PCDD AND PCDF LEVELS IN DIFFERENT ORGANS FROM INFANTS II

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INTRODUCTION

The investigation of samples of adipose tissue, liver, spleen, thymus and brain (gray and white matter) obtained from 3 infants had revealed a relatively low body burden with PCDDs and PCDFs [1]) compared with human milk. Human milk contributes most to the body burden of infants [2]), who are at the end of the food chain. To obtain a broader data base, samples from another 5 infants who had been, altogether, breast-fed for a longer period than the first 3 samples were analyzed.

EXPERIMENTAL

Samples: Samples of adipose tissue, spleen, thymus, liver and brain (gray and white matter) were collected by the Institute of Legal Medicine, Medical School Hannover, from 5 infants (D, E, F, G, H) who had died from the Sudden Infant Death Syndrome (SIDS) with the exception of sample G.

Extraction: Fat extraction from frozen tissue was carried out by grinding the material with sodium sulphate and sea sand followed by column extraction with hexane/acetone (2/1). An aliquot of the extract was used for gravimetric fat determination [3]). Results have been included in Table 1.

Clean-up and determination: The clean-up was performed following the procedure of Smith et al. [4]), with minor modifications [5,6]). The final extract was concentrated by evaporation to 30 µl and analyzed for PCDDs and PCDFs by HRGC-HRMS [5,6]) on the basis of ^{13}C-labelled internal standards.

RESULTS AND DISCUSSION

During the breast-feeding period intake of PCDDs and PCDFs by the infants is relatively high [2]). For this reason, the resulting body burden and the kinetics of these substances in different organs are of special interest under the aspect of preventive health care.
Infants who had died from SIDS had been selected since their nutritional status is entirely

normal whereas the fat depot of sick children may have become reduced within a short period so that concentrations are elevated. Table 1 shows some basic data for the samples examined. For a better overview, earlier data obtained from infants A, B, C [1]) were included in this paper.

Table 1: Basic data for 8 infants

Infant	A	B	C	D	E	F	G	H
Age (month)	9.7	3.8	4.8	4.8	3.0	3.8	23	6.2
Sex	male	male	male	male	male	female	male	female
Nutritional status	normal	normal	normal	slight u weight	normal	normal	normal	normal
Weight (kg)								
-at birth	3.7	4.2	2.6	1.7	3.2	2.5	3.7	1.5
-at death	9.8	5.8	5.9	5.5	6.2	5.1	12	5.7
Breast feeding								
-exclusively (days)	21	6	0	0	91	21	56	90
-partly (days)	63	0	0	0	0	0	28	0
Fat content (%) of:								
-adipose tissue	90	81	71	90	92	86	56	52
-spleen	2.5	2.9	3.5	1.4	1.6	2.3	1.7	2.1
-thymus	3.6	1.1	1.0		1.8	2.1		
-liver	6.4	4.2	4.3	6.7	3.4	4.7	5.9	4.4
-brain								
-gray matter	5.3	6.5	4.3	4.6	7.7	4.3	6.1	
-white matter	10	4.7	6.7	7.1	8.0	8.0	13	

u, under

The PCDD, PCDF and I-TEq concentrations in adipose tissue samples from the 8 infants examined have been listed in Table 2. A comparison of the PCDD and PCDF pattern in adipose tissue samples from the infants with the pattern in human milk [7,8]) demonstrates a good conformity. The concentrations in infants who had not been breast-fed or only over a short period (infants B, C, D, F, see Table 1) were the lowest and, for I-TEq concentrations, amounted to 12 % of the mean concentration in human milk fat [7]). For infants who had been breast-fed for a longer period (infants A, E, G, H, see Table 1), the I-TEq-levels were higher and reached, for infants E and G, the range of the mean I-TEq level in human milk fat [7]). The maximum level in adipose tissue was found in the only infant breast-fed up to his death (sampling date). Infants A, E and H had been breast-fed for about 3 months and had died at different ages. The shorter the interval between the end of breast-feeding and death (sampling date) the higher the body burden of the infants. Therefore, levels in infant A (breast-fed for 2.8 months) who had died at the age of 9.7 months do not differ much from

