RISK III

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	13.4-13.5	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	14.6-14.3	2.3	0.9-1.1	58-59
	British Columbia 92	20	2.97	15.7-15.3	2.5	1.0-1.2	70-71
	All provinces 92	100	2.96	14.5-14.5	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	16.5-16.5	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	12.5-12.5	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
	Szentes	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].