Dietary Intake and Fecal Excretion of 2378- and Non-2378-Substituted PCDDs and PCDFs in an 11-Month-Old Infant

Klaus Abraham[A], Olaf Päpke[B], Ulrich Wahn[A], and Hans Helge[A]
[A] Children's Hospital, Virchow-Klinikum, Humboldt-University Berlin, Augustenburger Platz 1, 13 353 Berlin, Germany
[B] ERGO Forschungsgesellschaft, Geierstr. 1, 22 305 Hamburg, Germany

Abstract

Dietary intake and fecal excretion of 2378- and non-2378-substituted PCDD/Fs were measured in an 11-month-old infant after weaning. As observed in other infants, concentrations in fecal fat (5.95 pg I-TEq/g fat) were clearly higher than in dietary fat (0.68 pg I-TEq/g fat), with concentration ratios increasing with the degree of chlorination. Fecal PCDD/F excretion as percentage of dietary intake was relatively high (61% for I-TEq), as expected for children after weaning with body fat concentrations (10.8 pg I-TEq/g fat) clearly higher than those in dietary fat. Non-2378-substituted isomers were found in dietary fat to about the same concentrations as the 2378-substituted isomers. In fecal fat, concentrations were in particular higher for 2378-substitited PCDD isomers. Fecal excretion for most tetra- and penta-chlorinated non-2378-substituted isomers was below 25% of the dietary intake. Concentrations of non-2378-substituted isomers below the limit of detection in human body fat are obviously not due to missing intestinal absorption, but may be due to relatively fast metabolism.

Introduction

Non-2378-substituted PCDD/Fs are of minor toxicological interest: In contrast to the 2378-substituted isomers, they do not accumulate in the mammalian organism and are not found in detectable concentrations in humans. However, the toxicokinetics of non-2378-substituted compounds is of interest in order to understand the various fates of PCDD/F isomers in mammals.
As a part of our studies of PCDD/F toxicokinetics in infants, we report here on the results of a 3-day balanced investigation of oral intake and fecal excretion which included the non-2378-substituted isomers.

Methods

The investigation was performed with an 11-month-old healthy girl (body weight 8.9 kg) who was fully breast-fed for 20 weeks and weaned at 34 weeks (equivalent to full breast-feeding for

about 29 weeks). She is second born and sister of infant "B-1" (1) who was breast-fed for about the same duration and in whom the same balanced investigation was performed at the age of 11 months without analyzing non-2378-substituted isomers.
During the investigation period of 3 days, diet consisted of pap, cow's milk and home-cooked food. The amount of all ingested food was recorded, and a 10% aliquot was frozen at -18°C. The whole stool corresponding to the 3-day dietary intake (identified by blueberries in the first and last meal) was collected in cotton diapers (pre-extracted first with ethanol, then with hexane/acetone). The pooled food and stool samples were and ground before analysis. In order to determine the PCDD/F body burden of the infant, a whole blood sample of 15 ml was taken before breakfast at the same age.
The lyophilized samples of food and stool were mixed with sodium sulfate and spiked with ^{13}C-labelled internal standards. The extraction was performed using a hexane/acetone mixture on a Soxhlet apparatus. The blood sample was spiked and then applied on a Chem Elute (modified Silicagel) column, and lipids were eluted with hexane/isopropanol.
All lipid extracts were determined gravimetrically. A multicolumn system was used for clean-up of the lipid extracts. The measurements were carried out by means of HRGC/HRMS involving a SP 2330 and/or DB 5 silica column coupled with a VG AutoSpec mass spectrometer. The quantification was performed by the isotope dilution method and by comparison with an external standard mixture.
All concentrations were based on extracted fat. I-TEq were calculated using I-TEFs (2) not including PCBs.

Results and Discussion

During the 3 days, average of infant's daily dietary consumption was 1250 g (wet weight) with a dry mass of 219 g (13.2 % extractable fat). Average of the daily fecal dry mass excretion was 23.1 g (8.63 % extractable fat). During the three days, the intestinal fat absorption rate was 93.1 % (taking excreted fat as unabsorbed fat and not considering endogenous fat losses) which is relatively low compared to our other infants investigated (1). Daily intake of PCDD/Fs was 2.2 pg I-TEq/kg body weight, including 0.36 pg T4CDD/kg body weight. This is within the range observed in our other infants at the end of the first year of life (1) and in 22 months to 5 years old children (3).
Concentrations of PCDD/Fs measured in dietary and fecal fat as well as in blood fat are compiled in Table 1. Additionally, concentration ratios (feces/diet) and fecal excretion as percentage of intake were calculated.
As reported previously for 2 breast-fed infants after weaning, the concentrations of 2378-substituted PCDD/Fs were clearly higher in fecal than in dietary fat. Concentration ratios were found to increase with the degree of chlorination, and (as net effect) a considerable amount of the PCDD/F intake was excreted with the feces, exceeding 100% for few higher chlorinated congeners. Our interpretation of these investigations after weaning and earlier ones during breast-feeding have been reported elsewhere (1) and can be summarized as follows:
- For the lower chlorinated 2378-substituted PCDD/Fs intestinal absorption of ingested congeners is almost complete.
- Despite much lower concentrations in dietary fat after weaning, concentrations in fecal fat do not decrease to the same extent and more or less reflect concentrations in body fat (as seen in this