levels in samples from infants who had not been breast-fed or only over a short period. Obviously, consumption of infant formula and conventional food results in a lower PCDD and PCDF intake. The overproportional increase of total body fat during the first months of life in relation to the total body weight increase has an effect of dilution [9]) and is the main reason why infants show lower PCDD and PCDF concentrations than adults. This is recognizable also in infants who had been breast-fed, weaned and subsequenty given conventional food.

PCDD and PCDF and I-TEq concentrations in different organs based on fat weight are listed in Tables 2-4. It can be easily seen that, on a fat weight basis, PCDD and PCDF levels in liver, thymus and spleen are, in general, higher compared with those in adipose tissue (see Table 2). Because of low sample weights and low fat contents (see Table 1) resulting in concentrations close to or below the detection limits, the I-TEq concentrations in spleen and thymus (see Table 3) were calculated with half the detection limits of many of the congeners and should therefore be seen as an estimation. On the other side it became clear that accumulation of OCDD in thymus is obviously higher by a factor of 10 (mean), on a fat weight basis, compared to adipose tissue. For spleen no such remarkable relationship can be derived. In liver, results for HxCDFs, HpCDF, HpCDD and OCDD are striking. In this organ, the levels for these congeners on a fat weight basis are higher by mean factors of 8, 9, 6 and 11, respectively, compared to adipose tissue (see Table 2).

In general, the amounts of PCDDs and PCDFs accumulated in adipose tissue correlate with those accumulated in liver and spleen, i.e. the highest levles in adipose tissue correspond to the highest levels in liver and spleen, a.s.o. Whether such a correlation exists also in in thymus and brain could not be verified since many of the levels determined in these organs were close to or below the detection limits.

Although PCDD and PCDF concentrations in brain were mostly below the detection limits and I-TEq concentrations were calculated with half the detection limits for most of the congeners, it is nevertheless obvious that the levels in brain are lower even on a fat weight basis compared to adipose tissue (see Table 4).

In the toxicological evaluation it should be considered that, on a wet weight basis, PCDD and PCDF do not accumulate in organs with some minor exceptions for liver. To give an overview, mean I-TEq concentrations (pg/g) in infant organs on a fat and wet weight basis are summarized in Table 4 and mean ratios of I-TEq concentrations (pg/g / pg/g) in infant organs (on a fat and wet weight basis) to the fat concentration of adipose tissue in Table 5. Compared to the levels in adipose tissue of the general population those in infants were the lowest [10]).

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Table 2: PCDD and PCDF concentrations (pg/g fat) in adipose tissue and liver

