Effects of Lactational Exposure to Chlorinated Dioxins and Related Chemicals on Lymphocyte Subpopulations in Japanese Babies

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Abstract

Effects of lactational exposure to polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar polychlorinated biphenyls (Co-PCBs) on lymphocyte subpopulations were investigated in the peripheral blood of 69 breast-fed Japanese babies. As a result, estimated total intakes of PCDDs, PCDFs and Co-PCBs in toxic equivalent quantity (TEQ) converted into 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) from the breast milk significantly and negatively correlated with the percentages of CD8-positive (suppressor/cytotoxic) lymphocytes in the blood of breast-fed babies ($p=0.043$). Consequently, the ratios of CD4-positive (helper/inducer) lymphocytes to CD8-positive lymphocytes in percentages in the peripheral blood showed increasing tendency with the estimated total TEQ intakes ($p=0.062$). Therefore, it is considered that exposure to background levels of PCDDs, PCDFs and Co-PCBs through breast milk may cause some immunologic disturbance like atopic dermatitis.

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Introduction

Human beings have been contaminated with extremely toxic PCDDs, PCDFs and Co-PCBs[1)2)3)] Consequently, these chemicals have been also determined in the human breast milk. According to their levels in the breast milk, breast-fed babies in Japan are considered to have relatively a large amount of these chemicals, namely, about 100 to 200 TEQ pg/kg body weight/ day[2)]. Babies seem more sensitive to the toxic chemicals, so we should give due attention to their possible health consequences in breast-fed babies.

In order to clarify the effects of lactational exposure to PCDDs, PCDFs and Co-PCBs on the immune system, we investigated the lymphocyte subpopulations in the peripheral blood of 69 babies in relation to their intakes from breast milk.

Experimental Methods

Eighty two mothers volunteered to participate in all in this study and they had a normal pregnancy without use of medicines. Breast milk (50~100mℓ), sampled 2 to 3 months after childbirth, was used to determine concentrations of PCDDs, PCDFs and Co-PCBs by high resolution GC-MS method and the amounts of TEQ were calculated in the breast milk using the international toxic equivalent factors (TEF) for PCDDs and PCDFs, and the TEFs by WHO-ECEH for Co-PCBs[2)3)].

About 1 year after birth, 5 to 10mℓ of peripheral blood samples were individually obtained from 69 breast-fed babies. These blood samples were used to measure lymphocyte subpopulations by indirect immunofluorescence using monoclonal mouse anti-human antibodies against CD3 (mature T-lymphocytes), CD4 (helper/inducer T-lymphocytes), CD8 (suppressor/cytotoxic T-lymphocytes), CD4 + CD8, CD16 (natural killer T-lymphocytes), CD20 (B-lymphocytes) and HLA-DR (activated T-lymphocytes), and their relative population densities were calculated[4)].

Total TEQ intakes (ng/kg body weight) were estimated by multiplying daily TEQ intakes (pg/kg body weight) of PCDDs, PCDFs and Co-PCBs as a whole from the breast milk, which were calculated with their TEQ levels in the breast milk times an expected intake of breast milk in Japanese baby, namely, 120g/kg body weight, by individual duration of breast feeding (days).

Analysis of variance (ANOVA) was applied to examine the relationship of the estimated total TEQ intakes of PCDDs, PCDFs and Co-PCBs from the breast milk to each variable of interest and statistical significance was evaluated by Student's *t*-test.

Results

1) Concentrations of PCDDs, PCDFs and Co-PCBs in the breast milk

Respective distributions in total concentrations of PCDDs, PCDFs and Co-PCBs as TEQ on the whole and fat weight bases are indicated in Fig. 1. Median concentrations on the whole and fat weight bases were 0.95 and 25.7ppt, respectively. The range of concentrations on the whole weight basis was 0.27 to 2.53ppt and that on the fat weight basis 8.6 to 48.5ppt.

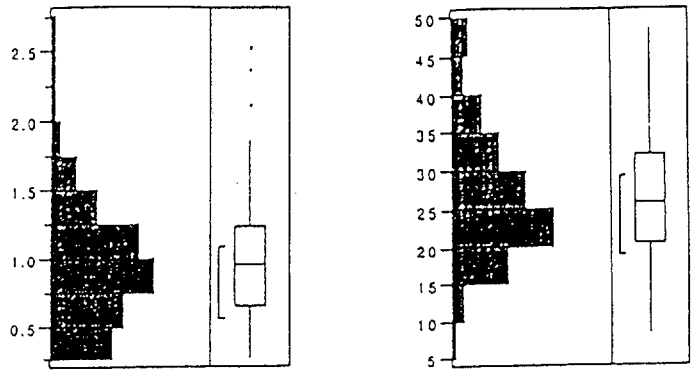

Fig. 1. Distributions in total concentrations (ppt) of PCDDs, PCDFs and Co-PCBs as TEQ on the whole (left) and fat (right) weight bases in the breast milk of 82 mothers

2) Estimated intakes of PCDDs, PCDFs and Co-PCBs from the breast milk

The distribution in estimated total intakes of PCDDs, PCDFs and Co-PCBs as TEQ in 75 breast-fed babies is shown in Fig.2. The median intake was 29.0 ng/kg and the range was 6.1 to 83.6 ng/kg.

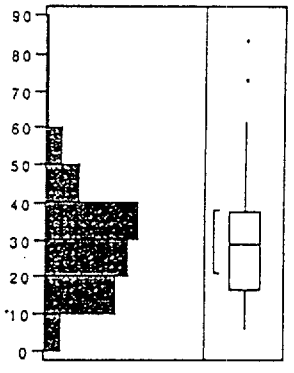

Fig. 2. Distribution in estimated total TEQ intakes (ng/kg) of PCDDs, PCDFs and Co-PCBs in 75 breast-fed babies

3) Percentages of lymphocyte subpopulations in the peripheral bloof of breast-fed babies

As shown in Table 1, median percentages of lymphocyte subpopulations in the blood of 69 breast-fed babies were as follows. Mature T-lymphocytes (CD3) was the highest, 60.4%, helper/inducer T-lymphocytes (CD4) : 39.5%, activated T-lymphocytes (HLA-DR) : 26.0%, B-lymphocytes (CD20) : 22.7%, suppressor/cytotoxic T-lymphocytes (CD8) : 19.1%, natural killer T-lymphocytes (CD16) : 8.6% and T-lymphocytes possitive to both CD4 and CD8 the lowest, 0.5%. The median ratio of CD4/CD8 was 2.05. Distributions in percentages of CD8-positive

lymphocytes and in the ratios of CD4/CD8 in percentages were shown in Fig. 3.

Table 1. Percentages of lymphocyte subpopulations in the peripheral blood of 69 breast-fed babies

Lymphocyte Subpopulation (Positive Cells)	Median (min. ~ max.) Percentage
CD3	60.4 (31.2 ~ 76.6)
CD4	39.5 (15.7 ~ 60.4)
CD8	19.1 (10.6 ~ 41.2)
CD4 + CD8	0.5 (0.2 ~ 2.1)
CD16	8.6 (1.7 ~ 23.3)
CD20	22.7 (5.5 ~ 56.2)
HLA-DR	26.0 (8.2 ~ 62.1)
CD4/CD8	2.05 (0.62 ~ 3.65)

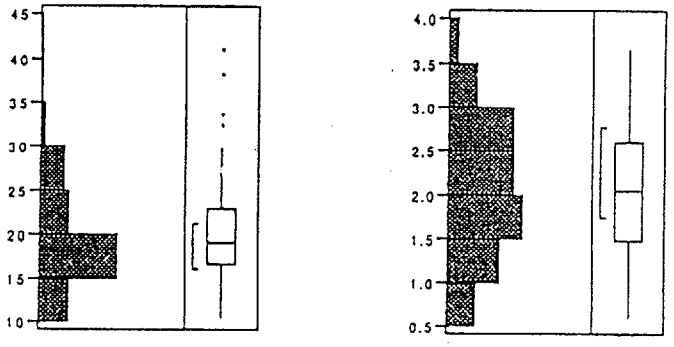

Fig. 3. Distributions in percentages of CD8-positive lymphocytes (left) and in the ratios of CD4/CD8 in percentages (right) in the peripheral blood of 69 breast-fed babies

4) Correlation between the estimated total intakes of PCDDs, PCDFs and Co-PCBs from the breast milk and the peripheral lymphocyte subpopulations in breast-fed babies.

Estimated total TEQ intakes of PCDDs, PCDFs and Co-PCBs from the breast milk significantly and negatively correlated with the percentages of CD8-positive lymphocytes in the blood of the babies ($p=0.043$). Consequently, as indicated in Fig. 4, the ratios of CD4 to CD8 in percentages in the peripheral blood showed increasing tendency with the estimated total TEQ intakes ($p=0.062$).

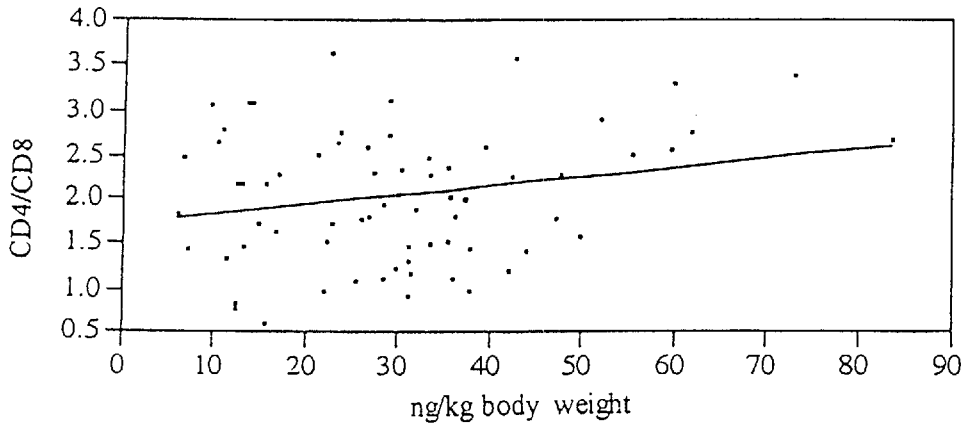

Fig. 4. Correlation between the ratios of CD4- to CD8-positive lymphocytes in percentages in the peripheral blood and the estimated total TEQ intakes in 69 breast-fed babies (p=0.062)

Discussion

The presence of PCDDs, PCDFs and Co-PCBs in the breast milk results in daily intakes of about 32 to 304 pg/kg body weight as TEQ with the median figure of 114 pg/kg body weight. Conse- quently, the babies have been estimated to take 6 to 84 TEQ ng/kg body weight with the median of 29 ng/kg body weight during whole breast-feeding periods (Fig.2).

Probably due to such kinds of relatively great TEQ intakes, we observed negatively significant correlation between the percentages of CD8-positive lymphocytes in the blood of the breast-fed babies and the estimated total TEQ intakes. Accordingly, as indicated in Fig. 4, the ratios of CD4 to CD8 seemed to proportionally increased with the estimated TEQ intakes.

Recently, no relationship between pre- and postnatal PCB/Dioxin exposure and upper or lower rispiratory tract symptoms or humoral antibody production was reported[5]. However, babies with higher CD4/CD8 ratios than 2.5 or 3.0 will be expected to become atopic dermatitis in future with high possibility (personal communication).Therefore, the results of this study suggest that exposure to background levels of PCDDs, PCDFs and Co-PCBs via breast milk may cause some immunologic disturbance such as atopic dermatitis.

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Effects of Lactational Exposure to Chlorinated Dioxins and Related Chemicals on Thyroid Functions in Japanese Babies

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Abstract

Effects of lactational exposure to polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar polychlorinated biphenyls (Co-PCBs) on thyroid functions were studied in the peripheral blood of 71 breast-fed Japanese babies. Estimated total intakes of PCDDs, PCDFs and Co-PCBs as a whole in toxic equivalent quantity (TEQ) converted into 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) from the breast milk significantly and negatively correlated with the levels of thyroxine (T_4) and positively correlated with the levels of thyroid stimulating hormone (TSH) in the blood of breast-fed babies. Therefore, exposure to background levels of PCDDs, PCDFs and Co-PCBs through breast milk may cause some effects on thyroid functions in Japanese babies.

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Introduction

Human beings have been contaminated with extremely toxic PCDDs, PCDFs and Co-PCBs[1)2)3)]. Consequently, these chemicals have been also determined in the human breast milk. According to their levels in the breast milk, breast-fed babies in Japan are considered to have relatively a large amount of these chemicals, namely, about 100 to 200 TEQ pg/kg body weight/day[2)]. Babies seem more sensitive to the toxic chemicals, so we should give due attention to their possible health consequences in breast-fed babies.

In order to clarify the effects of lactational exposure to PCDDs, PCDFs and Co-PCBs on thyroid functions, we investigated the levels of thyroid hormones, TSH and thyroxine binding globulin (TBG) in the peripheral blood of 71 babies in relation to their intakes from breast milk.

Experimental Methods

Eighty two mothers volunteered to participate in all in this study and they had a normal pregnancy without use of medicines. Breast milk (50~100mℓ), sampled 2 to 3 months after childbirth, was used to determine concentrations of PCDDs, PCDFs and Co-PCBs by high resolution GC-MS method and the amounts of TEQ were calculated in the breast milk using the international toxic equivalent factors (TEF) for PCDDs and PCDFs, and the TEFs by WHO-ECEH for Co-PCBs[1)2)3)].

About 1 year after birth, 5 to 10mℓ of peripheral blood samples were individually obtained from 71 breast-fed babies. These blood samples were used to determine serum concentrations of triiodothyronine (T_3), T_4, TSH and TBG by radioimmunoassay methods using commercially available kits[4)].

Total TEQ intakes (ng/kg body weight) were estimated by multiplying daily TEQ intakes (pg/kg body weight) of PCDDs, PCDFs and Co-PCBs from the breast milk, which were calculated with their TEQ levels in the breast milk times an expected intake of breast milk in Japanese baby, namely, 120g/kg body weight, by individual duration of breast feeding (days).

Analysis of variance (ANOVA) was applied to examine the relationship of the estimated total TEQ intakes of PCDDs, PCDFs and Co-PCBs from the breast milk to each variable of interest and statistical significance was evaluated by Student's *t*-test.

Results

1) Concentrations of PCDDs, PCDFs and Co-PCBs in the breast milk

Respective median concentrations of PCDDs, PCDFs and Co-PCBs as TEQ on the whole and fat weight bases were 0.95 and 25.7ppt as a whole in the breast milk of 82 mothers. The ranges of concentra- tions on the whole and fat weight bases were 0.27 to 2.53ppt and 8.6 to 48.5ppt, respectively.

2) Estimated intakes of PCDDs, PCDFs and Co-PCBs from the breast milk

The median estimated total intakes of PCDDs, PCDFs and Co-PCBs as TEQ was 29.0 ng/kg in 75 breast-fed babies and the range was 6.1 to 83.6 ng/kg.

3) Thyroid function tests in breast-fed babies

Results of the examination in thyroid functions in the serum of 71 breast-fed babies are summarized in Table 1.

Table 1. Results of thyroid function tests in the serum of 71 breast-fed babies

	Median (min. ~ max.)
Triiodothyronine (T_3, ng/mℓ)	2.00 (1.00 ~ 2.50)
Thyroxine (T_4, μg/dℓ)	11.3 (7.7 ~ 16.4)
Thyroid stimulating hormone (TSH, μU/mℓ)	2.59 (0.56 ~ 8.51)
Thyroxine binding globulin (TBG, μg/mℓ)	25.4 (17.2 ~ 37.9)
T_4/TBG	0.45 (0.30 ~ 0.61)

4) Correlation between the estimated total intakes of PCDDs, PCDFs and Co-PCBs from the breast milk and thyroid functions in breast-fed babies.