infant) due to secretion of endogenous fat and PCDD/F exchange between fat in intestinal lumen and intestinal wall. Therefore, it is not possible to simply calculate PCDD/F absorption rates as the 100% ingested amount minus the excreted amount (with the methods used, it is only possible to observe net effects).
- Ingested higher chlorinated 2378-substituted congeners are less completely absorbed. After weaning, fecal excretion can exceed the oral intake, possibly due to low reabsorption of endogenous losses. However, the possibility of other sources especially for OCDD has to be taken into account, as discussed earlier (4).

Compared to the first born brother (infant "B-1" in Ref. 1) investigated at the same age, concentrations in blood fat were clearly lower (10.8 vs. 29.2 I-TEq/g fat). This results from the high PCDD/F transfer from the mother to her first infant during breast-feeding for several months, as already observed in another infant pair (5). Compared to the balanced investigation in the brother, results of the fecal excretion (as percentage of the intake) were surprisingly similar for the different 2378-substituted congeners.

In dietary fat, a number of *non-2378-substituted PCDD/Fs* were found in the same range as the 2378-substituted isomers (Table 1). As for the latter, their concentrations in fecal fat were generally higher than in dietary fat, with concentration ratios increasing with the degree of chlorination.
More obvious for 2378-substituted PCDDs than PCDFs, fecal excretion rates (calculated as percentage of the intake) were higher than for the non-2378-substituted isomers. As stated above, this does not mean a less complete intestinal absorption. The results support the hypothesis that fecal PCDD/F concentrations are determined by their concentrations in body fat if these are clearly higher than those in dietary fat.
Only if concentrations in body fat are low compared to those in dietary fat, as for the non-2378-substituted compounds (and sources other than dietary intake or metabolism to other PCDD/F are excluded), is a calculation of absorption rates as 100 % of the intake minus fecal excretion possible. Doing this for the tetra- and penta-chlorinated non-2378-substituted congeners, intestinal absorption rates of more than 75% can be calculated (with a few exceptions). These values are higher than those for the higher chlorinated non-2378-substituted congeners, but lower than those calculated for the tetra- and penta-chlorinated 2378-substituted isomers in infants during breast-feeding (1).
As in other mammals, very low concentrations of non-2378-substituted isomers in human body fat are obviously not due to missing intestinal absorption, but may be due to a relatively fast metabolism. However, the mechanism which protects the 2378-substituted isomers from metabolic degradation is not yet understood.

References

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Table 1 Concentrations of the different 2378- and non-2378-substituted PCDD/F congeners in dietary and fecal fat. From these data, concentration ratios (dietary/fecal fat) and fecal excretion rates (as percentage of the intake) were calculated. Additionally, concentrations of the compounds in blood fat are shown. Bold face: 2378-substituted isomers.

Isomer	Conc. dietary fat *(pg/g fat)*	Conc. fecal fat *(pg/g fat)*	Conc. ratio	Fecal excretion (percentage of intake)	Conc. blood fat *(pg/g fat)*
T4CDDs					
1368	0.17	0.39	2.3	15.8	
1379	0.12	0.19	1.6	10.9	
1369	n.d.	n.d.			
1247, 1248, 1378, 1469	0.07	0.17	2.4	16.8	
1246, 1249, 1268, 1478	n.d.	n.d.			
1279	n.d.	n.d.			
1269, 1236, 1234, 1237, 1238	0.07	0.16	2.3	15.8	
2378	**0.11**	**0.77**	**7.0**	**48.3**	**2.8**
1239	n.d.	n.d.			
1278	n.d.	n.d.			
1267	n.d.	n.d.			
1289	n.a.	n.a.			
Sum T4CDDs	*0.53*	*1.67*			
P5CDDs					
12468, 12479	0.11	0.64	5.8	40.2	
12469	n.d.	0.13			
12368	0.11	0.26	2.4	16.3	
12478	0.07	0.13	1.9	12.8	
12379	0.07	0.13	1.9	12.8	
12369, 12489, 12467	0.08	0.17	2.1	14.7	
12347	n.d.	n.d.			
12346	n.d.	n.d.			
12378	**0.14**	**1.83**	**13.1**	**90.3**	**3.2**
12367	n.d.	n.d.			
12389	n.d.	n.d.			
Sum P5CDDs	*0.57*	*3.29*			
H6CDDs					
124679, 124689	0.16	0.95	5.9	41.0	
123468	0.14	0.57	4.1	28.1	
123679, 123689	0.34	1.24	3.6	25.2	
123469	n.d.	0.10			
123478	**0.14**	**1.42**	**10.1**	**70.0**	**2.5**
123678	**0.25**	**6.71**	**26.8**	**185.3**	**10.6**
123789, 123467	**0.14**	**1.70**	**12.1**	**83.9**	**2.6**
Sum H6CDDs	*1.16*	*12.69*			
H7CDDs					
1234679	0.72	8.29	11.5	79.5	
1234678	**1.30**	**14.61**	**11.2**	**77.6**	**28.8**
Sum H7CDDs	*2.02*	*22.90*			
OCDD	**6.86**	**189.87**	**27.7**	**191.1**	**230.2**

