Dioxin-like PCB in Food and Breast Milk Samples

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ABSTRACT

6 butter samples, 6 fish samples from the upper part of the Rhine River and 11 breast milk samples collected in the south-western part of Germany were analysed for contamination with non-ortho, mono-ortho and di-ortho PCB. If the TEQs are calculated according to the interim TEFs proposed by WHO-ECEH for dioxin-like PCBs, these PCB-derived TEQs exceed the PCDD/PCDF-derived I-TEQs for the butter samples with a factor of 1.9 (range 1.2 - 2.8), for fish with a factor of 8.7 (range 2.7 - 23.2) and for breast milk with a factor of 1.9 (range 1.3 - 3.5). The breast milk and fish samples have a relatively constant correlation between PCB 153 and PCB-TEQs, whereas the butter samples have higher PCB-TEQs than would be expected from the PCB 153-content. The results are given in detail and can contribute to a better data base in the controversial discussion of the applicability of PCB-TEF.

1 Introduction

In December 1993, the WHO-European Centre for the Environment and Health (WHO-ECEH) and the International Programme on Chemical Safety (ICPS) held a consultation during which the available data were discussed to derive TEF factors for dioxin-like PCB. TEFs were recommended for 3 non-ortho, 8 mono-ortho and 2 di-ortho substituted PCBs (1). Recently, several German toxicologists have regarded the applicability of this concept critically. They have voted for the risk assessment of the PCB intake on the base of a TDI of 1 μg PCB/kg body weight (b.w.)/day. Because a constant correlation between PCB 153 and some mono-ortho PCBs was found in breast milk and because non-ortho PCBs are not accumulated overproportionally in breast milk, they assume that the contamination of samples with dioxin-like PCB and the resulting intake could be estimated by the usually determined indicator PCB (2). A scientific discussion of this question is planned by WHO-ECEH for 1997. According to the German authors, only very limited data with complete determination of the dioxin-like PCB are available for food. However, for risk assessment it would be helpful to have the complete data of all individual PCB congeners available in order to recalculate the TEQs with different TEFs if TEFs are changed. Thus, our results can help to improve the data base for the controversial discussion.

2 Materials and Methods

The *butter samples* were collected as part of the official food inspection in the south-western part of Germany. 3 samples were from Germany, 3 samples from Ireland. - The *fish samples* were collected as part of the international programme for determination of contaminants in fish of the Rhine River. The fish was caught in the upper Rhine. Each individual fish got its own number. In order to get the mean contamination for different sorts of fish in selected sections of the Rhine and to reduce the number of samples for economic reasons, the edible parts of some individual fish samples of the same sort and from the same origin were mixed and analysed as homogenized samples: pike samples 5466 to 5467 and eel samples 5472 to 5476 from Sasbach, roach samples 5481 to 5483 from Taubergießen, pike samples 5498 to 5500 and eel samples 5504 to 5507 from Diersheim. Eel sample 5484 from Taubergießen was analysed as individual fish. - *Breast milk samples* were sent by the public health department Kehl and by the Ärzteinitiative Kehl from mothers from the Kehl area for evaluation of the contamination by sources in the neighbouring city of Strasbourg. All samples were collected in 1995.

The dioxin-like PCBs are determined along with PCDD/PCDF: Extraction and fat separation were done according to the successfully tested method for determination of PCDD/PCDF in eggs (3), with optimisation of the extraction for each sort of food.

3 g of the pure fat was spiked for determination of PCDD/PCDF and dioxin-like PCB with
- all 2,3,7,8-substituted $^{13}C_{12}$-labelled PCDD/PCDF,
- the non-ortho $^{13}C_{12}$-labelled PCB 77, 126 and 169,
- the mono-ortho $^{13}C_{12}$-labelled PCB 28, 105, 118 and 156 and
- the di-ortho $^{13}C_{12}$-labelled PCB 52, 101, 153, 138 and 180.

Fat was separated by gel chromatography (Bio-Beads S-X3, solvent ethyl acetate/cyclohexane [1+1]). Small amounts of remaining lipophilic and oxidizable substances were removed on a mixed column with layers of 1 g sulfuric acid impregnated silica gel and 1 g NaOH-impregnated silica gel (solvent: hexane).

Separation of PCB from PCDD/PCDF was performed on a florisil column (6 g, deactivated with 3 % water; 50 ml hexane elutes PCB, 60 ml toluene elutes PCDD/F).

The PCB fraction was purified on Carbopack B in 3 fractions:
- elution 1: di-ortho PCB (hexane)
- elution 2: mono-ortho PCB (hexane/7.5 % toluene)
- elution 3: non-ortho PCB (toluene).

This purification step was published for application of column chromatography with every sample to be purified on a separate column (4). We could automate this procedure using a modified ABIMED clean up-system with a single column for all samples that are run one after another in a sequence. The column was filled with 4 g Carbopack B/Celite (1 + 1). All fractions were eluted from top to bottom. The carbon column was automatically regenerated prior to use. Cross-contamination was checked carefully.