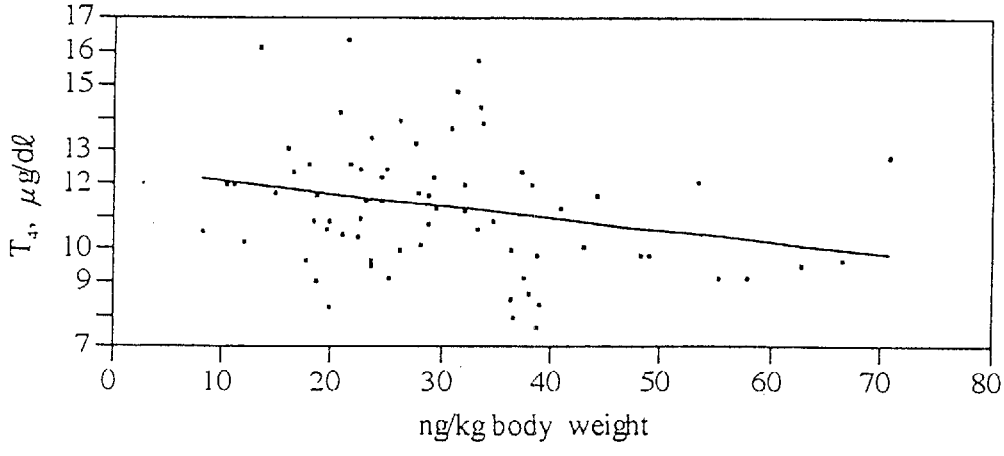

Fig. 1. Negative correlation between the estimated total TEQ intakes from the breast milk and the levels of T_4 in the serum of breast-fed babies (p=0.033)

Significant negative correlation was observed between estimated total intakes of PCDDs, PCDFs and Co-PCBs as TEQ and levels of T_4 in the serum of the babies (p=0.033), as shown in Fig. 1. Such a kind of tendency was also seen between the estimated total intakes and serum levels of T_3 (p=0.064) or TBG (p=0.057). On the contrary, as indicated in Fig.2, the levels of TSH in the serum of the breast-fed babies positively correlated with the estimated total intakes (p=0.022).

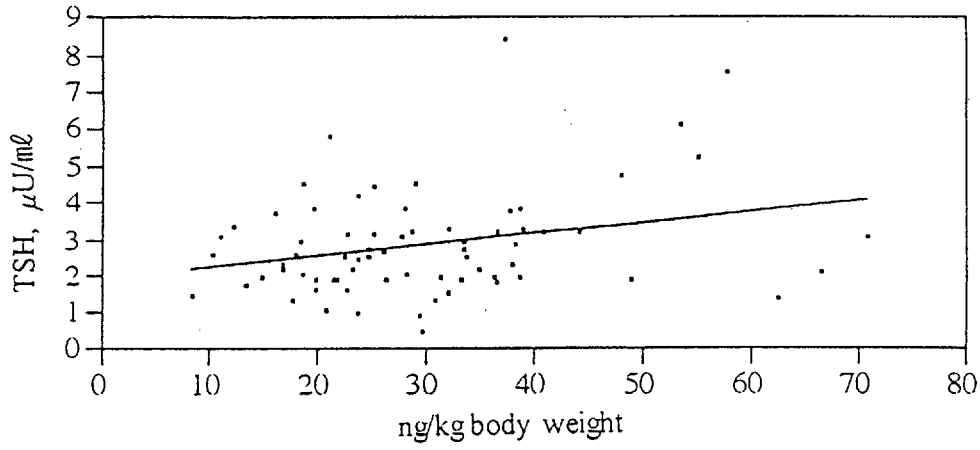

Fig. 2. Positive correlation between the estimated total TEQ intakes from the breast milk and the levels of TSH in the serum of breast-fed babies (p=0.022)

Discussion

The presence of PCDDs, PCDFs and Co-PCBs in the breast milk results in daily intakes of about 32 to 304 pg/kg body weight as TEQ with the median figure of 114 pg/kg body weight. Consequently, the babies have been estimated to take 6 to 84 TEQ ng/kg body weight with the median of 29 ng/kg body weight during whole breast-feeding periods.

Probably due to such kinds of relatively high TEQ intakes, we observed negatively significant correlation between the levels of T_4 in the serum of the breast-fed babies and the estimated total TEQ intakes. On the contrary, the levels of TSH in the serum proportionally increased with the estimated TEQ intakes. However, contrary to our results, T_4 and the ratio of T_4/ TBG were significantly higher in high-exposed babies than in low-exposed ones at 7 days and 11 weeks of age[5]. Anyhow, exposure to background levels of PCDDs, PCDFs and Co-PCBs via breast milk may have some effects on thyroid functions in breast-fed babies.

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Effects of Lactational Exposure to Organochlorine Pesticides on Lymphocyte Subpopulations and Thyroid Functions in Japanese Babies

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Abstract

Effects of lactational exposure to organochlorine pesticides such as hexachlorocyclohexanes (HCHs), 1,1,1-trichloro-2,2-bis-(4-chlorophenyl)-ethane (DDT), dieldrin and heptachlor-epoxide (HCE) on lymphocyte subpopulations and thyroid functions in the peripheral blood of 75 breast-fed Japanese babies.Estimated total intakes of DDT (p,p'-DDE + p,p'-DDT) from the breast milk significantly and negatively correlated with the percentages of CD3-positive (mature T-lymphocyte) and CD4-positive (helper/inducer) lymphocytes and also with the serum levels of triiodothyronine (T_3), thyroxine (T_4) and thyroxine binding globulin (TBG) in the peripheral blood of breast-fed babies. Therefore, exposure to background levels of DDT through the breast milk seems to cause some effects on both the immune system and thyroid functions in Japanese babies.

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Introduction

Japanese food has been contaminated with some organochlorine pesticides[1] and Japanese people have also been contaminated with these pesticides[2]. Consequently, some pesticides such as HCHs, DDT, dieldrin and HCE have been determinated in Japanese breast milk and their mean concentrations on fat weight basis were about 1300, 950, 20 and 9ppb, respectively[3]. Their levels were much higher than those of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar polychlorinated biphenyls (Co-PCBs) in Japanese breast milk[3]. Therefore, we should give due attention to possible health consequences of these organochlorine pesticides in breast-fed babies.

In order to clarify the biological and/or biochemical effects of lactational exposure to HCHs, dieldrin, DDT and HCE, we investigated the lymphocyte subpopulations and thyroid functions in the peripheral blood of 75 breast-fed babies in relation to their intakes.

Experimental Methods

Eighty two mothers volunteered to participate in all in this study and they had a normal pregnancy without use of medicines. Breast milk (50~100mℓ), sampled 2 to 3 months after childbirth, was used to determine concentrations of HCHs, dieldrin, DDT and HCE by ECD gas chromatography method[1].

About 1 year after birth, 5 to 10mℓ of peripheral blood samples were individually obtained from 75 breast-fed babies. These blood samples were used to measure the lymphocyte subpopulations by indirect immunofluorescence using monoclonal mouse anti-human antibodies against CD3, CD4 , CD8, CD4 + CD8, CD16, CD20 and HLA-DR, and their relative population densities were calculated[4]. They were also employed to determine serum concentrations of T_3, T_4, TSH and TBG by radioimmunoassay methods by using commercially available kits[5].

Total intakes (μg/kg body weight) were estimated by multiplying daily intakes (ng/kg body weight) of HCHs, dieldrin, DDT and HCE from the breast milk, which were calculated with their levels in the breast milk times an expected intake of breast milk in Japanese baby, namely, 120g/kg body weight, by individual duration of breast feeding (days).

Analysis of variance (ANOVA) was applied to examine the relationship of respective estimated total intakes of these pesticides from the breast milk to each variable of interest and statistical significance was evaluated by Student's *t*-test.

Results

1) Concentrations of the organochlorine pesticides in the breast milk

Among HCHs isomers, β-HCH only was detected and determined in the breast milk of 82 mothers. In DDT and it's metabolites, only two congeners, namely, p,p'-DDE and p,p'-DDT were detected and p,p'-DDE was dominant. Therefore, in this study DDT indicates combined concentrations of p,p'-DDE and p,p'-DDT in the breast milk.

Respective median, minimum and maximum concentrations of β-HCH, dieldrin, DDT and HCE on the whole and fat weight bases are shown in Table 1. Order of median concentration was β-HCH, DDT, HCE and dieldrin. Concentrations of β-HCH and DDT were two orders of magnitude higher than those of HCE and dieldrin.

Table 1. Concentrations of the organochlorine pesticides in the breast milk of 82 mothers

	Median (min. ~ max.) in ppb	
Organochlorine Pesticide	Whole Basis	Fat Basis
β-HCH	12.1 (0.7 ~ 51.1)	365 (39 ~ 1229)
Dieldrin	0.11 (0.02 ~ 1.04)	3.5 (1.0 ~ 27.0)
DDT	10.1 (1.0 ~ 61.4)	308 (52 ~ 1348)
HCE	0.13 (0.02 ~ 0.50)	4.0 (1.0 ~ 17.0)

2) Estimated intakes of the organochlorine pesticides from the breast milk

Estimated total median, minimum and maximum intakes of the organochlorine pesticides are indicated in Table 2. Total median intakes of β-HCH, dieldrin, DDT and HCE were 401, 3.5, 325 and 4.6 μg/kg body weight, respectively.

Table 2. Estimated total intakes of the organochlorine pesticides from the breast milk in 75 breast-fed babies

	Median (min. ~ max.)
Organochlorine Pesticide	μg/kg body weight
β-HCH	401 (24.5 ~ 2025)
Dieldrin	3.5 (0.22 ~ 33.7)
DDT	325 (18.5 ~ 2019)
HCE	4.6 (0.36 ~ 16.9)

3) Correlation between estimated total intakes of the organochlorine pesticides and peripheral lymphocyte subpopulations in breast-fed babies.

Percentages of lymphocyte subpopulations were examined in the peripheral blood of 69 brea-

st-fed babies and simple correlation coefficients of estimated total intakes of respective organochlorine pesticides with the percentages of lymphocyte subpopulations were calculated. The results are shown in Table 3.

Intakes of dieldrin and DDT from the breast milk showed significant negative correlation with the percentages of CD3-positive lymphocytes. Intake of DDT also negatively correlated with the percentages of CD4-positive lymphocytes and this relationship is indicated in Fig. 1.

Table 3. Simple correlation of estimated total intakes of the organochlorine pesticides from the breast milk with percentages of lymphocyte subpopulations in the peripheral blood of breast-fed babies

	Correlation Coefficient					
	CD3	CD4	CD8	CD16	CD20	HLA-DR
β-HCH	-0.091	-0.087	0.033	0.070	0.046	-0.025
Dieldrin	-0.272*	-0.207	-0.121	0.094	0.193	0.214
DDT	-0.325*	-0.307*	-0.119	-0.122	0.258	0.224
HCE	-0.125	-0.238	0.092	0.092	0.073	0.081

* : $p<0.05$

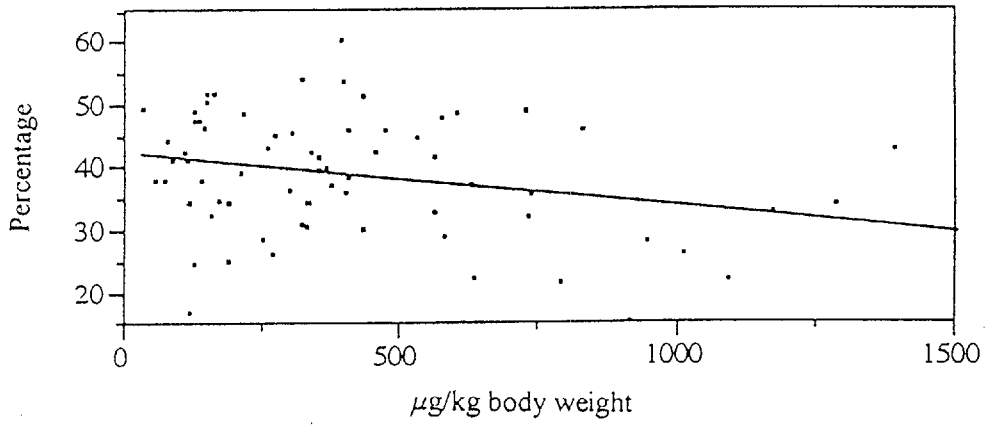

Fig. 1. Negative correlation between the estimated total intakes of DDT from the breast milk and the percentages of CD4-positive lymphocytes in the peripheral blood of 69 breast-fed babies ($p=0.021$)

4) Correlation between estimated total intakes of the organochlorine pesticides and thyroid functions in breast-fed babies

Serum levels of T_3, T_4, TSH and TBG were determined in the peripheral blood of 71 breast-fed babies and simple correlation coefficients of estimated total intakes of respective organochlorine pesticides with the serum levels related to thyroid functions were calculated. The results are shown in Table 4.

Table 4. Simple correlation of estimated total intakes of the organochlorine pesticides from the breast milk with serum levels related to thyroid functions in the peripheral blood of breast-fed babies

	Correlation Coefficient			
	T_3	T_4	TSH	TBG
β-HCH	-0.059	-0.159	-0.022	-0.076
Dieldrin	-0.179	-0.029	0.015	-0.008
DDT	-0.257*	-0.381**	0.146	-0.307*
HCE	-0.064	-0.183	0.045	-0.062

* : $p<0.05$, ** : $p<0.01$

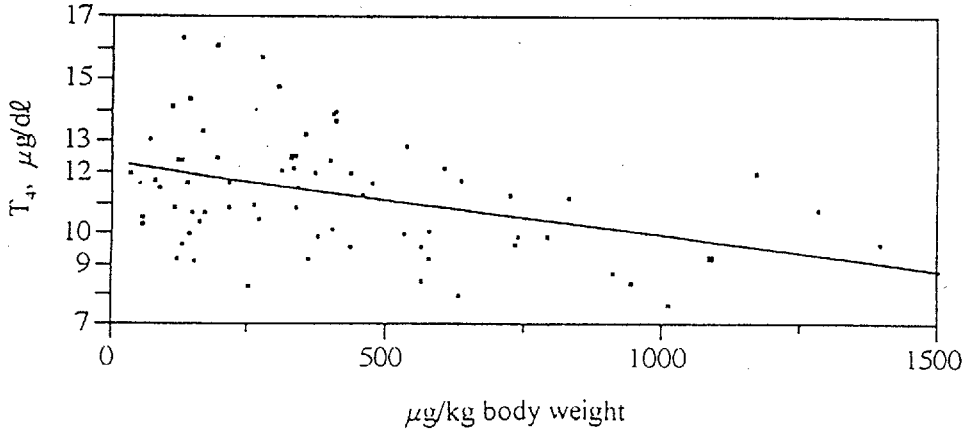

Fig. 2. Negative correlation between the estimated total intakes of DDT from the breast milk and the levels of T_4 in the serum of 71 breast-fed babies ($p=0.001$)

The estimated total intake of DDT from the breast milk showed significant negative correlation with the serum concentrations of T_3, T_4 and TBG. The relationship of the intake of DDT to the serum level of T_4 is indicated in Fig. 2.

Discussion

We have already determined these organochlorine pesticides in Japanese breast milk collected in 1990 to 1992[3]. The breast milk in this study was collected in 1994 and 1995. The concentra- tions of these pesticides in our former study were 2 to 6 times higher than those in this study and in addition to p,p'-DDE and p,p'-DDT, p,p'-DDD and o,p'-DDT were also determined. At the present time, the reason of this marked decrease in their concentrations is unknown and maybe human contamination with these pesticides was truly decreased during such a short period.

