Determinants Influencing Levels of Polychlorinated Organic Compounds in Human Milk.

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INTRODUCTION

Polychlorinated organic compounds (POCs) like organochlorine pesticides (OCPs), polychlorinated biphenyl's (PCBs), dibenzodioxins and dibenzofurans (PCDDs and PCDFs), are stable lipophilic pollutants. Although their sources and routes of environmental contamination may differ they are simultaneously present in various biological samples, including fish, wildlife, meat and dairy products[1]. Environmental contamination occurs via aerial transport from their sources and deposition on plants, soils, and in water. Because of their lipophilic nature they accumulate in animal fat. The main route of human exposure to POCs is from food (approximately 95%), the remainder is attributed to inhalation and dermal exposure[2]. In the Netherlands foods of animal origin, including dairy products, fish and fish products are the major sources of background human exposure[3]. According to Di Dominico relevant exposure also occurs through consumption of vegetables and fruits[4]. Because of their persisting and lipophilic nature POCs accumulate in the human body, especially in tissues with a high fat content. In lactating women, breast milk is a major route of POC excretion and can therefore be used as a biological exposure index for the human body burden. Previously, several factors have been suggested to influence the contamination levels of human milk, such as the mothers age, dietary habits, body mass, and parity[5].
In the Netherlands human milk surveys are performed at five years intervals to study trends in human exposure to polychlorinated compounds. The most recent sampling campaign took place in June 1993. Besides describing current levels and providing data for the trend, the survey was aimed at potential determinants of the POC levels in human milk (like the mothers, age, Quetelet Index (QI=weight/(height)^2), and dietary habits). Since dietary intake represents the common route of human exposure, associations with food products and nutrients were studied extensively. In the present paper, the results of levels and determinants for 103 mother-child pairs are briefly discussed.

METHODS

sampling strategy

The sampling strategy in 1993 was similar to the approach used in the former human milk surveys[5] 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 100 ml milk sample between day 6 and day 10 after delivery. In addition, they were asked to fill out an extensive food frequency questionnaire[6] and a questionnaire on personal characteristics (such as age, weight, height, education) and -habits (such as smoking, alcohol use, work and hobbies). The mothers completed the questionnaires, if needed with assistance of a maternity nurse, and returned them by post. A detailed description of the study methods is ascribed elsewhere[7].

Using the information obtained by the food frequency questionnaires and according to the current knowledge on contamination levels in (Dutch) dietary products[8], several product groups were defined: 'cheese', 'fish', 'meat (products)' (subdivided in beef, pork and chicken meat), dairy products, egg, and vegetables (subdivided in green vegetables and the rest). Furthermore, nutrient intake (fat, protein) was calculated using the Dutch nutrient database, NEVO 1993[9]. Finally, dietary intake was expressed by calculating the (toxic equivalents) TEQ values by adding up the known levels in dietary products[8] multiplied by the international toxic equivalency factors (TEFs) for the dioxins and furans, (I-TEQfood)[10,11] and the Interim WHO TEFs for the non-ortho PCBs (PCB-TEQfood)[12].

Laboratory analysis

The analytical programme consisted of compound specific determinations of ten OCPs, fifteen PCBs, and seventeen 2,3,7,8-substituted PCDDs and PCDFs. Details on analytical methods have been described previously[13].

Statistical analyses

The analyses were performed using SAS V610©. In this study levels and distributions of 42 different congeners were determined. To reduce the number of analyses, we studied the associations of determinants with combined parameters, instead of individual congeners. For each mother eight sumparameters were calculated adding up the levels of the individual organochlorine pesticides (sumOCPs), the dioxins and furans (sumD/Fs), the non-ortho PCBs (IUPAC[a] numbers: 77, 126, 169), the indicator PCBs (28, 52, 101, 138, 153, 180), and the other dioxin-like PCBs (60, 74, 105, 118, 156, 157, 167, 180, 189), and on TEQ basis: the TEQdioxin/furan, TEQnon-ortho, and the TEQother. For these 8 sumparameters associations with the determinants, such as age, QI, smoking, education level (all categorical), dietary products (or nutrients, or TEQ values based on food intake) were studied, first, by calculating spearman correlation coefficients, and thereafter by using multiple linear regression analyses. For futher details see Cuijpers et al.[7]. Part of the congeners were non-detectable (n.d.). To use this information, in the present analyses the nondetects were assumed to equal half of the level of detection.

[a] IUPAC = International Union of Pure and Applied Chemistry

RESULTS

The response was 77%. Of the 157 approached mothers, 121 were willing to participate. Of the 36 non-respondents, 32 mothers completed a non-response questionnaire. After combining questionnaire and human milk data, for 103 mother-child pairs complete information was available. Table 1 represents some general characteristics for the research population and the non-respondents. Both groups appear to be fairly good comparable, only the difference in age is borderline significant (p=0.08).