For determination of PCDD/PCDF, the PCDD/PCDF fraction of the florisil column was purified on Carbopack C/Celite.

As recovery standard, $^{13}C_{12}$-labelled 1,2,3,4-TCDD or $^{13}C_{12}$-labelled PCB 80 were used for PCDD/PCDF or PCB-determination. GC/MS-detection was performed on a VG Autospec at 10,000 resolution using a 60 m DB5-MS-column for PCDD/PCDF determination in butter and human milk samples, using a 60 m DB-Dioxin column for PCDD/PCDF determination in fish

samples and a chemical bound polysiloxane phase with 5 % phenyl/ 95 % methyl for PCB determination. The AS 200 autosampler injected 5 µl into the Multinjector of a Carlo Erba Mega GC. With every acquisition sequence, a 3 point-calibration curve was acquired in duplicate for PCB analysis.

3 Results and Discussion

For this preliminary study, we chose fish, butter and breast milk to reflect the different metabolisms of fish, cows and human beings. Table 1 a and table 1 c give the results of the mean contamination and the range of the contamination for butter and breast milk samples. Table 1 b gives the results of the fish samples in detail, because the wide range of different fat content doesn't allow to calculate the mean contamination.

Table 1 a: PCB in butter samples (ng/g fat)

	PCB 37	PCB 77	PCB 126	PCB 169	sum TEQ
mean	0.0022	0.0029	0.0070	0.0045	0.00075
minimum	0.0012	0.0014	0.0027	0.0014	0.00028
maximum	0.0040	0.0067	0.0130	0.0164	0.00134

	PCB 28	PCB 118	PCB 105	PCB 167	PCB 156	sum TEQ
mean	< 0.68	0.582	< 0.114	0.083	0.129	0.00013
maximum	< 1.17	0.345	< 0.096	0.031	0.049	0.00007
minimum	< 0.35	0.864	< 0.143	0.172	0.211	0.00020

	PCB 52	PCB 101	PCB 153	PCB 138	PCB 180	PCB 170	sum TEQ
mean	< 0.01	< 0.35	1.93	1.39	0.71	0.33	0.000040
minimum	< 0.01	< 0.22	0.73	0.56	0.24	0.07	0.000010
maximum	< 0.02	< 0.50	3.43	2.49	1.33	0.55	0.000075

Table 1 b: PCB in fish samples (ng/g fat)

sample no.	fat (%)	PCB 37	PCB 77	PCB 126	PCB 169	sum TEQ
5466-5467[1)]	0.1	2.23	8.24	2.51	0.26	0.257
5472-5476[1)]	32.5	0.11	0.10	0.42	0.06	0.042
5481-5483[1)]	1.2	3.94	4.50	0.62	0.05	0.064
5484	33.8	0.31	0.55	0.56	0.12	0.057
5498-5500[1)]	0.4	14.35	39.97	2.91	0.18	0.313
5504-5507[1)]	26.1	0.10	0.19	0.57	0.09	0.058

sample no.	PCB 28	PCB 118	PCB 105	PCB 167	PCB 156	sum TEQ
5466-5467[1)]	380	405	81.1	82.6	82.4	0.091
5472-5476[1)]	n.d.	103	32.8	14.3	21.9	0.025
5481-5483[1)]	109	108	30.8	15.2	20.6	0.024
5484	n.d..	226	82.4	22.6	34.8	0.048
5498-5500[1)]	2308	854	168.5	94.5	102.5	0.155
5504-5507[1)]	n.d.	213	73.2	26.9	37.6	0.048

[1)] mixed sample

sample no.	PCB 52	PCB 101	PCB 153	PCB 138	PCB 180	PCB 170	sum TEQ
5466-5467[1)]	271	1018	2104	1151	840	291	0.038
5472-5476[1)]	57	142	426	331	142	62	0.008
5481-5483[1)]	99	263	501	327	177	71	0.009
5484	59	253	748	676	187	90	0.011
5498-5500[1)]	914	2105	3928	2371	1126	445	0.056
5504-5507[1)]	132	230	807	655	290	139	0.017

[1)] mixed sample

Table 1 c: PCB in 11 breast milk samples (ng/g fat)

	PCB 37	PCB 77	PCB 126	PCB 169	sum TEQ
mean	0.0250	0.0172	0.1067	0.0684	0.0114
minimum	0.0055	0.0047	0.0706	0.0273	0.0075
maximum	0.0911	0.0431	0.1652	0.1112	0.0171

	PCB 28	PCB 118	PCB 105	PCB 167	PCB 156	sum TEQ
mean	5.76	22.65	3.72	6.64	20.31	0.0131
minimum	2.45	14.20	2.52	3.84	8.41	0.0060
maximum	9.45	32.52	5.16	10.87	40.99	0.0235

	PCB 52	PCB 101	PCB 153	PCB 138	PCB 180	PCB 170	TEQ
mean	0.30	0.87	223	122	108	64.9	0.0076
minimum	0.09	0.38	103	60	42,1	27.5	0.0032
maximum	0.54	1.50	399	203	258	159.0	0.0185

In the breast milk samples, we found non-ortho and mono-ortho PCBs slightly below the Dutch PCB/Dioxin study (5, 6) and about 20 - 50 % below the the background contamination of human blood in Germany, with the exception of PCB 126 (7, 8). PCB 77 and 126 are considerably lower than the values reported for breast milk determined by GC/ECD and lower than the values confirmed by GC/MS (9). The mono-ortho PCBs determined by GC/ECD (9) have a general tendency to higher values, as well.