Even in such decreased levels of the pesticides in the breast milk, their estimated intakes were 100 to 10,000 times greater than those of PCDDs, PCDFs and Co-PCBs as a whole in toxic equivalent quantity (TEQ) converted into 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) and lactational exposure to DDT in particular significantly decreased the percentages of CD3- and CD4-positive lymphocytes and also serum levels of T_3, T_4 and TBG in the peripheral blood of breast-fed babies. Therefore, exposure to background levels of DDT through breast milk may cause some effects on both immune system and thyroid functions in Japanese babies.

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PARTITIONING OF PCDDs, PCDFs, AND COPLANAR PCBs IN HUMAN MATERNAL TISSUES: BLOOD, MILK, ADIPOSE TISSUE AND PLACENTA

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ABSTRACT: To determine partitioning of dioxins, dibenzofurans, and the dioxin-like coplanar polychlorinated biphenyls (PCBs) in maternal tissues, we collected and analyzed the following tissues from a series of 5 American women having cesarean section deliveries during 1995-96: Whole blood, placenta, and adipose tissue at delivery, and whole blood and breast milk 4-8 weeks after delivery. A total of 25 samples, 5 from each woman, were collected from upstate New York hospitals, frozen and shipped to the dioxin laboratory. Preliminary data suggests that levels of dioxins in placental tissue may reflect levels in other maternal tissue. Also, lower levels of dioxins and furans were found in tissue samples analyzed thus far compared to earlier measurements of milk samples from American women taken in the late 1980's.

INTRODUCTION: The purpose of this pilot project is to build a database which will characterize the partitioning of dioxin (PCDD), dibenzofuran (PCDF), and the dioxin-like coplanar PCB congeners of interest in commonly sampled human tissues such as blood and breast milk, and also in less commonly sampled tissues, adipose and placenta. In addition, we wished to compare blood dioxin levels before and after delivery in order to better understand the effects of pregnancy on dioxin metabolism. Adipose tissue dioxin measurements are currently considered the "gold standard" in estimating body burden, but we as well as others have shown that congener-specific partitioning varies in different tissues, even when reported on a lipid normalized basis.[1-7] For example, in previous work reports we noted higher measured levels of PCDD/Fs and calculated dioxin toxic equivalents (TEQs) in blood than in milk and subtle congener differences in autopsy obtained organs from the same individual even when reported on a lipid basis.

One study objective is to begin compiling a series of data on maternal tissue dioxin levels prior to delivery in order to better characterize the relationship between maternal dioxin levels during pregnancy and after delivery. These values may be of use in clinical medicine for predicting levels in milk from maternal blood or placenta. In previous work with American and with Taiwanese Yucheng placentas we found that the high levels of PCDD/Fs in Yucheng mothers were reflected in the elevated placental levels.[8] This suggests that placental tissue may be useful for estimating dioxin exposure.

METHODS: Tissues samples were collected from five American women, mean age 21.6 years (range 21-34), residing in upstate New York, and undergoing cesarean section deliveries between 9/95 and 1/96. Blood, placenta, and fat were collected at time of delivery. The milk and second blood was collected about 4-8 weeks afterwards. Specimens were placed in chemically clean containers, frozen, and shipped to the dioxin laboratory for

analysis. The analytic methodology has been previously described.[9-10]

Sample Collection: The following tissues were collected from each mother: Maternal blood (100 ml) on the day of delivery; maternal adipose tissue, collected during C-section (10 grams); placenta (400 grams) at delivery; maternal blood (100 ml) collected during first 3 months of nursing; maternal milk (25 ml) collected at the same time as the second maternal blood.

RESULTS: Table 1 reviews our previous finding for American and Yucheng placentas and for American milk and blood from the general population. The elevated PCDF level characteristic of Yucheng can be seen in the measured placental levels (20,659 ppt) and in the TEQ, 3901 ppt, as compared with American PCDF levels of 23.5 ppt and 2.9 ppt TEQ. Measured PCDD/F and coplanar PCB congeners and calculated dioxin TEQ values for the mean of the five individually analyzed new breast milk samples are presented in Table 2. Total PCDD/Fs and TEQs are 189.3 ng/kg (ppt) (range 168.5-221) and 8.16 ppt, (range 6.25-9.7) respectively. Mean coplanar PCB values are 30.9 ppt and 2.02 ppt TEQ. The 20 remaining new samples are at ERGO laboratory awaiting analysis at the time of manuscript preparation.

DISCUSSION: Dioxin levels in adipose tissue, milk, and blood tissue have been used to estimate body burden in the general population and in those with special exposures. We, as well as others, have reported congener-specific differences in dioxin levels between various tissues when analyzed on a lipid normalized basis. In this study we address the question of PCDD/F/PCB partitioning in several tissue samples from the same women obtained during pregnancy and after delivery. This study adds to the database for dioxin levels in various maternal tissues before and after delivery. These values can be of use in clinical medicine for predicting levels in milk from maternal blood or placenta. Finally, it appears, from the newly collected and analyzed breast milk samples, that current measured levels of PCDD/Fs in American breast milk (189 ppt) may be declining as compared with samples collected in the late 1980's (398 ppt).[6]

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Table 1. Dioxin and Dibenzofuran Levels and Dioxin Toxic Equivalent Values in Human Placentas, Breast Milk, and Blood (ppt, lipid)

CONGENER	TEF	American Placentas (n=14)(a)		Yu-Cheng Placentas (n = 6)(b)		American Milk (n = 43)(c)		American Blood(d) (n=44)	
		Measured	TEQ	Measured	TEQ	Measured	TEQ	Measured	TEQ
2,3,7,8-TCDD	1	2.4	2.4	2.1	2.1	3.3	3.3	3.8	3.8
1,2,3,7,8-PeCDD	0.5	4.0	2.0	16.81	8.40	6.7	3.35	9.3	4.63
1,2,3,4,7,8-HxCDD	0.1	2.4	0.2	0.22	0.02	6.0	0.60	9.8	0.68
1,2,3,6,7,8-HxCDD	0.1	15.9	1.6	210	20.99	6.2	0.62	72	7.21
1,2,3,7,8,9-HxCDD	0.1	3.2	0.3	22.5	2.25	30.5	3.05	12	1.19
1,2,3,4,6,7,8-HpCDD	0.01	36.2	0.4	44.1	0.44	42	0.42	119	1.19
OCDD	0.001	282	0.3	599	0.60	233	0.23	794	0.79
2,3,7,8-TCDF	0.1	1.9	0.2	3.61	0.36	2.9	0.29	2.3	0.23
2,3,4,7,8-PeCDF	0.5	3.6	1.8	4.19	0.21	7.3	3.65	1.2	0.06
1,2,3,7,8-PeCDF	0.05	<1.0	0.0	4679	2339	0.5	0.03	8.8	4.38
1,2,3,4,7,8-HxCDF	0.1	4.0	0.4	15405	1540	5.6	0.56	10.6	1.06
1,2,3,6,7,8-HxCDF	0.1	2.0	0.2	167.5	16.7	3.2	0.32	6.9	0.61
2,3,4,6,7,8-HxCDF	0.1	nd (1.0)	0.1	0.22	0.02	1.9	0.19	2.8	0.28
1,2,3,7,8,9-HxCDF	0.1	1.7	0.2	0.18	0.02	--	--	2.8	0.28
1,2,3,4,6,7,8-HpCDF	0.01	6.3	0.1	355.7	3.56	4.1	0.04	19.6	0.20
1,2,3,4,7,8,9-HpCDF	0.01	<1.0	0.005	39.1	0.39	4.1	0.04	3.1	0.03
OCDF	0.001	<5.0	0.003	5.35	0.01	4.1	0.004	9.3	0.01
Total PCDDs		**346**	**7.2**	**895**	**35**	**367**	**12**	**1020**	**19**
Total PCDFs		**23.5**	**2.9**	**20659**	**3901**	**31**	**8**	**67**	**7**
Total PCDD/Fs		**370**	**10.1**	**21554**	**3936**	**398**	**20**	**1087**	**27**

(a) American placentas were combined for one analysis (ERGO July, 1994), (n = 14) = 1.33% lipid
(b) Average of 6 Individual Yu-Cheng placentas, collected between October, 1984 and June 1985 for Dr. George Lucier with the National Institute of Environmental Health Sciences
(c) Average of two pooled samples: Binghamton , NY (n=21); Los Angeles, CA (n = 22), collected and analyzed in the late 1980's
(d) Blood measured is a mean of 44 individual analyses and TEQs for 44 males

Table 2. PCDD/Fs and Coplanar PCBs in Human Breast Milk from Five Binghamton, NY Women 1995-6 (ng/kg (ppt), lipid)

CONGENER		MEAN (N = 5)*	
	TEF**	LEVEL	TEQ
DIOXINS			
2,3,7,8-TCDD	1	1.45	1.45
1,2,3,7,8-PeCDD	0.5	2.48	1.24
1,2,3,4,7,8,-HxCDD	0.1	3.01	0.30
1,2,3,6,7,8-HxCDD	0.1	20.10	2.01
1,2,3,7,8,9-HxCDD	0.1	3.50	0.35
1,2,3,4,6,7,9-HpCDD	0.01	.--	0.00
1,2,3,4,6,7,8-HpCDD	0.01	34.03	0.34
OCDD	0.001	104.28	0.10
DIBENZOFURANS			
2,3,7,8-TCDF	0.1	0.91	0.09
2,3,4,7,8-PeCDF	0.5	2.81	1.40
1,2,3,7,8,-PeCDF	0.05	0.51	0.03
1,2,3,4,7,8-HxCDF	0.1	3.88	0.39
1,2,3,6,7,8-HxCDF	0.1	2.40	0.24
1,2,3,7,8,9-HxCDF	0.1	0.15	0.02
2,3,4,6,7,8-HxCDF	0.1	1.41	0.14
1,2,3,4,6,7,8-HpCDF	0.01	5.43	0.05
1,2,3,4,7,8,9-HpCDF	0.01	0.53	0.01
OCDF	0.001	2.46	0.002
COPLANAR PCBs			
77 3,3',4,4'-Te-PCB	0.0005	5.92	0.003
126 3,3',4,4',5-Pe-PCB	0.1	19.59	1.96
169 3,3',4,4',5,5'-Hx-PCB	0.01	5.37	0.05
TOTAL PCDDS		**168.84**	**5.79**
TOTAL PCDFS		**20.48**	**2.37**
TOTAL PCDD/Fs		**189.32**	**8.16**
TOTAL COPLANAR PCBs		**30.89**	**2.02**
TOTAL PCDD/Fs and PCBs		**220.21**	**10.17**

* Mean of five individual samples analyzed

** PCB TEFs from Ahlborg UG, Becking, GC, Birnbaum LS et al. Toxic equivalent factors for dioxin-like PCBs. Chemosphere 28:1049-1067, 1994.

Effects of Lactational Exposure to Chlorinated Dioxins and Related Chemicals on Lymphocyte Subpopulations and Thyroid Functions in Japanese Babies

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Introduction

We have reported the concentrations of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar polychlorinated biphenyls (Co-PCBs) in breast milk[1), 2), 3)]. According to levels of these compounds in breast milk, it is estimated breast-fed babies in Japan consume from about 100 to 200 pg/kg/day as the Toxic Equivalents (TEQ) of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD)[2)]. These TEQ values are much greater than the Acceptable Daily Intake (ADI) of 2,3,7,8-TCDD, namely, 1 ~ 10 pg/kg/day for adults. Babies are considered more sensitive to such kinds of chemicals. Therefore, we should give due attention to their possible health consequences to sucklings.

In order to clarify biological and/or biochemical effects of PCDDs, PCDFs and Co-PCBs, we

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investigated the lymphocyte subpopulations and thyroid functions in the blood of 37 healthy breast-fed babies in relation to their concentrations of the breast milk.

Methods

Forty healthy women volunteered to participate in this study. They had a normal pregnancy without use of medicines. Breast milk (50~100 mℓ), sampled about 3 months after childbirth, was used to determine concentrations of PCDDs, PCDFs and Co-PCBs by GC-MS and the amounts of TEQ of 2,3,7,8- TCDD were calculated with Toxic Equivalency Factor (TEF) values proposed by NATO Committee on Challenge to Modern Society (1988) for PCDDs and PCDFs, and those by WHO-ECEH and IPCS (1994) for Co-PCBs[1), 2), 3)].

About 1 year after birth, 5 to 10 mℓ of peripheral blood samples were individually obtained from 37 breast-fed babies. The blood samples were used to measure the lymphocyte subpopulations by indirect immunofluorescence using monoclonal mouse anti-human antibodies against CD3, CD4, CD8, CD16, CD20 and HLA-DR, and the relative population densities of the lymphocyte subpopulations were calculated[4)]. They were also employed to determine serum concentrations of triiodothyronine (T_3), thyroxine (T_4), thyroid stimulating hormone (TSH) and thyroxine binding globulin (TBG) by radio-immunoassay methods using commercially available kits[5)].

Analysis of variance (ANOVA) was applied to fit the concentrations of PCDDs, PCDFs and Co-PCBs in TEQ of 2,3,7,8-TCDD as a function of variables of interest and statistical significance was evaluated by Student's *t* -test.

Results

1) Concentrations of PCDDs, PCDFs and Co-PCBs in breast milk

Medians and ranges in total concentration of PCDDs, PCDFs and Co-PCBs as TEQ of 2,3,7,8-TCDD are given in Table 1. Median concentrations on the whole and fat weight bases were 1.00 and 25.7ppt, respectively. Range was 0.34 to 2.37ppt on the whole weight basis and 15.2 to 48.5ppt on the fat weight basis.

2) Percentages of peripheral lymhocyte subpopulations in breast-fed babies

As shown in Table 2, median percentages of peripheral lymphocyte subpopulation in 37 breast-fed babies were as follows. Mature T-cell expressing CD3 was the highest (54.6%), helper/inducer T-cell (CD4) : 34.5%, human leucocyte antigen expressing DR domain (HLA-DR) : 31.0%, B-cell (CD20) : 27.1%, suppressor/cytotoxic T-cell (CD8) : 19.3%, Natural killer T-cell (CD16) : 7.9% and T-cell reactive to both CD4 and CD8 the lowest (0.5%). T-cell subpopulation expressing CD4 had a tendency to show higher percentage than that for CD8 in the peripheral blood of 37 babies and their ratio (CD4/CD8) was 1.8 in median.

Table 1. Medians and ranges in total concentration of PCDDs, PCDFs and Co-PCBs as TEQ of 2,3,7,8-TCDD in the breast milk of 40 mothers

Compound	Median (min.~ max.) in TEQ of 2,3,7,8-TCDD (ppt)	
	Whole Basis	Fat Basis
PCDDs PCDFs Co-PCBs	1.00 (0.34~2.37)	25.7 (15.2~48.5)

Table 2. Percentages of lymphocyte subpopulations in the peripheral blood of 37 breast-fed babies

Lymphocyte Subpopulation (Positive Cells)	Median (min.~ max.) Percentage
CD3	54.6 (31.2~69.8)
CD4	34.5 (15.7~51.4)
CD8	19.3 (10.6~38.4)
CD4+CD8	0.5 (0.2~1.3)
CD16	7.9 (2.1~23.3)
CD20	27.1 (12.8~56.2)
HLA-DR	31.0 (15.5~62.1)
CD4/CD8	1.8 (0.8~3.3)

3) Thyroid function tests in breast-fed babies

Results of the examination in thyroid functions in the serum of 37 breast-fed babies are summarized in Table 3. We could not find any apparent abnormal figure in all the tests.