The levels and distribution of the calculated sumparameters are depicted in table 2. The data for the individual congeners have been presented before[13]. Spearman correlation coefficients were calculated between sumparameters and potential determinants, among the sumparameters and among the determinants. Significant correlations were found between one or more of the sumparameters and the mother's age, the QI, the education level, smoking, the consumption of fish, pork, meat products, vegetables, and the PCB-TEQfood. Among the sumparameters high, statistically significant correlations were observed ranging from 0.37 ($p \leq 0.001$) up to 0.97 ($p \leq 0.0001$). Significant associations were also observed among the determinants, especially among the defined product groups, like for instance a negative correlation between the consumption of cheese and meat, or a positive correlation between de consumption of fish and vegetables. Like expected, the I-TEQfood and the PCB-TEQfood were significantly correlated with the investigated groups of dietary products. No significant correlations were observed among the mother's age, QI, smoking, and education level.

The results of the multiple linear regression models are presented in table 3. For each dependent variable (=sumparameter), four different regression models were calculated. The models differed in the way exposure by food intake was quantified: as 1) dietary products, 2) nutrients, 3) I-TEQfood, or as 4) PCB-TEQfood. Table 3 summarises the results of the regression analyses. Here only the direction and p-value of the statistically significant regression coefficients will be reported. A full paper with a more complete overview of the results will be reported elsewhere[7].

The most consistent finding is the (positive) association between the sumparameters and the mother's age. POC-levels appeared to be significantly lower in mothers younger then 30 years then in mothers of 30 years and older. The QI was significantly related to part of the sumparameters. In general the levels were higher in mothers with a QI $\leq$ 19 compared to mothers with a QI $\geq$ 25. Current smoking was compared to ex-smoking as well as never smoking associated with significantly lower levels of the sumparameters. Of the investigated dietary products the consumption of fish, beef, meat products, and dairy products were significantly, positive, associated with (part of) the sumparameters. A significant, negative association was observed for some of the sumparameters with the consumption of pork and egg. When dietary intake was quantified by nutrients, significant associations with part of the sumparameters were observed with the consumption of animal protein only. No significant associations were observed with 'fat' or 'fat-groups'. For the I-TEQfood and PCB-TEQfood significant associations were found for a few sumparameters, only.

DISCUSSION

The POC levels in this study were fairly good comparable to recently reported Dutch figures[14,15], although for individual congeners differences were observed which may be due to analytical variance. Compared to international data, the observed POC levels are relatively high but similar

to those reported for Belgium, Germany and Spain[16]. A trend analysis of German and Dutch data on dioxins and furans in the period 1986-1993, shows a decrease up to 30% of the I-TEQ level[13,17]. The (international) database for dioxin like PCBs is rather limited, since methods for the determination of these compounds have only recently been implemented. As reported before, the OCP levels indicate a further decrease[13]. The results of an extended trend-analysis of the Dutch figures will be reported in the near future.

The results of the present study confirm that the mother's age, body mass (QI), and dietary intake are important determinants of POC levels in human milk. The most consistent association was observed with the mother's age. The higher concentrations observed in 'older' mothers (≥ 30 years) may be due to differences in accumulation period. On the other hand, differences in historical exposure may have occurred. In agreement with previous results[5], a significant negative association was found between the QI and part of the sumparameters, suggesting lower POC levels in women with more fat tissue. These results again support the theory of dilution[18] resulting in a lower POC concentration per unit. A striking observation was that current smoking appeared to be related to lower levels of PCBs and dioxins. Lower dioxin and furan levels in smoking women compared to non-smoking or passive smoking women have been reported before[19]. Theoretical smoking may interfere with the metabolism of congeners, and thereby enhance excretion. However, this proposition is purely speculative, but interesting for further research.

As stated before food intake is the main route of human POC exposure. In agreement with what was expected the present results demonstrate positive, although not in all instances significant, associations between POC levels and the consumption of fish, beef, meat products and dairy products. The results for the investigated 'nutrients' were less clear, since significant associations were observed with animal protein, only. If the food intake was expressed on TEQ basis, (I-TEQfood and PCB-TEQfood) positive, in a few instances statistically significant, regression coefficients were found. For calculating the TEQ values, average POC levels in dietary products, as known for the Dutch situation[8], were used. Since the POCs accumulate in the body, the levels measured in human milk are the result of exposure over years. Associations between dietary intake and congener levels therefore are subject to changes in dietary habits or to changing levels in dietary products. In additon, differences in personal characteristics, such as absorption, metabolism, and distribution may influence the association between 'exposure' and the measured contamination levels. According to the present results, future human milk studies should at least obtain information on the mother's age, QI, smoking-, and dietary habits, in order to keep study populations comparable. Moreover, studies aimed at specific associations which are at present not fully understood (like with smoking) or unexpected (like with 'fat consumption) will still be necessary.