Infant	A	B	C	D	E	F	G	H	MIN	MEAN	MAX
Congener					**Adipose Tissue**						
2378-TCDF	<0,5	<0,5	<0,5	1,4	1,2	1,0	1,1	3,1	<0,5	1,1	3,1
2378-TCDD	1,0	0,7	<0,5	<0,2	3,9	0,5	2,1	<0,5	<0,2	1,1	3,9
12378-PeCDF	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5
23478-PeCDF	5,5	4,5	3,9	1,6	33	1,7	13	6,0	1,6	8,7	33
12378-PeCDD	2,5	2,0	1,9	0,5	14	0,4	12	2,9	0,4	4,5	14
123478-HxCDF	1,0	1,8	1,1	2,3	12	1,7	7,5	7,7	1,0	4,4	12
123678-HxCDF	0,7	1,6	1,3	0,7	9,3	1,3	4,8	2,4	0,7	2,8	9,3
234678-HxCDF	0,6	<0,5	<0,5	0,5	3,0	1,2	2,0	0,3	<0,5	1,0	3,0
Total HxCDFs	2,3	3,7	2,7	3,5	25	4,2	14	10	2,3	8,3	25
123478-HxCDD	1,7	1,3	0,8	0,3	9,2	1,3	6,2	2,6	0,3	2,9	9,2
123678-HxCDD	9,1	6,0	5,7	2,1	37	3,4	37	13	2,1	14	37
123789-HxCDD	1,5	1,6	0,9	0,8	9,5	2,3	7,1	3,6	0,8	3,4	9,5
Total HxCDDs	12	8,9	7,4	3,2	56	7,1	50	19	3,2	21	56
1234678-HpCDF	2,5	2,6	1,8	2,2	8,5	1,0	12	11	1,0	5,2	12
1234678-HpCDD	5,9	5,8	6,5	5,1	32	11	57	20	5,1	18	57
OCDF	<0,5	1,2	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	1,2
OCDD	55	43	81	59	150	62	341	120	43	114	341
I-TEq	**6,6**	**5,4**	**4,4**	**2,1**	**36**	**3,0**	**22**	**8,4**	**2,1**	**11**	**36**
					Liver						
2378-TCDF	<1,0	1,3	1,2	<2	<3	<1	<2	<2	<1		<3
2378-TCDD	1,1	0,8	0,4	<1	4,6	<1	2,3	<2	<1	1,4	4,6
12378-PeCDF	1,0	1,3	1,1	<2	<2	<1	<2	<2	<1		<2
23478-PeCDF	25	7,7	5,3	3,3	82	3,5	39	15	3,3	23	82
12378-PeCDD	4,0	2,3	1,8	<2	14	<1	10	6,7	<1	5,0	14
123478-HxCDF	21	8,1	7,5	<2	107	4,2	45	22	<2	27	107
123678-HxCDF	31	9,3	10	2,9	85	4,9	66	32	2,9	30	85
234678-HxCDF	6,1	1,4	2,0	<2	15	<1	11	7,9	<1	5,6	14
Total HxCDFs	58	19	20	4,9	206	9,6	122	62	4,9	63	206
123478-HxCDD	4,8	1,6	1,3	<2	17	2,0	16	8,3	<2	6,5	17
123678-HxCDD	14	5,5	5,0	6,5	50	3,0	54	14	3,0	19	54
123789-HxCDD	4,0	1,4	1,6	<2	16	<2	11	9,9	<2	5,7	16
Total HxCDDs	23	8,5	7,9	8,5	83	6,0	81	32	6,0	31	83
1234678-HpCDF	34	13	18	<4	112	8,6	169	40	<4	50	169
1234678-HpCDD	78	30	46	20	215	29	396	108	20	115	396
OCDF	11	6,4	6,4	6,1	13	4,5	15	9,9	4,5	9,1	15
OCDD	1129	531	777	375	2916	416	2546	1076	375	1221	2916
I-TEq	**26**	**9,7**	**8,3**	**4,7**	**88**	**4,9**	**56**	**24**	**4,7**	**28**	**88**

MIN, Minimum; MAX, Maximum

Means, total HxCDFs, total HxCDDs and I-TEq calculated using half the detection limits