4) Correlation between the concentrations of PCDDs, PCDFs and Co-PCBs in breast milk and percentages of peripheral lymphocyte subpopulations in breast-fed babies

We observed positive correlation between the total levels of TEQ as 2,3,7,8-TCDD in the breast milk and the percentages of CD4-positive cells in the blood of the babies ($p=0.086$) and also negative

correlation between the 2,3,7,8-TCDD TEQ levels and the percentages of CD8-positive cells (p=0.070). Consequently, as indicated in Fig. 1, the ratios of CD4 to CD8 showed a significant increasing tendency with the total TEQ levels in the breast milk (p=0.015).

Table 3. Results of thyroid function tests in the serum of 37 breast-fed babies

	Median (min. ~ max.)
Triiodothyronine (T_3, ng/mℓ)	2.00 (1.00~2.50)
Thyroxine (T_4, μg/dℓ)	11.4 (7.7~16.4)
Thyroid stimulating hormone (TSH, μU/mℓ)	2.69 (0.92~5.86)
Thyroxine binding globulin (TBG, μg/mℓ)	25.1 (17.8~31.8)
T_4/TBG	0.45 (0.30~0.61)

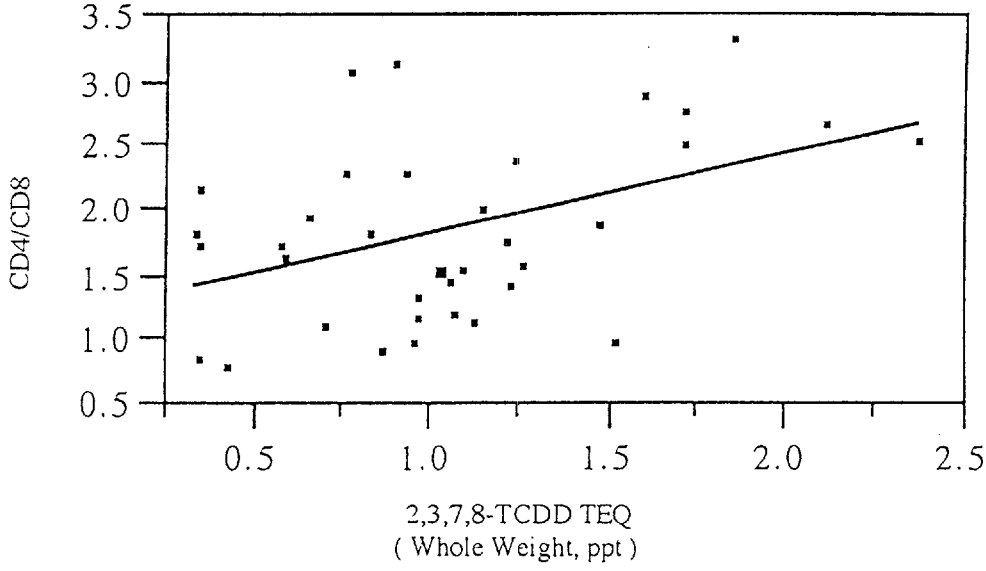

Fig. 1. Correlation between 2,3,7,8-TCDD TEQ levels in the breast milk and the ratios of percentages of CD4-positive to CD8-positive cells in the blood of breast-fed babies (p=0.015)

5) Correration between the concentrations of PCDDs, PCDFs and Co-PCBs in breast milk and thyroid functions in breast-fed babies

Significant negative correlation was observed between the total levels of TEQ as 2,3,7,8-TCDD in the

breast milk and the levels of T_4 in the serum of the babies (p=0.035). The result is shown in Fig. 2. Other thyroid functions did not indicate any significant correlation with the total TEQ levels.

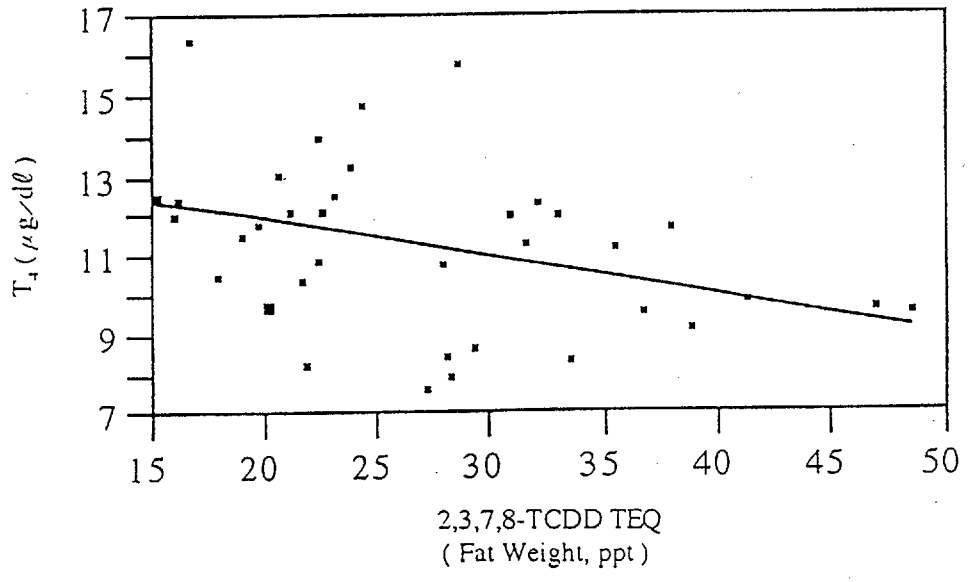

Fig. 2. Correlation between 2,3,7,8-TCDD TEQ levels in the breast milk and the levels of T_4 in the serum of breast-fed babies (p=0.035)

Discussion and Conclusions

The presence of PCDDs, PCDFs and Co-PCBs in breast milk results in daily intakes from 40 to 280 pg/kg body weight as TEQ of 2,3,7,8-TCDD with the median figure of 120pg/kg for breast-fed babies (Table. 1). These intakes far exceed the ADI of various countries.

Possibly due to such kinds of great intakes of Dioxin-like chemicals from breast milk, significant correlations were observed between peripheral lymphocyte subpopulations in the breast-fed babies and 2,3,7,8-TCDD TEQ levels of the breast milk (Fig. 1), and also between serum T_4 levels of the babies and the 2,3,7,8-TCDD TEQ levels (Fig. 2). Recently, no relationship between pre- and postnatal PCB/Dioxin exposure and upper or lower respiratory tract symptoms or humoral antibody production was reported[6]. However, at 11 weeks of age, calculated cumulative intakes of Dioxins by breast milk consumption were significantly correlated with aminotransferase activities in plasma and negatively with platelet counts in newborns[7]. Concerning effects on thyroid hormone regulation, contrary to our results, T_4 and the ratio of T_4/TBG were significantly higher in high-exposed babies than in low-exposed ones at 7 days and 11 weeks of age[8].

The results of studies mentioned above and our present investigation suggest that exposure to background levels of Dioxins and related chemicals via breast milk may have some effects in breast-fed babies.

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Levels of PCBs, PCDDs and PCDFs in human milk. Results from the Second Round of a WHO-coordinated Exposure Study.

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1 Introduction

It is nowadays widely known that chlorinated aromatic hydrocarbons such as polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) can be detected in human adipose tissues because of their global distribution in the environment and subsequent exposure of people through numerous sources with foodstuffs being the most important. As a result of the extreme lipophilic properties and the slow elimination behaviour these compounds are accumulated in human adipose tissues. They can pass through the placenta causing exposure of the foetus, and their existence in human milk exposes infants during the lactation period.

Since the first findings of these chemicals in human milk were published, the WHO Regional Office for Europe (WHO/EURO) has been coordinating a comprehensive programme aiming to evaluate the possible health risks especially for infants and to control and prevent exposure to these toxic chemicals. Because the analytical data on exposure levels through breast milk was rather limited WHO/EURO initiated a series of international studies on levels of PCBs, PCDDs and PCDFs in human milk. The purpose of these studies is to collect data on levels in human milk from different areas and countries at five years' intervals to detect possible trends in exposure and to provide data to assess health risks for breast-fed infants on the basis of available epidemiological and toxicological data. The first round took place in 1987-1988 [1].

In this paper the results are presented from the second round in which nineteen countries participated. Sampling strategy was based on a standardized study protocol and determinations of levels of PCBs, PCDDs and PCDFs were carried out at recommended laboratories to assure the comparability of the results from different areas. The presented data have been discussed at a consultation in Berlin in 1994. A detailed WHO/EURO report will become available in 1996 [2].

2 Materials and methods

Study design

A standardized study protocol was developed based on recommendations of the previous consultations within this programme. The protocol was developed to allow comparison of results with those from the first round of the studies. Instructions were provided on the type of samples (either pooled or individual samples), on the sampling areas (at least two different groups to distinguish highly polluted/unpolluted areas), the selection of donors (primiparae, healthy mother/child pairs, residential factors) and on methods for collecting, storing and transporting of samples. In addition, a standard questionnaire was provided to be used for the individual interviews of the mothers to collect data on selected determinants (e.g., age, environmental factors, smoking, dietary habits) potentially influencing levels of the PCBs, PCDDs and PCDFs in the human milk. It was requested to collect samples between 2 weeks and 2 months after delivery.

Chemical analyses

Analyses of the human milk samples were carried out by the Chemisches Landes- und Staatliches Veterinäruntersuchungsamt (P. Fürst) in Münster, Germany, the Food Research Division of the Bureau of Chemical Safety, Health Protection Branch, Tunney's Pasture (J.J. Ryan) in Ottawa, Canada, the Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin (W. Mathar) in Berlin, Germany, the National Institute of Public Health (G. Becher) and the Norwegian College of Veterinary Medicine (J.U. Skåre) in Oslo, Norway, the CSL Food Science Laboratory (J.R. Startin) in Norwich, United Kingdom and the RIVM Laboratory for Organic-analytical Chemistry of the National Institute of Public Health and the Environment (A.K.D. Liem) in Bilthoven, the Netherlands. For those countries unable to perform the analyses the samples were sent to a reference laboratory selected by WHO/EURO, i.e., the RIVM Laboratory for Organic-Analytical Chemistry in Bilthoven, the Netherlands.

All samples were analysed for the seventeen 2,3,7,8 chlorine substituted PCDDs and PCDFs, three non-*ortho* (IUPAC nos. 77, 126 and 169) and two mono-*ortho* chlorine substituted PCBs (IUPAC nos. 105 and 118), as well as the six marker PCBs (IUPAC nos. 28, 52, 101, 138, 153 and 180) most commonly analysed in measurement programmes. In addition, some laboratories also provided data on other PCB congeners. A detailed description of the analytical methods of the different laboratories have been published elsewhere [3-13].
In summary, extraction most frequently followed a method proposed by the AOAC [14]. In some cases, alternative extraction methods were used such as acetone/hexane and on-column extraction following freeze drying. Several ($^{13}C_{12}$-labeled) internal standards were added prior to extraction. The fat content was in all cases determined gravimetrically by accurate weighings of the extracted fat after evaporation of (aliquots of) the extract to dryness. For clean-up, all laboratories applied modifications of the method proposed by Smith *et al.* [15], involving activated carbon as key step to separate fractions containing the planar PCDDs, PCDFs and non-*ortho* PCBs from interfering non-planar compounds. For PCB analysis, either hexane/sulphuric acid partitioning, an automated normal phase HPLC or GPC system was used. Prior to gas chromatographic analysis, varying compounds were added as injection standards.
Analyses of PCDDs, PCDFs and non-*ortho* PCBs were carried out by use of GC/HRMS with the MS operating in the EI/SIM mode at a mass resolution of 5,000 to 10,000. GC/ECD was used to determine mono-*ortho* and the six marker PCBs.

Quality of data

Applied methods have been tested in terms of within-lab repeatability and between-lab reproducibility by means of internal method validation studies [3-13] and interlaboratory quality control studies organised by WHO/EURO [1,16]. During the course of the analytical programme of this exposure study, the performance of the applied methods was verified by conducting recovery studies and repeated analyses of quality control samples of milk. In addition, a cross check was performed with regard to the determination of the fat content. Results from these within-laboratory and between-laboratory quality control checks will be reported elsewhere [2].

3 Results

A full report of the levels for each single congener will be presented elsewhere [2]. A summary of the levels of PCDDs, PCDFs, non-*ortho* PCBs with IUPAC nos. 77, 126, 169 ("no-PCBs") and mono-*ortho* PCBs with IUPAC nos. 105 and 118 ("mo-PCBs") expressed in TEQs as well as the sums of the marker PCBs with IUPAC nos. 28, 52, 101, 138, 153 and 180 is presented in Table 1. TEQ values have been calculated using the International Toxic Equivalency Factors (ITEF) for the PCDDs and PCDFs as proposed by NATO/CCMS [17] and the interim WHO-TEFs for dioxin-like PCBs [18]. With the exception of the Netherlands and Russia, human milk data represent analyses of pooled samples composed of a varying amount of individual samples. A comparison of the mean and the range of levels observed for individual samples may indicate the degree of representativity of reported levels for pooled samples. A statistical analysis of observed levels in individual samples from Denmark and the Netherlands revealed that large variations (by a factor of 3 to 5) should be taken into account between levels of PCBs, PCDDs and PCDFs in the individual samples from which a pooled sample is composed. These variations are much higher than the analytical repeatability (RSDs generally below 10%) [2]. An overall relative standard deviation of 30 to 40% should be considered when comparing levels expressed in TCDD equivalents and sums of the marker PCBs [2].

A few regions and countries have been identified where levels in human milk are consistently higher or lower than those found in human milk from the other countries. In this regard, the sample from the Hudson Bay region in Canada appears to have relatively high levels of all compounds investigated. Levels of all compounds were significantly lower in Albania, Hungary and Pakistan. No consistent rankings apply for the other countries with respect to levels of the different compounds analysed. Different regions in several countries can be identified in which higher body burdens of specific component groups (i.e. PCDDs and PCDFs, dioxin-like PCBs, marker PCBs) are found than in other areas and countries. Besides some particular regions in different countries, generally higher levels can be observed for Belgium and the Netherlands (PCDDs and PCDFs), and Lithuania (non-*ortho* and mono-*ortho* PCBs). Exceptionally high levels of the six marker PCBs have been found for particular regions in the Czech Republic, Slovak Republic and Canada.

An important part of this study was to compare the data with those collected in the first round that took place in 1987-1988 [1]. This comparison was only possible for levels of PCDDs, PCDFs and the marker PCBs, as other compounds were not determined in the first round (table 2). It appeared that levels of PCDDs and PCDFs are not increasing. In some countries levels tend to decrease and some countries even show a dramatic decrease up to 50%, in comparison with the 1987 study. Using several assumptions an overall annual decrease was estimated of 7.2% with a standard deviation of 0.9% on the basis of the data for eleven countries participating in both rounds. For the PCB situation, this is not so clear, since many countries used different, and sometimes less reliable analytical methods (i.e., the packed column technique) in the first of the two studies.

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Table 1

Results from the second round of WHO-coordinated exposure studies on levels of PCBs, PCDDs and PCDFs (on fat basis) in human milk. In calculating sums of the six marker PCBs and levels of PCDDs, PCDFs, non-*ortho* and mono-*ortho* PCBs expressed in toxic equivalents (TEQ), both data are shown when nd-values are equal to zero and nd-values are equal to the LOD. If no differences appeared, a single value is presented.

