6], a significant positive correlation was found between concentrations of TCPMe and DDTs in the breast milk of this study ($p<0.001$), supporting the hypothesis that human exposure to TCPMe was derived likely from the use of technical DDTs [6]. Positive correlations were also observed between concentrations of TCPMe and other OCs, indicating that the bioaccumulative nature of TCPMe is similar to other OCs, such as DDTs and PCBs [7]. Occurrence of TCPMe in almost all the samples analyzed indicate widespread contamination by these compounds in the general population of Indonesia.

Table 1: Concentrations of organochlorines (ng/g lipid wt.) in breast milk from Indonesia[a].

Location	Age (Years)	Fat (%)	PCBs	DDTs	HCHs	CHLs	*HCB*	TCPMe
Jakarta (*n*=16; 2001)[b]	26 (15-35)[c]	2.5 (0.63-4.9)	33 (6.7-250)	630 (20-7100)*	14 (1.6-120)	2.0 (0.24-5.4)	2.2 (0.53-7.0)	2.8 (nd-27)
Purwakarta (*n*=18; 2002)	27 (21-40)	1.7 (0.56-3.9)	24 (4.4-99)	1300 (39-15000)	30 (1.6-130)	7.7 (0.33-48)	1.8 (0.42-3.8)	3.3 (nd-16)
Bogor (*n*=15; 2003)	26 (18-38)	3.1 (1.1-5.8)	21 (9.3-53)	1100 (120-5800)	11 (0.61-31)	1.8 (0.27-3.5)	1.8 (0.41-4.5)	4.8 (0.72-27)
Lampung (*n*=6; 2003)	29 (27-32)	2.3 (0.96-4.3)	27 (12-54)	1000 (350-2000)	11 (2.2-18)	2.7 (0.90-6.4)	2.3 (0.67-3.7)	9.3 (4.8-15) *

Note: [a] *arithmetic means;* [b] sampling year and *n* indicates number of samples; [c] values in parenthesis indicates range concentrations*;* DDTs = *p,p'*-DDE+ *p,p'*-DDD+ *p,p'*-DDT; CHLs = oxychlordane+*trans*-nona+*cis*-nona; HCHs =β-HCH; *$p<0.05$.

In comparison with worldwide OCs (see Minh *et al.*; Table 2) [7], human breast milk from Indonesia contained apparently higher levels of DDT but low levels of PCBs, HCHs, CHLs and HCB almost similar to those in other developing countries. This result may suggest continuing DDT usage in Indonesia. In fact, higher *p,p'*-DDT proportion than other derivatives of DDTs in some samples from Purwakarta may suggest recent exposure of DDT in general population of Indonesia.

Table 2: Comparison of OCs levels (ng/g lipid wt) in human milk over the world [7].

Country	Year	*n*	PCBs	DDTs	CHLs	HCHs	HCB
Germany	1995-1997	246	550	240	-	40	80
Sweden	1997	40	324	143	-	-	12
UK	1997-1998	168	-	470	-	103	43
Canada	1992	497	238	244	34	24	15
Australia	1995	60	500	1200	-	350	-
Japan	1998	49	200	290	85	210	14
Russia	1996-1997	140	380	1040	37	280	91
Ukraine	1993-1994	197	594	2700	38	730	168
India	2000	8	30	420	0.9	650	1.0
China	1999-2000	186	37	3200	-	1000	-
Cambodia	2000	49	25	1500	1.6	5.2	1.7
Philippines	2000	10	72	190	15	4.7	-
Vietnam	2000-2001	48	76	2200	4.5	36	3.2
Indonesia*	2001-2003	55	26	1000	3.6	17	2.0

n: number of samples, -: data not available, *: the present study

Dependence accumulation on parity and age: In lactating women, several factors have been reported to have association with the concentrations of OCs in human breast milk such as parity, age of mother, food intake preferences, period of breast-feeding and several other external factors [8]. In this study, we examined the association of OCs concentrations with the corresponding number of children and age of mothers. Concentrations of DDTs significantly decrease ($p<0.05$) in human milk with increasing number of children (Figure 2a). This result suggests number of previous children as an important factor for the OCs reduction in nursing women. Furthermore, significant positive correlation between concentration of DDTs and age of mothers ($p<0.05$) was also observed (Figure 2b), suggesting age as an important factor on bioaccumulation of OCs during women's life. Although other OC compounds were relatively low in breast milk of this study, the similar association trends were also observed (data not shown). Thus, number of children and age of mother were important factors controlling OCs body burden in lactating women of this study.

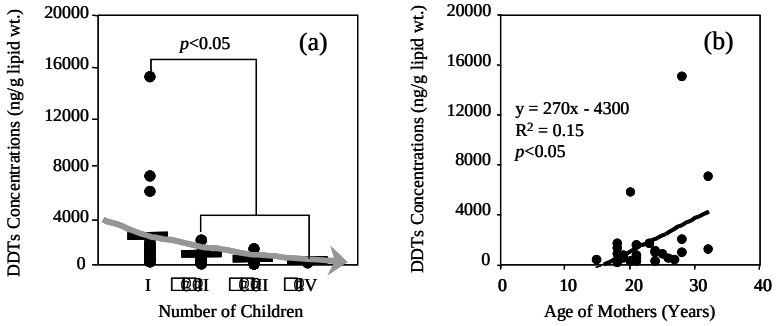

Figure 2: Relationships between number of children (a), age of mother (b) and DDTs concentrations.

Infant's health risk: Epidemiological studies conducted in Netherlands [9] and Germany [10] found that PCBs residues in human breast milk (400 ng/g lipid wt.) were associated with adverse health effect in children, such as neurodevelopment impairments. In this study, PCBs in Indonesian

human breast milk are still far below those reported as the harmful threshold limits for infants. However, daily intake (DI) of some OCs in some individuals were close to or exceeded the guideline standards proposed by WHO and Health Canada [5] (Figure 3). This fact may raise greater concern on infant health because they are highly susceptible to effects imposed by various environmental contaminants.

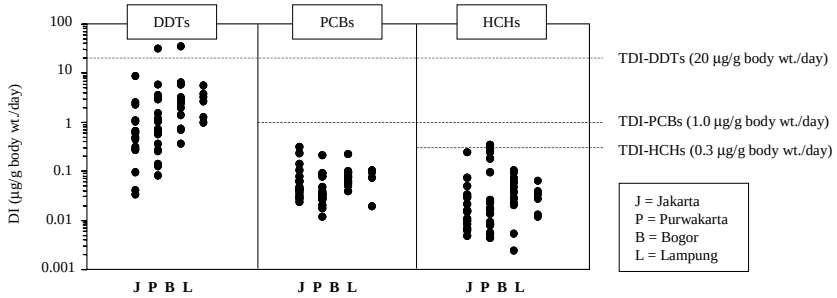

Figure 3: Individual daily intake (µg/g body wt./day) of DDTs, PCBs and HCHs by infants.

Overall, these results highlight the need for more detailed investigation on the levels of POPs in human as well as food, and other major routes of exposure of POPs for the general Indonesian population.

Acknowledgements
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