The PCB 77, PCB 126, PCB 169 and PCB 153 levels of the butter samples are about 30 to 40 % below retail milk samples from the UK (values converted into ng/g fat) (10). GC/ECD determination of coplanar PCB in different food samples lead to considerably higher values (11).

If the TEQs of the non-ortho, mono-ortho and di-ortho PCB are calculated according to the TEFs proposed by WHO-ECEH, the resulting PCB-TEQs exceed the dioxin-related I-TEQ-values for the butter samples with a factor of 1.9 (range 1.2 - 2.8), for fish with a factor of 8.7 (range 2.7 - 23.2) as well as for breast milk with a factor of 1.9 (range 1.3 - 3.5) (table 2).

An interesting point is the correlation between PCB 153 and TEQs calculated from dioxin-like PCBs. German toxicologists summarised results of the analyses of Arochlor 1242, Arochlor 1254 and Arochlor 1260. These results indicate that the content of non-ortho, mono-ortho and di-ortho PCBs and their respective TEQs varies considerably between these different PCB

products, but the total PCB-TEQ would be more or less in a similar range (3.5 - 11.5 μg TEQ/g Arochlor 1242, 12.1 - 47.8 μg TEQ/g Arochlor 1254 and 5.3 - 24.9 μg TEQ/g Arochlor 1260) (2, 12). Thus, a constant correlation between indicator PCBs and PCB-TEQs could be expected. If this assumption proved to be true for all matrices, there would be no need for further costly determination of dioxin-like PCB (2).

The mean contamination of the different Arochlor products is about 15 μg TEQ/g PCB product. Assuming that PCB 153 makes up about 10 % of Arochlor 1260 as for Clophen A 60 (13) and that there are no substantial differences in PCB-TEQs between these Arochlor and Clophen products, the theoretical total PCB-TEQ, which is to be expected from the PCB 153-content, can be derived for matrices with Arochlor 1260- or Clophen A60-pattern. These values are given in table 2 („expected PCB-TEQ (pg/g)"). If this assumption proves true, the factor between the determined PCB-TEQ and the expected PCB-TEQ should be „1". As table 2 demonstrates, this assumption is approximately true for the breast milk samples and the fish samples from the upper part of the Rhine River, whereas the butter samples have higher total PCB-TEQs than was expected from this assumption. Because of these differences, further data are necessary for different types of food samples to evaluate the correlation between PCB indicator congeners and TEQs.

Table 2a: Butter samples (results in pg/g fat)

	sum PCB: pg TEQ/g	PCDD/F: pg I-TEQ/g	quotient PCB-TEQ: dioxin-TEQ	expected PCB-TEQ (pg/g)	factor determ./ expected
mean	0.91	0.46	1.9	0.29	3.8
minimum	0.38	0.33	1.2	0.12	2.1
maximum1	1.57	0.59	2.8	0.51	6.7

Table 2b: Fish samples (results in pg/g fat)

sample	fish	fat (%)	sum PCB: pg TEQ/g	PCDD/F: pg I-TEQ/g	quotient PCB-TEQ: dioxin-TEQ	expected PCB-TEQ (pg/g)	factor determ./ expected
5466-5467	pike[1)]	0.1	385.4	62.1	6.2	316	1.2
5472-5476	eel[1)]	32.5	74.6	12.0	6.2	64	1.2
5481-5483	roach[1)]	1.2	97.5	36.0	2.7	75	1.3
5484	eel	33.8	116.4	23.0	5.0	112	1.0
5498-5500	pike[1)]	0.4	523.6	59.8	8.7	589	0.9
5504-5507	eel [1)]	26.1	122.4	5.2	23.3	121	1.0

[1)] Mixed samples

Table 2c: Breast milk samples (results of 11 samples in pg /g fat)

	sum PCB: pg TEQ/g	PCDD/F: pg I-TEQ/g	quotient PCB-TEQ: dioxin-TEQ	expected PCB-TEQ (pg/g)	factor determ./ expected
mean	31.7	16.7	1.9	33.4	0.9
minimum	16.8	9.4	1.3	15.5	0.6
maximum	53.2	24.9	3.5	59.8	0.9

ACKNOWLEDGEMENT

I'd like to thank Mrs. Tritschler and Mr. Huber for their reliable preparation of the samples and Mr. Winterhalter for running the high resolution mass spectrometer.

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