Country	Area	Indiv. samples in pool	Fat (wt%)	PCDD/F (pg TEQ/g)	no-PCBs (pg TEQ/g)	mo-PCBs (pg TEQ/g)	Σ [PCBs] (ng/g)
ALBANIA	Tirana	10	5.84	4.8	1.3	1.1	63
	Librazhd	10	4.72	3.8	1.0	0.7	43-46
AUSTRIA	Vienna (urban)	13	4.10	10.7	8.3	3.4	381
	Tulln (rural)	21	3.80	10.9	9.4	3.0	303
	Brixlegg (industrial)	13	3.40	14.0	15.1	3.8	449
BELGIUM	Brabant Wallou	8	3.79	20.8	3.8	3.6	275-277
	Liege	20	2.98	27.1	1.7	3.1	306-308
	Brussels	6	2.81	26.6	4.0	3.9	260-261
CANADA	Maritimes 92	20	2.76	10.8-11.0	2.9	1.2-1.4	86-87
	Québec 92	20	3.06	13.4-13.6	5.1	1.7-1.9	137-138
	Ontario 92	20	3.09	18.1-18.3	5.8	1.8-2.0	128-129
	Prairies 92	20	3.20	14.6-14.8	2.3	0.9-1.1	58-59
	British Columbia 92	20	2.97	15.7-15.8	2.5	1.0-1.2	70-71
	All provinces 92	100	2.96	14.5-14.6	3.8	1.5-1.7	112-113
	Gaspe	12	3.52	23.2-23.4	9.5	3.2-3.4	220-221
	Basse Côte-Nord	4	3.63	14.6-14.7	19.6	5.7-6.0	559-560
	Ungave Bay	4	3.31	14.3-14.5	9.8	4.3-4.6	576
	Hudson Bay	5	3.26	20.9-21.1	13.3	8.0-8.3	1361
CROATIA	Krk	10	3.80	8.4	3.8	2.2	218-219
	Zagreb	13	3.26	13.5	5.2	2.7	219
CZECH	Kladno	11	5.41	12.1	2.5	3.5	532-533
	Uherske Hradiste	11	4.92	18.4	4.1	5.7	1068
DENMARK	7 different cities	48	3.61	15.2	2.3	2.2	209-210
FINLAND	Helsinki	10	4.14	21.5	1.9	2.7	189
	Kuopio	24	4.49	12.0	1.0	1.4	133-135
GERMANY	Berlin	10	5.00	16.5-16.6	9.0	2.7	375
HUNGARY	Budapest	20	4.97	8.5-8.6	0.8	0.8	61-65
	Scentes	10	4.97	7.8	0.9	0.5	45-47
NETHERLANDS	Whole country	17	2.73	22.4-22.5	8.8	2.5	253-256
NORWAY	Tromsø (coastal)	10	2.56-2.70	10.1	16.1	3.4	273
	Hamar (rural)	10	2.51-2.76	9.3	7.4	3.0	265-266
	Skien/Porsgrunn (ind)	10	2.75-3.00	12.5-12.6	6.7	2.9	302
LITHUANIA	Palanga (coastal)	12	4.00-4.83	16.6	12.8	7.6	361
	Anykshchiai (rural)	12	3.56-4.10	14.4	12.9	7.8	287
	Vilnius city (urban)	12	2.69-2.87	13.3	11.6	8.9	322
PAKISTAN	Lahore	14	4.31	3.9	1.9	0.4	19-20
RUSSIA	Arkhankelsk	1	5.17	15.2	2.9	5.7	197
	Karhopol	1	3.64	5.9	2.0	2.9	102
SLOVAK	Michalovce	10	4.77	15.1-15.2	6.4	7.0	1015
	Nitra	10	3.61	12.6	3.6	2.5	489-490

Table 1 (Cont'd)

Country	Area	Indiv. samples in pool	Fat (wt%)	PCDD/F (pg TEQ/g)	no-PCBs (pg TEQ/g)	mo-PCBs (pg TEQ/g)	Σ [PCBs] (ng/g)
SPAIN	Bizkaia	19	3.75	19.4	6.7	3.9	461
	Gipuzkoa	10	3.86	25.5	3.8	4.4	452-453
UKRAINE	Kiev nr.1	5	3.40	11.0	9.3	5.6	264
	Kiev nr.2	5	3.76	13.3	6.0	5.6	191-192
UNITED KINGD.	Birmingham	20	3.09-3.10	17.9	2.5	1.8	129-131
	Glasgow	23	3.40-3.45	15.2	2.6	1.3	131-133

Table 2
Comparison of results from the first and second round of WHO-coordinated human milk study. Results are expressed onm fat basis. Σ (marker PCBs) and TEQs are calculated assuming nd-values are equal to zero.

Country	Area	PCDDs and PCDFs (pg TEQ/g)				Σ [marker PCBs] (ng/g)			
		1987/88[b]	n	1992/93	n	1987/88	n	1992/93	n
AUSTRIA	Vienna (urban)	17.1	54	10.7	13			381	13
	Tulln (rural)	18.6	51	10.9	21			303	21
BELGIUM	Brabant Wallou	33.7		20.8	8	558	12	275	8
	Liege	40.2		27.1	20	609	21	306	20
	Brussels	38.8		26.6	6			260	6
CANADA	All provinces 1981			28.6	200			212	200
	All provinces 1992			14.5	100			112	100
	Maritimes	15.6	19	10.8	20			86	20
	Québec	18.1	34	13.4	20			137	20
	Ontario[c]	17.6	76	18.1	20			128	20
	Prairies	19.4	31	14.6	20			58	20
	British Columbia	23.0	23	15.7	20			70	20
CROATIA	Krk	12.0	14	8.4	10	500[a]	14	218	10
	Zagreb	11.8	41	13.5	13	450[a]	41	219	13
DENMARK	Several regions/cities	17.8	42	15.2	48	830[a]	10	209	48
FINLAND	Helsinki	18.0	38	21.5	10	150	38	189	10
	Kuopio	15.5	31	12.0	24	203	31	133	24
GERMANY	Berlin	32.0	40	16.5	10			375	10
	North Rhine-Westphalia	31.6	79	20.7[e]		762	143		
HUNGARY	Budapest	9.1	100	8.5	20			61	20
	Scentes	11.3	50	7.8	10			45	10
NETHERLANDS	rural area	37.4	13			416	10		
	urban area	39.6	13			392	10		
	all regions	34.2	10	22.4	17	272	96	253	17
NORWAY[d]	Tromsø (coastal)	18.9	11	10.1	10	562[a]	10	273 (536[a])	10
	Hamar (rural)	15.0	10	9.3	10	507[a]	10	265 (483[a])	10
	Skien/Porsgrunn (industr.)	19.4	10	12.5	10	533[a]	8	302 (468[a])	10
UNITED KINGD.	Birmingham	37.0		17.9	20			129	20
	Glasgow	29.1		15.2	23			131	23

[a] Analysed using packed column technique.
[b] Calculated using Nordic TEF-model.
[c] Ontario-1988 denotes proportional mean of two pooled samples analysed in the first round.
[d] To compare results between first and second round, samples from 1992/93 have been re-analysed using (old) packed column technique (Becher and Skåre, personal communication).
[e] Dioxin levels in human milk samples from North Rhine-Westphalia collected in 1992 as reported by Fürst [19].

PCDD/ Fs in mother's milk may cause developmental defects in childrens' teeth

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1. Introduction

Dental hard tissues, like bone, are mineralized connective tissues. Unlike bone, enamel and dentin are not remodeled. Thus disturbances in the function of odontoblasts (dentin forming cells) and/or ameloblasts (enamel forming cells) lead to definite morphological consequences, by which the time and sometimes the nature of the damage can be determined. This makes the dental hard tissues unique compared with other tissues in humans. The etiology behind the dental disturbances can be genetic, acquired (e.g. nutritional deficiencies, high fluoride intake, drugs such as cyclophosphamide and tetracyclines) or, most often, idiopathic.

Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) can be detected in human milk and thus infants may be exposed to high cumulative doses via breast feeding[1]. Dental changes after accidental exposure of human infants to polychlorinated biphenyls or other dioxin-like compounds have been reported[2,3]. These include the presence of erupted teeth in newborn babies, mottled, chipping and carious teeth, altered eruption of permanent teeth, and abnormally shaped tooth roots. Animal studies on the continuously growing incisors of rats and mice have also indicated dental changes after exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). These include accelerated tooth eruption[4] and impaired dentin and enamel formation [5]. The aim of the present study was to investigate whether any correlations between PCDD/F exposure and changes in the dentition of a normal breast-fed child population are found. Especially we were interested in seeing whether PCDD/F compounds could be among the

causative agents in enamel hypomineralization, often found in newly erupted permanent first molars. The permanent first molars were chosen as the target teeth since they are mineralized during the first two years of life, the time when the child is exposed to PCDD/Fs via mother's milk.

2. Methods

Milk Sample Collection

Women were recruited between January and May 1987 from one of the maternity hospitals in Helsinki, the capital, and the maternity clinic of the hospital serving the surroundings of the city of Kuopio, Eastern Finland. The study is part of a WHO/EURO coordinated follow-up studies on levels of PCDDs and PCDFs in human milk. All women giving birth to a child during that time period were invited and about 150 mothers from both areas promised to collect a milk sample after four weeks provided they were still nursing. Altogether 168 milk samples were obtained, 77 samples from Helsinki and 91 samples from Kuopio.

Determination of PCDDs and PCDFs

About 40–80 mL of each breast milk sample, equivalent to 1.4 g fat, was spiked with 100 pg of ^{13}C-labelled PCDD and PCDF standards (ED-998 tetra-octa chlorodioxin standard solution and EF-999 tetra-octa chlorofuran standard solution, Cambridge Isotope Laboratories). Milk fat was extracted with diethyl ether/hexane and the fat content determined. The extract was defatted in a silica gel column and cleaned up with activated carbon column (Carbopack C, 60/80 mesh) containing Celite (Merck 2693) to separate PCDD/Fs from PCBs and further cleaned with an activated alumina column (Merck 1097, standardized, activity level II–III). The quantitation was performed by selective ion recording using a VG 70 SE mass spectrometry (resolution 10,000). Levels of 17 most toxic PCDD/Fs were expressed in TCDD toxic equivalents (TEQ) calculated by using the international equivalency factors (NATO/CCMS, 1988). The laboratory reagent and equipment blank samples were treated and analyzed by the same method as the proper samples, one blank for five samples. Detection limits for the different PCDD/F congeners were 0.3 – 1.0 pg/g in fat samples. Recoveries for internal standards were more than 60% for all congeners. The laboratory has participated successfully in international quality control studies for the analysis of PCDDs and PCDFs in cow milk samples organized by EU/BCR-project in 1993[6,7].

The total PCDD/F exposure of a child via mother's milk was calculated using the formula: I-TEQ/0.2877 x (1-exp(0.2877 x duration of lactation months/12)) which takes into account the duration of breast feeding and the milk I-TEQ value with 25% decrease per year in the concentration.

Dental examination

Six years later 136 children of mothers whose milk was analyzed and who still lived in Helsinki or Kuopio area were invited to a dental examination. Of those invited 91 participated the study at the age of 6 (mean age 6.3 years, range 6.1–6.3 years). Mineralization changes in the primary first and second molars and permanent first molars (provided that they were erupted) were recorded. Those children who had not their permanent first molars erupted at the age of 6, as well as those who had not responded to the first invitation were invited one year later. Altogether data on mineralization changes of the primary teeth were available from 91 children and of the permanent teeth from 102 children.

3. Results

A total of 17 children (17%) showed mineralization changes in the permanent first molars and 32 children (35%) in the primary first and/or second molars (Table 1). One out of the four permanent first molars was affected in eight children, two in three, three in four, and four molars in two children. Of the affected molars 16 were in maxilla and 19 in mandible. In the primary dentition, one or two out of eight primary molars were affected in 21 children, two to five molars in seven children and six to eight molars in four children, respectively. Most lesions in the permanent and primary molars were white chalky enamel lesions of moderate size (Table 1).

In the mother's milk, I-TEQs ranged from 3.8 to 99.4 pg/g milk fat with the mean being 19.8 pg/g (S.D. 10.9). The duration of breast feeding ranged from 1 to 36 months, the mean was 10.5 months (S.D. 5.5). Mineralization changes in the permanent first molars occurred more often (Mann-Whitney U-test, P=0.017) and they were more severe (regression analysis, r=0.3, P=0.003, Mantel Hänszel chi square P=0.010, Table 2) in children who were exposed to a greater amount of PCDD/Fs via mother's milk than in those who were exposed to a lesser extent. The duration of breast feeding alone was not associated with mineralization changes (Mann-Whitney U-test, P=0.17). Neither were I-TEQs or the log(I-TEQ) values significantly associated with the severity of mineralization changes in primary molars which are mineralized before birth.

None of the mothers reported an exposure to putative harmful compounds in their work environment. Twelve mothers had a history of cigarette smoking during the last 12 months before the delivery. All children of these mothers had normally mineralized first permanent molars and two children had mineralization defects in primary molars.

Table 1. Number of children with different hypomineralized teeth: severity and extent of enamel hypomineralization in the permanent first molars and primary molars.

	Number of children (%)	Chalky lesions	Chalky lesions with loss of enamel	Chalky lesions with affected dentin	Moderate-sized lesion(s)	Large lesion(s)
Hypomin-eralized permanent first molar(s)	17/102 (17%)	14	1	2	10	7
Hypomin-eralized primary molar(s)	32/91 (35%)	26	6	0	25	7

Table 2. Number of children with mineralization defects of the permanent first molars in relation to the total PCDD/F exposure of the child via mother's milk.

Mineralization of the permanent first molars	Low exposure (<8.0)*	Moderate exposure (8.0–16)*	High exposure (>16)*
Normal	22	41	22
Mild defect in only one tooth	1	5	2
Moderate defect or mild defect in more than one tooth	0	3	4
Severe defect	0	0	2

* Exposure value is calculated from the formula I–TEA/0.2877 x (1–exp(0.2877 x duration months/12))

4. Conclusions

The results suggest that PCDD/Fs may be a causative agent of enamel hypomineralization and that the prevailing levels of PCDD/F compounds in human milk may cause enamel hypomineralization in the developing teeth of children. As PCBs and other chlorinated compounds found in human milk may also be involved in disturbing tooth development, further studies are needed to evaluate their role.

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Time to pregnancy and miscarriages in women with a high dietary intake of fish contaminated with persistent organochlorine compounds

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Introduction

Consumption of fatty fish from the Baltic Sea, on the east coast of Sweden, is the main source of exposure for persistent organochlorine compounds (POC) for the general Swedish population (1, 2). Previous studies have shown an association between dietary intake of POC-contaminated fish and both low birthweight for infants born to exposed women (3-6) and reduction of the menstrual cycle length (7).

The main objective of this study was to assess the relationship between high dietary intake of POC-contaminated fish and decreased fertility, measured as increased time to pregnancy (TTP), and risk for miscarriage, in a cohort of fishermen's wives from the Swedish east coast. This group of women was chosen as they and their husbands have been found to eat more than twice as much fish on an average as subjects from the general population (8, 9). For relevant comparisons a similar cohort from the Swedish west coast (Skagerrak and Kattegatt), where the contamination of fish is considerably less (10), was used.

Materials and Methods

Cohorts of women married to fishermen from the Swedish east and west coasts have been established previously (11). Women from these cohorts, born in 1945 or later, were defined as the study population. A self-administered questionnaire was sent to all women in the two cohorts, asking about background information and relevant risk factors on their five first pregnancies. The primary outcome variable, TTP, was assessed by the question "How long did it take you to get pregnant?", allowing for an open-ended answer. Each woman's first planned pregnancy was thereafter chosen for the analysis to avoid interference from correlation between TTP on succeeding pregnancies.