Table 3: PCDD and PCDF concentrations (pg/g fat) in spleen and thymus

Infant	A	B	C	D	E	F	G	H	MIN	MEAN	MAX
Congener						**Spleen**					
2378-TCDF	<1	<1	<1	<5	<10	<5	<5	<5	<1	2,1	<10
2378-TCDD	<1	<1	1,2	<5	<10	<5	<5	<5	<1	2,2	<10
12378-PeCDF	<1	<1	<1	<5	<10	<5	<5	<5	<1	2,1	<10
23478-PeCDF	11	3,6	5,8	<5	68	<5	28	<5	<5	15	68
12378-PeCDD	2,4	1,1	1,3	<5	20	<5	12,0	<5	1,1	5,5	20
123478-HxCDF	5,6	1,4	3,6	<5	28	<5	10,0	<5	1,4	7,0	28
123678-HxCDF	3,1	1,3	5,2	<5	16	<5	6,6	<5	1,3	5,0	16
234678-HxCDF	3,9	1,0	1,5	<5	20	<5	<5	<5	1,0	4,6	20
Total HxCDFs	13	3,7	10,3	<15	64	<15	19	<15	3,7	17	64
123478-HxCDD	3,7	2,0	1,6	<5	50	<5	22	<5	1,6	11	50
123678-HxCDD	19	4,1	4,8	<5	113	<5	47	<5	<5	24	113
123789-HxCDD	2,2	0,8	0,9	<5	18	<5	10	<5	0,8	4,9	18
Total HxCDDs	25	6,9	7,3	<15	181	<15	79	<15	6,9	40	181
1234678-HpCDF	3,3	2,8	1,9	<5	31	<10	29	<10	1,9	10	31
1234678-HpCDD	42	16	31	26	236	22	188	48	16	76	236
OCDF	<1	<1	2,0	n.a.	n.a.	<5	n.a.	<5	<1	1,6	<5
OCDD	281	110	107	n.a.	n.a.	n.a.	n.a.	n.a.	107	166	281
I-TEq	**12**	**4,3**	**7,0**	**7,2**	**77**	**7,1**	**35**	**7,4**	**4,3**	**20**	**77**
						Thymus					
2378-TCDF	3,2	3,3	6,4		<15	<15			3,2	5,6	7,5
2378-TCDD	<1	<1	<2		<15	<15			<1	3,4	7,5
12378-PeCDF	<1	<1	<2		<15	<15			<1	3,4	7,5
23478-PeCDF	10	6,1	10		37	<15			6,1	14	37
12378-PeCDD	3,6	2,6	<2		<15	<15			<2	4,4	<15
123478-HxCDF	2,6	2,2	5,9		<15	<15			2,2	5,1	<15
123678-HxCDF	3,5	3,6	5,9		<15	<15			3,5	5,6	<15
234678-HxCDF	2,9	2,1	5,2		<15	<15			2,1	5,0	<15
Total HxCDFs	13	11	17		<45	<45			7,9	16	<45
123478-HxCDD	5,8	2,4	4,6		<15	<15			2,4	5,6	<15
123678-HxCDD	24	11	24		<15	<15			7,5	15	24
123789-HxCDD	2,4	1,5	4,0		<15	<15			1,5	4,6	<15
Total HxCDDs	32	15	33		<45	<45			15	25	33
1234678-HpCDF	3,0	2,6	9,4		19	<15			2,6	8,3	19
1234678-HpCDD	24	19	75		155	28			19	60	155
OCDF	<1	<1	4,9		<15	<15			<1	4,2	<15
OCDD	660	705	446		1500	600			446	782	1500
I-TEq	**13**	**8,4**	**13**		**39**	**22**			**8,4**	**19**	**39**

MIN, Minimum; MAX, Maximum; n.a., not analyzed

Means, total HxCDFs and I-TEq calculated using half the detection limits

Table 4: Mean I-TEq concentrations (pg/g) in infant organs on a fat and wet weight basis

	Adipose tissue	Liver	Spleen	Thymus	Brain, white m.	Brain, gray m.
Fat weight	11	28	20	19	(1)	(1)
Wet weight	8.5	1.3	0.4	0.4	(0.08)	(0.06)

m., matter

Table 5: Mean ratios of I-TEq concentrations (pg/g / pg/g) in infant organs (on a fat and wet weight basis) to the fat concentration of adipose tissue

	Adipose tissue	Liver	Spleen	Thymus	Brain, white m.	Brain, gray m.
Fat weight	1	2.4	1.8	3.0	(0.2)	(0.2)
Wet weight	(1)	0.1	0.04	0.06	(0.01)	(0.01)

m., matter

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