Table 1 (continued)

Isomer	Conc. dietary fat *(pg/g fat)*	Conc. fecal fat *(pg/g fat)*	Conc. ratio	Fecal excretion (percentage of intake)	Conc. blood fat *(pg/g fat)*
T4CDFs					
1368	0.08	0.18	2.3	15.5	
1468	0.09	0.20	2.2	15.3	
2468	0.09	0.20	2.2	15.3	
1247, 1347, 1378, 1346, 1246	0.34	0.73	2.1	14.8	
1379, 1367, 1348, 1248	0.28	0.66	2.4	16.3	
1268, 1478, 1467	0.34	0.98	2.9	19.9	
237, 1369	0.16	0.98	6.1	42.3	
2368, 2467, 1238, 1236	0.17	0.50	2.9	20.3	
1469, 1678, 1234	0.16	0.22	1.4	9.5	
1278	0.32	0.50	1.6	10.8	
1349, 1267	0.10	0.22	2.2	15.2	
1249, **2378**, 1279, 2346, 2347, 2348	**0.59**	**0.97**	**1.6**	**11.4**	**< 4.8 (m)**
2367	0.20	0.44	2.2	15.2	
3467, 1269	0.09	0.21	2.3	16.1	
1239	n.d.	n.d.			
1289	n.a.	n.a.			
Sum T4CDFs	*2.99*	*7.00*			
P5CDFs					
12468, 13468	0.12	0.36	3.0	20.7	
13678	n.d.	0.10			
12368, 12478, 13478, 13467, 12467	0.40	2.17	5.4	37.5	
14678, 13479	0.05	0.12	2.4	16.6	
13469, 12479	n.d.	n.d.			
23468, 12347, 12346, 12469	0.09	0.30	3.3	23.0	
12348	n.d.	0.10			
12378	**0.09**	**0.28**	**3.1**	**21.5**	**< 1.5**
12367	0.06	0.12	2.0	13.8	
12678, 12379	0.06	0.12	2.0	13.8	
12679, **23478**, 12369	**0.41**	**3.80**	**9.3**	**64.0**	**6.1**
23467, 12489	0.08	0.24	3.0	20.7	
12349	n.d.	n.d.			
12389	n.d.	n.d.			
Sum P5CDFs	*1.35*	*7.71*			
H6CDFs					
123468	0.08	0.66	8.3	57.0	
134678, 124678	0.34	1.33	3.9	27.0	
134679	n.d.	n.d.			
124679	n.d.	n.d.			
124689	0.21	0.52	2.5	17.1	
123467, **123478**	**0.76**	**3.30**	**4.3**	**30.0**	**4.7**
123678	**0.41**	**2.04**	**5.0**	**34.4**	**2.9**
123479	n.d.	n.d.			
123469, 123679	n.d.	n.d.			
123689	n.d.	n.d.			
234678	**0.26**	**0.89**	**3.4**	**23.6**	**1.5**
123789	n.d.	n.d.			**n.d.**
123489	n.d.	n.d.			
Sum H6CDFs	*2.07*	*8.75*			

Table 1 (continued)

Isomer	Conc. dietary fat *(pg/g fat)*	Conc. fecal fat *(pg/g fat)*	Conc. ratio	Fecal excretion (percentage of intake)	Conc. blood fat *(pg/g fat)*
H7CDFs					
1234678	**0.95**	**25.68**	**27.0**	**186.7**	**8.3**
1234679	n.d.	n.d.			
1234689	0.41	5.24	12.8	88.3	
1234789	**0.19**	**0.71**	**3.7**	**25.8**	**< 1.0**
Sum H7CDFs	*1.56*	*31.63*			
OCDF	**1.68**	**45.26**	**26.9**	**186.0**	**< 4.3 (m)**
I-TEq	**0.68**	**5.95**	**8.8**	**60.6**	**10.8**

m maximum value, due to possible contribution of a contaminant
n.d. not detected, limit of detection: 0.05 pg/g for dietary fat, 0.10 pg/g for fecal fat, 1.1 pg/g for blood fat
n.a. not analyzed