Data were also collected on whether the pregnancy ended in a miscarriage or a stillborn baby, and in which gestational week the pregnancy ended. For the TTP analysis, information on 399 east coast and 936 west coast pregnancies was available. The corresponding numbers in the miscarriage analysis were 443 and 992. Analyses were also performed on subfertility, defined as at some occasion having tried to get pregnant for more than 12 months without succeeding, and infertility, defined as being subfertile and never have

had being pregnant. For the analysis of subfertility there were 378 east and 819 west coast observations, and for the infertility analysis there were 407 east and 905 west coast observations. In both the subfertility and infertility analyses women who were sterile due to medical or surgical treatment were excluded.

The primary outcome variable, TTP, was analysed by using Cox regression and hereby calculating Success Rate Ratios (SuRR). For analysis of miscarriages, sub- and infertility, Odds Ratios (OR) were calculated.

Results

In a univariate analysis of TTP an increase, although not statistically significant, in time to first planned pregnancy was found in the east coast cohort compared to the west coast cohort (SuRR 0.89, 95% CI 0.77, 1.02, table 1). Since interaction was found between cohort and smoking, with a negative correlation between the number of cigarettes smoked and SuRR, Cox regression was also performed separately for non/light smokers and heavy smokers (> 10 cigarettes/day). The results indicated that the decreased SuRR for the east coast women was present only among the heavy smokers (SuRR 0.66, 95% CI 0.49, 0.89).

Analyses were also performed adjusted for maternal age as a continuous variable, but this did not alter the results. The effects of the woman's year of birth, the partner's smoking habits, parity, and the woman's working hours were not statistically significant, neither did they change the estimate for the cohort comparison. These covariates were therefore not included in the model. The cohorts did not differ in partner's age at conception, the woman's coffee consumption, use of oral contraceptives before conception, working hours, shift work and heavy lifts. The model was therefore not adjusted for these covariates.

In the analysis of subfertility and infertility a statistically significant increase in risk was found for the east coast women (OR 2.49, 95% CI 1.05, 5.90, table 2). Even after adjusting for smoking habits the infertility ratio stayed significantly higher (OR 2.58, 95% CI 1.07, 6.22).

The analysis of miscarriage rate was performed stratified for gestational week (table 3). Each analysis was based on the number of pregnancies at risk at that time period. An increase in miscarriage rate was found for the west coast cohort for early miscarriages (OR 2.09, 95% CI 1.11, 3.95). For late miscarriages and stillbirths no differences between the two cohorts were found. Analyses were also performed adjusted for smoking habits, but this did not alter the results.

Discussion

The results of the present study indicate an increase in TTP for women dietary exposed to contaminated fish from the Baltic Sea, and especially among women smoking more than 10 cigarettes per day. This difference between exposure effect on reproductive outcome between non/light smoking and heavy smoking women has been seen previously when heavy smokers exposed for PC was shown to have a higher OR for giving birth to a low weight child (3000 g) than did exposed non- and light smokers (3).

In the analysis of infertility, a statistically significant higher OR was found for the exposed women. In a study on Swedish midwives 3.9% of the responders reported having

tried to achieve pregnancy without success (12). Infertility of an even larger magnitude has also found in other studies on Swedish women (13, 14). This points to the possibility that there might be a low infertility frequency among the unexposed women, rather than a high one among the exposed women.

To sum up, the results of the present study indicate an association between exposure for POC and increased time to pregnancy, but not necessarily with infertility rate. Further analysis with individual exposure data needs to be done to more thoroughly investigate the effect of dietary exposure to POC on time to pregnancy.

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TABLE 1. Cox-regression models of the effect, measured as Success Rate Ratio (SuRR), of cohort on time to first planned pregnancy in an east coast vs. a west coast cohort of fishermen's wives.

All women n = 1086 SuRR (95% CI)	Non/light smokers n = 871 SuRR (95% CI)	Heavy smokers n = 214 SuRR (95% CI)
0.89 (0.77, 1.02)	0.99 (0.84, 1.16)	0.66 (0.49, 0.89)

**Light smoker = 1-9 cig/day, Heavy smoker = 10+ cig/day*

TABLE 2. Subfertility, defined as at least once having failed to achieve pregnancy within one year of trying, and infertility, defined as being subfertile and never have had being pregnant, in two cohorts of fishermen's wives. Odds Ratios (OR) comparing east coast vs. west coast women.

Cohort	Subfertility		
	All women n (%)	Non/light smokers n (%)	Heavy smokers n (%)
East Coast	88 (23)	53 (20)	33 (32)
West Coast	164 (20)	133 (19)	27 (22)
OR (95% CI)	1.21 (0.90, 1.63)	1.16 (0.86, 1.58)	

Cohort	Infertility		
	All women n (%)	Non/light smokers n (%)	Heavy smokers n (%)
East Coast	11 (3)	8 (3)	3 (3)
West Coast	10 (1)	9 (1)	1 (1)
OR (95% CI)	2.49 (1.05, 5.90)	2.58 (1.07, 6.22)	

**Light smoker = 1-9 cig/day, Heavy smoker = 10+ cig/day*

TABLE 3. Miscarriages and stillbirths in two cohorts of fishermen's wives compared to pregnancies under risk at the time of the miscarriage. Odds Ratios (OR) comparing east coast vs. west coast women.

Cohort	Before week 12	Week 12-28	After week 28
East Coast	12 (3%)	11 (3%)	5 (1%)
West Coast	54 (6%)	27 (3%)	8 (1%)
OR (95% CI)	0.48 (0.25, 0.90)	0.87 (0.43, 1.78)	1.34 (0.44, 4.13)

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Lowered birthweight among infants born to women with high intake of fish contaminated with persistent organochlorine compounds

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1. Introduction

Persistent organochlorine compounds (POC) are lipophilic pollutants which accumulate in the food chain. These compounds have been associated with various health hazards, such as cancer, and immunotoxic and reproductive effects[1,2]. In certain geographical areas the main route for human exposure is through consumption of contaminated fish. In Sweden, a high intake of fatty fish from the Baltic Sea (on the eastern coast) constitute such exposure[3-5].

The hypothesis of an association between exposure to POC and decreased birthweight was examined among cohorts of infants born to fishermen's wives from the Swedish east and west coasts[6]. The fish from the Swedish west coast is much less contaminated with POC[7]. Infants from the east coast cohort had an increased risk for lower birthweight compared with infants from the west coast cohort. A nested case-control study within the east coast cohort indicated an increased risk of lower birthweight among infants born to mothers who reported a relatively high current intake of fish from the Baltic Sea[8], as well as among infants born to mothers with a relatively high concentration of 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) in plasma[9]. Moreover, an increased risk for lower birthweight was also observed among infants born to mothers who had grown up in a fishing village[8]. This latter observation suggested that the sisters of the fishermen, who to a high proportion had grown up in a fishing village, may constitute a proper alternative study population for assessing the association between exposure to POC and birthweight.

The aims of the present study were to investigate whether fishermen's sisters from the Swedish east coast had a relatively high intake of fish from the Baltic Sea and to assess their reproductive outcome.

2. Material and Methods

By linkage to the national Swedish population register, we identified sisters to fishermen from the Swedish east and west coasts. The cohort of sisters were linked

to the Swedish Medical Birth Register (MBR). These linkages resulted in 1719 infants born to 1030 sisters to fishermen from the Swedish east coast during the period 1973-1993, and 2682 infants born to 1537 corresponding women from the Swedish west coast.

Telephone interviews were performed with randomly selected women from the east and west coast cohorts and with randomly selected women from the general population. The interviews were focused on the women's fish consumption.

3. Results

Both the interviewed east and west coast cohort women reported a more frequent intake of locally caught fish as compared with their referents (table 1). Forty-four percent of the interviewed sisters from the east coast cohort and 46 percent of the interviewed sisters from the west coast cohort reported a higher intake of locally caught fatty fish 10-20 years ago as compared with their current consumption. The corresponding figures among the referents were significantly lower (16 and 22 percent, respectively). The sisters were to a significantly higher degree than the referents born in a fisherman's family and grown up in a fishing village (table 1).

A direct comparison between the cohorts showed a significant difference between the birthweight distributions. The median birthweight was 3500 g in the east coast cohort and 3560 g in the west coast cohort. The adjusted cohort difference in mean birthweight was 72 g (95% CI 39-105 g). Moreover, east coast affiliation was an indicator of having an infant with low birthweight (figure 2). When 2500 g was used as the cutpoint the adjusted OR was 1.6 (95% CI 1.1-2.3), whereas for 3000 g as the cutpoint the adjusted OR was somewhat lower (OR 1.3, 95% CI 1.1-1.5). These calculations were performed after the exclusion of multiple births and infants with major malformations. For the outcome small for gestational age (SGA) the results were similar.

4. Discussion

The main findings in the present study were increased risks among fishermen's sisters from the Swedish east coast of having infants with low birthweight and SGA. The quality of the MBR birthweight data is considered to be good, whereas it is more difficult to get reliable estimates of the gestational length[10)]

A reasonable explanation to the increased risk of having an infant with low birthweight among fishermen's sisters from the Swedish east coast was an increased intake of fish from the Baltic Sea contaminated with POC. The interviewed sisters from the cohorts reported a somewhat higher current consumption of locally caught fish as compared with women from the general population. However, fish consumption before and during pregnancy is a more

relevant exposure measure than the current consumption. The interviews showed that the sisters from both the east and west coast cohorts had had a higher consumption earlier (10 to 20 years ago), whereas the women from the general population had not changed their dietary habits. Moreover, a high consumption of locally caught fish during early life for the fishermen's sisters, is supported by that a greater fraction of them were grown up in fishing village or in a fisherman's family than their referents.

To sum up, the results from the present study is in accordance with the results obtained among infants born to fishermen's wives, and thereby support the hypothesis of an association between exposure for POC and low birthweight.

5. Acknowledgements

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Table 1. Current consumption of fish for randomly selected women from the east and west coast cohorts and referent women from the general population. Moreover, the fractions of women grown up in a fishing village and with a fisherman as the father, respectively, are reported.

	Fishermen's sisters			Referents			
	East (N=99)	West (N=116)	p[a]	East (N=106)	p[b]	West (N=117)	p[b]
Locally caught fish (meals/month)							
median	4	5	0.02	3	0.02	4	0.03
(quartiles)	(1, 7)	(2.2, 9)		(0, 5)		(1, 7)	
Living in a fishing village during childhood and adolescence (percent)	34	74	<0.001	10	<0.001	3	<0.001
Born in a fisherman's family (percent)	50	64	0.05	8	<0.001	3	<0.001

[a] P-values for comparison between the cohorts of fishermen's sisters (Mann-Whitney test)
[b] P-values for comparison between the cohort of fishermen's sisters and the referents (Mann-Whitney test)

Table 2. Crude and adjusted odds ratios (OR) with 95% confidence intervals (CI). Multiple births and major malformations were excluded from the analyzed data.

		Crude		Adjusted[a]	
	N_{case}	OR	95% CI	OR	95% CI
<2500 g versus ≥2500 g					
West coast cohort	65	1.0		1.0	
East coast cohort	70	1.7	1.2-2.4	1.6	1.1-2.3
<3000 g versus ≥3000 g					
West coast cohort	293	1.0		1.0	
East coast cohort	246	1.3	1.1-1.6	1.3	1.1-1.5
SGA versus not SGA[b]					
West coast cohort	51	1.0		1.0	
East coast cohort	49	1.5	1.0-2.2	1.4	0.9-2.1

[a] Odds ratios adjusted for maternal age (3 categories: ≤24, 25-29, ≥30 years), parity (2 categories: 1 and ≥2), smoking habits (4 categories: nonsmokers, 1-9 cigarettes a day, ≥10 cigarettes a day, and unknown), and gender of the infant.
[b] Small for gestational age

Birth weight and growth in Dutch newborns exposed to background levels of PCBs and Dioxins

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Abstract

Two hundred and seven healthy newborns were followed from birth until pre-school age. Anthropometric measurements were collected at birth, 10 days, 3-, 7-, 18- and 42 months of age. Growth was defined as change in standard deviation score (SDS) for weight and length between two successive measurements. Prenatal PCB exposure was estimated from cord plasma PCB levels. After adjustment for covariates, cord plasma PCB levels had a significant negative effect on birth weight as well as growth from birth to 3 months of age. Growth beyond 3 months of age was not related to prenatal PCB exposure. We conclude that in utero exposure to PCBs has a negative effect on birth weight as well as growth until 3 months of age.

Introduction

Polychlorinated biphenyls (PCBs), and Polychlorinated dibenzo-p-dioxins (PCDDs) and -dibenzo-furans (PCDFs), summarized as dioxins, are widespread environmental contaminants. Intrauterine growth retardation was reported in the Yusho and YuCheng incidents in which pregnant women consumed cooking oil accidently contaminated with PCBs and related compounds[1]. Reduced birth weight and a shorter gestation was observed in infants whose mothers consumed contaminated fish from Lake Michigan, USA[2]. We investigated the influence of pre- and postnatal exposure to background PCB and dioxin levels on birth size and postnatal growth. Results from the Rotterdam part of the "Dutch/PCB dioxin study" are presented in this paper.

Methods

From 1990 to 1992, infants born at term (37-42 weeks of gestation), without congenital anomalies or diseases were recruited for this study. Pregnancy and delivery had to

be without complications or serious illnesses. All mother-infant pairs included were of the Caucasian race. To study the effects of prenatal and postnatal exposure to PCBs and dioxins, women were included who intended to give formula-feeding (formula-fed group) next to women who intended to breast-feed their child for at least 6 weeks (breast-fed group).In the formula, concentrations of both PCBs and dioxins were negligible. Participants lived in Rotterdam or its immediate surroundings, a highly industrialized and densely populated area in the western part of the Netherlands.

Maternal and umbilical cord plasma samples were obtained in the last month of pregnancy and shortly after delivery. Four non-planar PCB congeners, IUPAC n's 118, 138, 153 and 180 were analysed in maternal and cord plasma. The PCB sum (∑PCB) was calculated by adding up the 4 congeners in each plasma sample. A 24-hour representative breast milk sample was collected 2 weeks after delivery from each breast feeding mother, and analysed for 17 dioxin and 26 PCB congeners. Prenatal PCB exposure was estimated from PCB levels measured in maternal and cord plasma. Postnatal PCB and dioxin exposure was estimated by PCB and dioxin TEQ (toxic equivalents) levels measured in breast milk multiplied by the number of weeks of breast-feeding[3].

Birth weight and weight, length and head circumference were collected at 10 days, 3, 7, 18 and 42 months of age. Weight, length or height, and head circumference were converted into standard deviation scores (SDS) using weight, height and head circumference standards of healthy Dutch children as reference data. The SD Scores were calculated with the following formula; SDS = (X - Mean)/SD, X = Weight or Height or Head circumference, Mean = mean for age and sex of reference data and SD = standard deviation for age and sex of reference data[4]. Growth rate was defined as change in SD score of weight (ΔwSDS), length (ΔhSDS) and head circumference (ΔhcSDS) between 2 successive measurements, e.g. birth/10 days and 3 months.

Potential confounding variables for birth size and growth were selected from data on socio-economic background, maternal (health) history, pregnancy and delivery, gestational age, parity, gender and fetal exposure to alcohol and cigarette smoking. Parent's height served to calculate the target height (cm) (TH) as predicting variable for birth size and growth. Linear regression analysis was carried out to study the influence of prenatal PCB exposure on birth weight. The influence of pre- as well as postnatal PCB and dioxin exposure on growth per interval, e.g. from 0-3 months was also studied by linear regression analysis. Results were significant if $p \leq 0.05$.

Results and Discussion

In this study 207 mother-infant pairs were enrolled, of whom 105 were in the breast-fed group (BF) and 102 in the formula-fed group (FF). Linear regression analysis showed that after adjustment for gestational age, parity, target height, smoking and alcohol use during pregnancy, the ∑PCB in cord plasma negatively influenced birth weight (β (SE) = -119 (54), p=0.03, n=179). Similar results were found when maternal ∑PCB levels was entered in the regression analysis as a measure for prenatal exposure.