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Table 1: Population characteristics of the 103 mother-child pairs and the 32 non-respondents.

characteristics	group	N	min.	mean	max.	median	90-perc.
age mother (years)	R	103	18	29,3*	38	29	35
	NR	31	23	28,1	34,2	27,3	31,6
duration of pregnancy (weeks)	R	103	37	40,2	43	40	42
	NR	29	38	40,1	43	40	42
birth weight (grams)	R	101	2375	3412,5	4730	3460	3980
	NR	31	2700	3478	4720	3400	4060
[a]postnatal QI	R	96	19,1	23,9	35,9	23,4	27,2
[a]prenatal QI	R	102	17,2	22,2	30,5	21,8	25,6

* t-test p=0.08, N=number of observations, R=respondents, NR=non-respondents, min., max.: lowest, highest value, 90-perc. = 90-percentile, a= Quetelet Index or Body Mass index: by definition calculated as weight/(length)^2

Table 2: Distribution of the sumparameters, on weight and TEQ-base.

Parameters	N	min.	25-perc.	50-perc.	75-perc.	max.
sumOCPs[a]	85	0.22	0.40	0.49	0.69	4.62
sumDF[b]	99	131.5	293.5	392.65	553.65	1399.7
sumPCBindicator[c]	81	102.5	203.2	262.2	333	606.7
somPCBnon-ortho[b]	100	49.8	109.8	142.45	187.3	312.7
somPCBother[c]	79	53.3	98	121.05	157.2	295.9
TEQd/f[d]	99	8.4	17.7	21.9	28.1	63.1
TEQnon-orhto[d]	100	2.8	6.25	7.75	11.2	21.7
TEQother[d]	79	4.7	9.6	11.5	14.9	27.9

a = mg/kg fat b= pg/g fat c=ng/g fat d=pg TEQ/g fat
N=number of observations, min, max =lowest, highest, perc. =percentile

Table 3: Associations (direction of the regression coefficient and their p-values) between determinants and POC levels in human milk, as determined for 103 mothers.

Determinants	POC-coeff.	Regr. coeff.	p value	N	R^2
Age (<30 yr vs ≥30 yr)	ln(sumOCP)[a]	-	***	82	0.42
	sumD/F	-	**	96	0.29
	sumPCBindicator	-	***	78	0.50
	sumPCBnon-ortho	-	**	97	0.39
	sumPCBother	-	***	77	0.52
	TEQd/f	-	**	96	0.43
	TEQnon-ortho	-	•	97	0.36
	TEQother	-	***	77	0.50
Fish	sumD/F	+	*	96	0.29
	sumPCBnon-ortho	+	*	97	0.39
	sumPCBother	+	•	77	0.52
	TEQd/f	+	**	96	0.43
	TEQother	+	•	77	0.50
Beef	sumPCBnon-ortho	+	•	97	0.39
	TEQd/f	+	•	96	0.43
	TEQnon-ortho	+	•	97	0.36
Meat products	sumPCBindicator	+	•	78	0.50
	sumPCBnon-ortho	+	*	97	0.39
	sumPCBother	+	*	77	0.52
	TEQother	+	*	77	0.50
Dairy products	sumPCBindicator	+	•	78	0.50
	sumPCBother	+	*	77	0.52
	TEQd/f	+	•	96	0.43
	TEQother	+	•	77	0.50
Pork	sumPCBnon-ortho	-	•	97	0.39
Egg	TEQnon-ortho	-	*	97	0.36
	TEQother	-	•	77	0.50
QI (≤19 vs ≥25)	sumPCBindicator	+	*	78	0.50
	sumPCBother	+	**	77	0.52
	TEQd/f	+	*	96	0.43
	TEQother	+	*	77	0.50
QI (20-25 vs ≥25)	TEQd/f	+	*	96	0.43
Smoking (never/current)	sumPCBnon-ortho	+	**	97	0.39
	sumPCBother	+	*	77	0.52
	TEQnon-ortho	+	**	97	0.36
	TEQother	+	*	77	0.50
Smoking (ex-smok./current)	sumPCBnon-ortho	+	**	97	0.39
	sumPCBother	+	•	77	0.52
	TEQnon-ortho	+	**	97	0.36
Education (low vs high)	TEQd/f	-	*	96	0.43
Education (middle vs high)	sumPCBother	-	•	77	0.52
	TEQd/f	-	*	96	0.43
Animal protein[b]	sumPCBother	+	*	77	0.52
	TEQd/f	+	•	96	0.34
I-TEQfood[c]	TEQd/f	+	*	96	0.33
PCB-TEQfood[d]	sumD/F	+	•	96	0.22
	TEQd/f	+	**	96	0.36

***p≤.001 ** p≤.01 * p≤.05 •p≤.10. Unless stated otherwise the results come from the regression model in which food intake was quantified by dietary products. a=no normal distribution of residuals, therefore a logarithmic transformation was performed b=from the model in which food was quantified as nutrient intake, c and d= from the models with the TEQ-values; I-TEQ respectively PCB-TEQ.