The effect of pre- as well as postnatal PCB and dioxin exposure on growth between 0-3, 3-7, 7-18 and 18-42 months was studied by multiple linear regression analysis. Cord plasma

PCB levels and PCB- and dioxin-TEQ multiplied by breast feeding weeks, were entered as pre- respectively, postnatal exposure variables. Cord plasma PCB levels were negatively associated with the change in SD score of body weight (ΔwSDS) between 0-3 months (p=0.03), however the influence on change in SD score of length (ΔhSDS, p=0.06) and head circumference (ΔhcSDS, p=0.17) were not significant. Postnatal PCB and dioxin exposure were not related to growth after birth, nor was prenatal PCB exposure related to growth beyond 3 months of age (see table).

Our findings are consistent with the results found in animal[5] as well as human studies[1,2], showing that prenatal exposure to PCBs is associated with reduced birth weight. We also found that weight gain (growth) during the first 3 months after birth, is negatively influenced by prenatal PCB exposure. Although lactational exposure to PCBs and dioxins is much higher, the effects described are associated with prenatal PCB exposure. We must keep in mind that the effects are small and within the normal range, however they are described in a population exposed to background PCB and dioxin levels.

Table: Linear regression analysis for growth from 0-3 months of age.

	Weight growth*		Length growth**		Head growth***	
	β (se)†	p-value	β (se)	p-value	β (se)	p-value
Constant	3.5 (2.33)		-0.69 (1.84)		5.6 (1.9)	
Ln(∑PCB)cord#	-0.27 (0.13)	0.03	-0.18 (0.10)	0.06	-0.14 (0.10)	0.17
Total TEQ@ ng/g fat x weeks	2.07 (1.53)	0.89	1.34 (1.24)	0.28	-6.55 (1.25)	0.60
weight-SDS at birth	-0.38 (0.07)	<0.001				
height-SDS at 10 days			-0.32 (0.06)	<0.001		
head-SDS at 10 days					-0.23 (0.05)	<0.001

*: (ΔwSDS) change in standard deviation score of body weight **:(ΔhSDS) change in standard deviation score of body length ***:(ΔhcSDS) change in standard deviation score of head circumference. #: Sum of Polychlorinated Biphenyls, IUPAC nrs 118, 138, 153 and 180 in cord blood @: Postnatal exposure; sum of toxic equivalents (TEQs) of 8 dioxin like PCB and 17 dioxin congeners measured in breast milk, multiplied by the number of weeks of breast-feeding until 3 months. †: β (se)=Regression coefficient (standard error)= change in ΔSD score per unit change in variable. All regression analyses are adjusted for alcohol and cigarette smoking during pregnancy and gestational age.

Acknowledgments

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Pre- and postnatal exposure to PCBs and dioxins and cognitive development of Dutch children at 3½ years of age.

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Abstract

The effect of pre- and postnatal exposure to PCBs and dioxins on cognitive development at 3½ years of age was investigated. We studied 395 of the original 418 mother-infant pairs recruited for the "Dutch PCB/dioxin study". Their cognitive development was assessed with the Dutch version of the Kaufmann Assessment Battery for Children (K-ABC). After adjustment for covariates, prenatal PCB exposure, estimated from maternal plasma PCB levels measured during pregnancy, was significantly associated with a lower score on the cognitive scale of the K-ABC. Postnatal PCB and dioxin exposure measured from maternal milk samples, and PCB exposure at 3½ years measured from PCB levels in plasma of children, were not related to the cognitive development at 3½ years. We conclude that prenatal exposure to 'background' PCB levels influences the cognitive development of Dutch pre-school children negatively.

Introduction

Polychlorinated biphenyls (PCBs), chlorinated dibenzo-para-dioxins (PCDDs), -di-benzo-furans (PCDFs) are widespread and persistent environmental pollutants. The Netherlands belong to countries with high environmental levels of PCBs and PCDD/Fs (dioxins) as measured for instance in human milk. The "Dutch PCB/dioxin" project was therefore initiated to investigate possible adverse effects of background exposure to PCBs and dioxins on growth and neuro-development of young children. Healthy term newborns were studied until pre-school age. We have reported previously that subtle signs of neurological dysfunctioning, small delay in psychomotor development, alterations in thyroid hormone and immunological status are associated with perinatal PCB and dioxin exposure[1]. We report results of background PCB and dioxin exposure in relation to cognitive development of Dutch children at 3½ years of age.

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Methods

At 3½ years of age we examined 395 children, 94 % from the original 418 mother-infant pairs recruited in the period from 1990-1992. At that time healthy pregnant women were asked to volunteer for a prospective follow-up study in Rotterdam and Groningen. Subjects were included in the study, if they met the following criteria: (1) Pregnancy and delivery had to be without complications or serious illnesses. (2) First or second term born infants (37-42 wks of gestation). (3) No congenital anomalies or diseases. (4) Caucasian race. Women were included who intended to breast-feed their child for at least 6 weeks (breast-fed group) next to women who intended to give formula-feeding (formula-fed group). The medical ethics committees of both University Hospitals approved the study protocol.

Maternal and umbilical cord plasma samples were obtained in the last month of pregnancy and shortly after delivery. Plasma samples of children were collected at 3½ of age. Four PCB congeners, IUPAC n's 118, 138, 153 and 180 were analysed in maternal, cord and 3½-year plasma samples. The PCB sum (∑PCB) was calculated by adding up the 4 congeners in each plasma sample. Maternal milk samples were collected 2 weeks after delivery and analysed for 17 dioxin and 26 PCB congeners. The total toxic potency of all dioxins and 8 dioxin-like PCBs (IUPAC n's. 77, 126, 169, 105, 118, 156, 170 and 180) was calculated using the toxic equivalent factor (TEF) approach. The toxic equivalent (TEQ) was calculated by multiplying the concentration and the TEF value. The sum of all TEQ values yielded the total PCB/dioxin TEQ. Prenatal PCB exposure was estimated from PCB levels measured in maternal or cord plasma. Postnatal PCB and dioxin exposure was estimated by PCB and dioxin TEQ levels measured in breast milk multiplied by the number of weeks of breast-feeding. PCB levels at 3½ years of age are a measure of current exposure.

The cognitive abilities at 3½ years of age were assessed with the Dutch version of the Kaufmann Assessment Battery for Children (K-ABC). The Dutch version is standardized on a large sample of normal children, ages from 2.5 to 4.5 years of age[2]. This battery yields standard scores in 11 subtests measuring Sequential and Simultaneous processing. The scores of the sequential (SEQ) and simultaneous (SIM) scale result in an overall score the Cognitive scale (COG). These scores have a mean of 100 and a SD of 15.

Potential confounding variables for developmental outcome at pre-school age were selected from a list pertaining data on study centre, socio-economic background, maternal age, parents' educational level, birth order, gender and fetal exposure to alcohol and cigarette smoking, feeding type and period of breast-feeding. The child's home environment was assessed by the Dutch version of the home observation for the measurement of the environment[3]. The verbal IQ of the mother was assessed by 2 subtests of the verbal scale of the Wechsler Adult Intelligence Scale[4].

To investigate the effect of pre- and postnatal and current exposure to PCBs (and dioxins) on developmental outcome at 3½ years of age, we performed a multiple linear regression analysis. Each outcome variable was evaluated in three regression analyses, one for prenatal, one for postnatal, and one for current exposure, after adjustment for confounding variables. Results were significant if $p \leq 0.05$.

Results and Discussion

At 3½ years of age 395 children (94%) were examined from the original cohort of 418 children. From the remaining group, 203 were breast-fed (BF) and 193 were formula-fed (FF) in infancy. Finally, 380 children completed the Dutch version of the K-ABC test battery. None of the children had an abnormal score, below the -2 SD, all scores are within the normal range (Table). Mean scores were significantly, higher in the breast-fed group, however after adjustment for maternal education and HOME score, this effect did not remain significant.

In the final model all potential confounding variables mentioned in the methods section were entered as covariates next to the exposure variable. Results from multiple regression analyses showed that, prenatal PCB exposure, measured from maternal plasma ∑PCB levels, was significantly associated with lower scores on the Cognitive scale (n=373; β (SE) = -4.6 (1.6), $p<0.01$). This was also true for the Sequential as well as the Simultaneous scale of the Dutch K-ABC. Postnatal exposure to PCBs and dioxins measured from PCB- and dioxin-TEQs in breast milk and breast feeding period, and current exposure estimated from 3½-year plasma ∑PCB levels, were not associated with poorer performances on the three outcome variables.

Table: Test results at 3½ years of age, given for both feeding groups

	All n=380		Formula-Fed Group n=183		Breast-Fed Group n=197	
Dutch version K-ABC*	Mean	(SD)	Mean	(SD)	Mean	(SD)#
Cognitive Scale	111	(14)	108	(15)	114	(13)
-Sequential Scale	109	(14)	107	(14)	111	(14)
-Simultaneous Scale	109	(13)	106	(14)	112	(12)

*: Dutch version of the Kaufmann Assessment Battery for Children (Z&Z)
#: Student t-test, significantly higher than the formula-fed group, all p-values < 0.01.

These findings are in accordance to what Jacobson and colleagues have found in the "fish exposure cohort"[5,6], and are consistent with the reports of reduced IQ scores in children born from mothers exposed to accidental high levels of PCBs (and PCDFs) in Taiwan (Yucheng incident)[7]. However, when we compare our results with those of the North Carolina cohort by Gladen and Rogan, who also studied background exposure to PCBs, we found an effect of in utero exposure to PCBs on cognitive development at 3½ years, whereas the effects they found until 2 years of age was no longer apparent at 3, 4 and 5 years[8]. This difference could be due to higher background PCB levels found in the Netherlands compared to the USA[9], or due to different laboratory techniques used in measuring PCB exposure.

In conclusion, prenatal exposure to background PCB levels, is significantly associated with lower scores on cognitive abilities in Dutch pre-school children. Postnatal exposure to

PCBs and dioxins as well as current exposure to PCBs measured at 3½ years were not related with the cognitive outcome. Future studies in "background" exposed cohorts are needed to investigate what the long-term implications for later intellectual functioning will be.

Acknowledgments

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Exposure to Polychlorinated Organic Compounds and Thyroid Hormone Plasma Levels of Human Newborns

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Abstract

Thyroid hormones are essential for the normal neurological development of humans. Polychlorinated organic compounds (POCs) like organochlorine pesticides (OCPs), polychlorinated biphenyl's (PCBs), dibenzodioxins and dibenzofurans (PCDDs and PCDFs) are suspected to interfere with the thyroid hormone system because of their structural similarity to thyroid hormones.
In the present study, the association of prenatal exposure to POC-compounds and thyroid hormone levels in a group of 93 Dutch human newborns was investigated. Exposure was estimated by means of OCP-, PCB-, and PCDD/F-levels in mother's milk, an indirect measure of prenatal exposure. Plasma thyroxin (T4) levels of the infants were determined 5-7 days after birth. Univariate analyses demonstrated significant negative associations between plasma T4 levels of newborns and exposure to different POC-compounds. In the multivariate analyses these associations changed substantially. It appeared that Body Mass Index of the mother and smoking during pregnancy by the mother should be considered as potential confounding factors studying the association between POC-exposure and thyroid hormone levels.

Introduction

In the last few years polychlorinated organic compounds (POCs) like organochlorine pesticides (OCPs), polychlorinated biphenyl's (PCBs), dibenzodioxins and dibenzofurans (PCDDs and PCDFs), have been suspected to interfere with the endocrine system of both humans and wildlife. Unborn or newborn children are expected to be in particular at risk, because exposure to relatively high levels is occurring in a crucial period of the child's development[1,2]. One of the hormone systems which could be affected is the thyroid hormone system[1-3]. Because of the structural similarity of PCBs and dioxins to thyroid hormones, they are suspected to either decrease or mimic their biological action. This might result in irreversible neurological damage, as thyroid hormones are essential for normal neurological development in humans[3]. The main route of human exposure to POCs is food (approximately 95%). POCs are stable, lipophilic pollutants and therefore accumulate in the human body. Newborns can already be contaminated at birth with these substances, as transplacental transport takes place[4].
Altered thyroid hormone levels following (prenatal) exposure to POCs have been found in several experimental animal studies[5-9]. Effects of (prenatal) exposure to POCs on the thyroid hormone

system in humans have been examined only in a few studies. Most of these studies indicate that alterations in plasma thyroid hormone levels might also occur in humans and human neonates[10-14]. In the Netherlands, human milk surveys are performed at five years intervals to study trends in human exposure to POC-compounds. The most recent sampling campaign took place in June 1993. Current levels and determinants of POC levels in human milk have been reported earlier[15]. Parallel to the 1993-campaign, data on thyroid hormone plasma levels of newborns were collected. The aim of the present report is to study associations between prenatal exposure to POCs and thyroid hormone levels of human newborns.

Methods

The sampling strategy in 1993 was similar to the approach used in the former human milk surveys[16] with the exception that at this time the population was restricted to primiparae. In co-operation with 20 maternity centres scattered all over the country, finally 157 mothers were approached for participation. Each respondent was asked to collect a milk sample between day 6 and day 10 after delivery as breast milk POC-levels were used as an indirect measure of prenatal exposure. In addition, they were asked to fill out a questionnaire by which information was obtained on maternal characteristics (age, weight, height, education), personal habits (smoking and alcohol use) and on pregnancy characteristics (length of pregnancy, birthweight).
The analytical programme consisted of compound-specific determinations in the milk sample of ten OCPs, fifteen PCBs, and seventeen 2,3,7,8-substituted PCDDs and PCDFs. Details on analytical methods have been described previously[17]. Thyroid hormone plasma levels were obtained from the national PKU/CHT-screening programme. In this program each newborn child in the Netherlands is screened for phenylketonuria (PKU) and congenital hypothyroidism (CHT). Serum thyroxin (T4) and serum thyroid stimulating hormone (TSH) concentrations are determined 5-7 days after birth by using radioimmunochemical methods.
Data-analysis is performed using SAS V611©. In this study levels and distributions of 42 different congeners were determined. To reduce the number of analyses, the associations of thyroid hormone levels with combined parameters, instead of individual congeners have been studied. For each mother ten sumparameters were calculated adding up the levels of the OCPs, dioxins and furans, indicator PCBs, other dioxin-like PCBs, non-ortho PCBs, total POCs (OCPs, dioxin/furans and PCBs) and on a TEQ-basis[a] : TEQdioxin/furan, TEQnon-ortho, TEQother and TEQtotal (PCBs and dioxins/furans). Part of the samples were below the level of detection for some congeners. To reduce information loss in the present analyses the nondetects were assumed to equal half of the level of detection. Distributions of both exposure parameters and thyroid hormone plasma levels were examined and when parameters where not normally distributed, natural logarithm transformation took place.
To study the association between POC-exposure and plasma T4 levels, first Pearson correlation coefficients were calculated. Furthermore, the differences in mean plasma levels of low and high to POCs exposed groups of newborns were tested. Multivariate linear regression analysis was used to examine possible confounders like maternal and child characteristics. Variables which appeared to be statistically significant associated with thyroid hormone levels at the 0.10 level and of special interest from the literature (smoking of mother during pregnancy [18]) were considered in multivariate regression models.

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[a] TEQ values by adding up breast milk levels multiplied by the international toxic equivalency factors (TEFs) for the dioxins and furans[19,20] and the Interim WHO TEFs for the non-ortho PCBs[21]

Results

Of the 157 approached mothers, 121 were willing to participate. After combining questionnaire data, breast milk data and thyroid hormone plasma levels a complete set of data was available of 93 mother-child pairs. Tables 1 and 2 show population characteristics and distribution characteristics of both thyroid hormone parameters and exposure of the study population.

Table 1: Distribution of population characteristics of both mothers and infants (N=93)

		min.	median	max.
CHILD	Plasma T4 (nmol/l)	123	201	313
	Birthweight (grams)	2375	3460	4730
	Duration of pregnancy (weeks)	37	40	42
MOTHER	Age (years)	18	29	38
	Prenatal BMI [b]	18	22	30

[a] below level of detection, [b] Body Mass Index or Quetelet-Index: by definition calculated as weight/(length)^2, min=lowest, max=highest

Table 2: Distribution of the exposure parameters among the study population (N=93)

Parameters	N	min.	25-perc.	50-perc.	75 perc.	max.
sumOCP[a]	76	0.22	0.39	0.50	0.69	2.21
sumDioxin/Furan[b]	90	131.5	293.5	385.0	546.6	1125.2
sumPCBindicator[c]	72	102.5	203.6	263.4	331.8	606.7
sumPCBother[c]	70	53.3	99.6	121.2	157.1	295.9
sumPCBnon-ortho[b]	90	49.8	110.2	142.5	187.4	312.7
sumPOCtotal[a]	64	0.39	0.70	0.91	1.08	3.08
TEQdioxin/furan[d]	90	8.4	17.8	21.6	28.1	47.5
TEQnon-ortho[d]	90	2.8	6.3	7.9	11.3	21.1
TEQother[d]	70	4.7	9.8	11.5	14.7	27.9
TEQtotal[d]	66	19.9	34.9	42.8	54.4	87.2

[a] mg/kg fat, [b] pg/g fat, [c] ng/g fat, [d] pg TEQ/g fat; min = lowest, max = highest, perc.=percentile

Univariate analyses pointed out that all the exposure parameters were negatively associated with plasma T4 levels of the infants. Correlation coefficients ranged from -0.06 to -0.20. A borderline significant correlation was observed between TEQdioxin/furan and T4 (p=0.055, N=90). Dividing the mother-child pairs into low and high to POCs exposed groups (by means of the median of the exposuregroup of interest, table 2) confirmed these negative associations. Mean plasma T4 levels in high-exposed infants were lower than in low-exposed infants. These differences ranged from 0.9 to 7.4% for the different exposure groups (figure 1). Dividing the mother child-pairs in tertiles or quartiles demonstrated similar trends.

Body Mass Index of the mother (BMI) was associated with both plasma T4 and exposure. Smoking of the mother during pregnancy was associated with exposure and combined with BMI also with plasma T4 levels. When these factors were considered in multivariate analyses, the significant negative univariate association between TEQdioxin/furan and plasma T4 disappeared (table 3) and both a significant negative association between OCPs and plasma T4 and significant interactions between BMI, smoking and OCPs appeared (table 4). Similar effect patterns were demonstrated for the other pollutant groups.

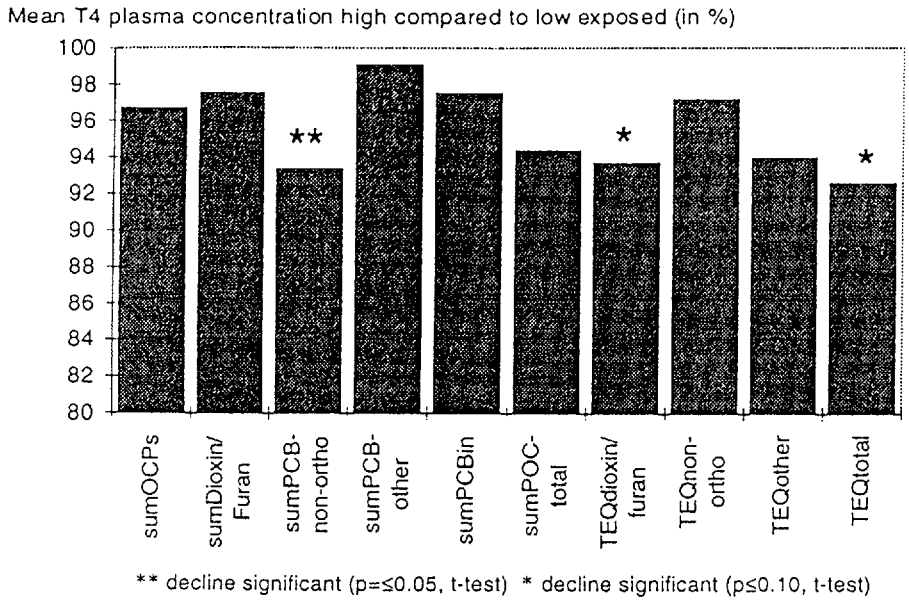

Figure 1 Mean plasma T4 levels in high to POC-compounds exposed groups of newborns as a percentage of the means of low exposed groups (median of exposure is cut-off point). Axis scale starts at 80%

Table 3 Association (regression coefficients and p-values) between prenatal exposure to dioxin/furan-TEQ[1] and plasma T4 levels of newborns (N=89)

	Univariate		Multivariate	
	rc	p-value	rc	p-value
TEQdioxin/furan	-19.1	0.07	-11.6	NS
BMI	3.9	0.008	3.5	0.03
Smoking	3.7	NS	2.4	NS

[1] log-transformed values

Table 4 Association (regression coefficients and p-values) between prenatal exposure to organochlorine pesticides (OCPs)[1] and plasma T4 levels of newborns (N=75)

	Univariate		Multivariate	
	rc	p-value	rc	p-value
OCPs	-4.4	NS	-126.8	0.06
BMI	4.0	0.02	6.5	0.02
OCPs*BMI	-	-	6.1	0.05
Smoking	8.5	NS	-98.2	NS
OCPs*Smoking	-	-	-65.5	0.002
BMI*Smoking	-	-	3.1	NS

[1] log-transformed values

Discussion

Measured POC levels in the present study are reasonably well comparable to other reported Dutch values[13,14], differences might be due to analytical variance. Plasma T4 levels of the study population appeared to be in the normal range, none of the children had an abnormal PKU-CHT test. A significant reduction of plasma T4 in high to dioxin-TEQ, PCB-dioxin-TEQ and non-ortho PCBs exposed groups of newborns was found, as well as a significant negative univariate correlation between dioxin-TEQ and plasma T4 levels. Multivariate analyses in this study showed that these univariate associations were modified by BMI of the mother and smoking during pregnancy. Furthermore, in some models complex interactions between these variables and exposure existed.

So far, other studies which have been performed on the association between POC-exposure and the thyroid hormone status of human(newborn)s reported contradictory results. In accidentally or occupationally to PCBs and PCDFs exposed persons, both increased and decreased serum T3 and T4 concentrations have been reported, compared to non-exposed persons[11,12]. Pluim et al.[13] reported elevated T4 plasma levels in a group of Dutch newborns exposed to high dioxin-TEQ concentrations (29.0-62.7 pg TEQ/g fat, N=19), compared to a group infants exposed to low dioxin-TEQ concentrations (8.7-28.0 pg TEQ/g fat, N=19). A comparable study by Koopman-Esseboom[14] demonstrated dioxin-TEQ, total PCB-dioxin-TEQ, planar and non-planar PCB-TEQ in human milk to correlate negative (Spearman correlation) with maternal plasma T3 and T4 levels and positive with plasma TSH levels in a group of newborns (N=79). A significant correlation of TEQ-levels with plasma T4 levels of the infants has not been demonstrated. In contrast to the Pluim data, infants of mothers in high to dioxin-TEQ (>30.75 pg TEQ/g fat) and PCB-dioxin-TEQ (>72.43 pg TEQ/g fat) exposed groups had significantly lower plasma T4 levels than infants in low dioxin-TEQ (≤30.75 pg TEQ/g fat) and PCB-dioxin-TEQ (≤72.43 pg TEQ/g fat) exposed groups.

Likewise, experimental animal studies demonstrated inconsistent findings as species-dependent associations between POC-exposure and plasma thyroid hormone levels were found. Elevated plasma T3, T4 and TSH levels were found in hamsters and guinea pigs after exposure to 2,3,7,8-TCDD[5,6]. Decreased plasma T3 and T4 levels and increased plasma TSH levels were observed after exposure to PCBs and dioxin/furans in rats and monkeys[7-9].

Unlike most of the epidemiological studies, in the present study findings were adjusted for potential confounders. The results of the multivariate linear regression analyses show that performing only univariate analysis appeared to be insufficient. Little is known about factors which influence plasma T4 levels of newborns. Infants of mothers who smoked during pregnancy are reported to have higher cord serum T4 levels[18]. Smoking is already known to correlate with lower POC breast milk levels probably through an effect of smoking on the fat metabolism[22]. Body Mass Index of the mother was in this study associated with higher plasma T4 levels of the infants. The mechanism underlying this association is, yet, not fully understood but might in part be explained by the important role of thyroid hormones on the fat metabolism.

It can be concluded that when studying the association between prenatal exposure and the thyroid hormone status of newborns, adjusting for confounders is necessary. Future studies will be needed to gain more insight in potential confounders and their interactions to clarify the inconsistencies reported so far on this issue.

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Perinatal exposure to PCBs and dioxins and the neurological status at 3½ years of age

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Abstract

We investigated the effect of perinatal exposure to PCBs and dioxins on the neurological condition at 3½ years of age. PCB levels were determined in cord and maternal plasma, and used as a measure of prenatal exposure. To measure postnatal exposure, breast milk was analyzed for PCBs and dioxins. In addition, PCBs were determined in child's 3½-year plasma. The neurological condition was assessed in 394 children by means of the Touwen/Hempel method. Neither prenatal exposure to PCBs nor lactational exposure to PCBs and dioxins nor the child's body burden at 3½ years was found to be related to the neurological condition at 3½ years of age after adjustment for covariates.

Introduction

As part of the 'Dutch PCB/Dioxin Study', we have studied the effects of 'background' levels of perinatal exposure to PCBs and dioxins on the neurological condition in the second week after birth[1)], and at 1½ years of age[2)]. Therefore, we followed a group of healthy term infants from birth. In the second week after birth, it was found that a high prenatal exposure together with a high lactational exposure resulted in an adverse effect on the neurological condition and a higher incidence of hypotonia. At 1½ year, higher levels of prenatal PCB exposure were found to be related to a less optimal neurological condition, and no effect of lactational exposure to PCBs and dioxins was found.

In the present abstract, we report on the effect of prenatal exposure to PCBs, exposure to PCBs and dioxins through breast milk, and the PCB level measured in the child's 3½-year plasma on the neurological condition at 3½ years of age.

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Methods

Healthy pregnant women were asked to participate in the study. The study was performed in two study centres, Groningen and Rotterdam, The Netherlands. It was planned to include 50% breast-feeding and 50% formula-feeding mother/infant pairs in each study centre. Only healthy, first or second born term children who did not suffer from complications during fetal life and birth were included in the final study group. Moreover, in addition to the formula-feeding mother/infant pairs, we included only those who could sustain breast-feeding for at least six weeks. For each mother, the duration of breast-feeding was noted. We used a 72-item questionnaire to record information on the obstetrical, socioeconomic, pre-, intra-, and immediate postpartum conditions. A compound score of this, the obstetrical optimality score, was made by counting the number of items that fulfilled pre-set optimality criteria.

We collected both maternal and cord blood. Child's blood was sampled at 3½ years. In plasma we determined the non-planar PCB congeners 118, 138, 153, and 180. The sum of the levels of these four congeners (PCBsum) was calculated for cord, maternal, and child's plasma. PCB levels in cord and maternal plasma are considered to be a reflection of prenatal exposure. To asses lactational exposure, human milk samples were collected, and analyzed for three planar and 23 non-planar PCB congeners and 17 ubiquitous 2,3,7,8-substituted dioxins. We calculated a total PCB/dioxin TEQ, a dioxin TEQ, a planar PCB TEQ (nrs. 77, 126, and 169), a mono-ortho PCB TEQ (nrs. 105, 118, and 156), and a di-ortho PCB TEQ (nrs. 170, and 180).

At 3½ years, the neurological condition was assessed according to Touwen/Hempel[3]. This age-adequate technique is directed at the observation of the motor functions prehension, sitting, crawling, standing and walking. It is conducted in a free-field situation. The neurological examination led to a clinical diagnosis: normal, mildly abnormal, or abnormal. In addition, we evaluated the neurological findings in terms of optimality by means of a list of 56 predefined criteria for optimality. A neurological optimality score (NOS) was calculated for each child. The quality of movements was scored as a 15-item fluency cluster score. The examiners were unaware of the levels of pre- and postnatal exposure to PCBs and dioxins and the type of feeding during early life.

To investigate the effect of pre- and postnatal exposure to PCBs and dioxins on the neurological condition at 3½ years, we performed a linear regression analysis. Results were considered to be significant if $p \leq 0.05$.

Results and Discussion

The final study group consisted of 418 mother infant pairs, of which 209 were in the breast-feeding group and 209 in the formula-feeding group. At 3½ years, the neurological condition was assessed in 394 (94%). Twelve (3%) children were considered to be mildly abnormal. One child was diagnosed as abnormal. The remaining 3½-year-olds (n=381; 97%) were considered to be neurologically normal. The children classified as mildly abnormal or abnormal did not differ from the group of neurological normal children in terms of PCB levels in plasma, and PCB and dioxin levels in breast milk. The median NOS at 3½ years was 52 (range: 30-56), whereas the mean fluency cluster score was 13 ± 2. Table 1 shows the PCB levels in plasma, and table 2 presents the PCB and dioxin levels in breast milk.

Table 1: PCB levels (μg/l) in plasma.

PCBsum*	Maternal plasma (n=394)	Cord plasma (n=352)	3½-year plasma (n=298)
Median	2.0	0.4	0.4
5th percentile	1.0	0.2	0.1
95th percentile	3.8	0.9	1.9

* PCBsum = sum of the levels of the PCB congeners 118, 138, 153, and 180.

Table 2: PCB and dioxin levels (ng TEQ/kg fat) in breast milk.

PCB/dioxin levels	Dioxins (n=170)	Planar PCBs (n=186)	Mono-ortho PCBs (n=186)	Di-ortho PCBs (n=186)
Median	28.8	14.5	14.2	4.2
5th percentile	14.9	6.8	6.9	2.1
95th percentile	51.5	31.9	24.8	7.8

Neither the PCBsum in cord plasma nor the PCBsum in maternal plasma nor the PCBsum in child's 3½-year plasma was found to be significantly related to the 3½-year NOS. Adjustments were made for the study centre and the obstetrical optimality score. No significant effect of the levels of dioxins, planar-, mono-ortho, di-ortho PCBs, and the total PCB/dioxin TEQ on the NOS was found. The final model consisted of the study centre and the obstetrical optimality score. Neither the PCBsum in cord plasma nor the PCBsum in maternal plasma nor the PCBsum in child's 3½-year plasma nor the levels of dioxins, planar-, mono-ortho, and di-ortho PCBs nor the total PCB/dioxin TEQ was significantly related to the fluency cluster score.

Our results are in accordance with the findings by Rogan and Gladen who, in the United States, studied the effect of pre- and postnatal exposure to PCBs on cognitive development; deficits seen through two years of age were no longer apparent at three, four or five years of age[4]. On the other hand, also in the US, Jacobson and co-workers[5] found that higher levels of prenatal exposure predicted poorer cognitive functioning at four years. It should be kept in mind that cognitive tests measure the child's abilities in a quantitative fashion, whereas the neurological examination is a qualitative measure of brain function.

In conclusion, adverse effects of prenatal exposure on the neurological condition could not be detected at 3½ years. Moreover, postnatal exposure to PCBs and dioxins was not found to be related to the neurological condition at 3½ years.

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