Partitioning of PCDD/Fs between blood and adipose tissue in 9 former chemical workers

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1 Introduction

As polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) are lipophilic substances the body burden in humans has to be determined in compartments with high lipid content. Besides analysis in human milk restricted only to women earlier determinations were based on samples of adipose tissue while nowadays the sensitivity of analytical methods is good enough to use blood (serum or whole blood) on a lipid basis.

For part of a cohort of former chemical workers having been exposed to PCDD/Fs corresponding measurements are available, partly determined in adipose tissue, but mainly in whole blood. For the assessment of past exposure those from adipose tissue should be made comparable to the remaining by a corrective factor if necessary. Similar situations might occur in other unplanned studies with retrospective design.

Determinations of 2,3,7,8-substituted dioxins and furans from both whole blood and adipose tissue within a maximum space of time of 6 months are available for a small subgroup (n=9) of the workers cohort. Measurements from both matrices were highly correlated. Just as from Schecter et al.[1)] an increasing ratio between blood and fat with chlorine substitution was observed, but in contrast to those authors' results of a maximum ratio of 2 for OCDD we found the proportion tending towards balance. For 2,3,7,8-TCDD the estimated ratio was 0.83 and differed significantly from 1.

2 Materials and methods

2.1 Study population

The original cohort of workers from a plant producing herbicides until 1984 is described elsewhere[2)]. During a health investigation program by the Institute of Occupational and Social Medicine (University of Mainz) in 1985/86 blood samples were withdrawn from 188 participants and kept frozen at -20°C. For 45 volunteers PCDD/F-levels were determined 1986/87 in adipose tissue on a lipid basis and were described by Beck et al.[3)]. A subgroup (n=15) also was part of a half life analysis' study group. In the course of this study[4)] blood samples from 1985/86 had been analyzed 1994 for 2,3,7,8-substituted dioxins and furans.

Thus determinations in adipose tissue and whole blood on a lipid basis were available for 15 persons. As for six persons the time between sampling of blood and adipose tissue exceeded 6 months, they were excluded from statistical analysis.

The nine persons included were men in the age of 37 to 57 (mean 47). From the health investigation it was known that there were 6 smokers and 2 ex-smokers among them. Mean percentage of body fat (calculated from height and weight following a formular by Knapik et al.[5]) was 22.8 (range 14.5-32.4), mean level of total cholesterol 220 (155-271). Mean time between end of occupational exposure to PCDD/F and health investigation was 2.3 years and during that one man had suffered from liver cirrhosis while no cancer, diabetes or thyroid dysfunction had occured. Another one had reported an unintentional weight loss of more than 5 kg since leaving the plant.

2.2 Analytical methods

Sampling, storing and measuring of adipose tissue had been carried out as described in Beck et al.[3]. In brief, 5-10 g of subcutaneous abdominal fat had been removed and stored at -20°C. Afterwards fat had been extracted and PCDD/Fs had been determined by HRGC/HRMS. Blood samples also had been kept frozen at -20°C until analysis by the ERGO laboratory, Hamburg. The analytical method was nearly identical to that used in round II and III of WHO's interlaboratory validation studies[6][7]. The HRGC/HRMS measurements in blood had been performed in duplicate.

2.3 Statistical analysis

In order to examine the existence of a linear relation Pearson's correlation coefficient was calculated. The empirical distribution of lipid based levels in whole blood and adipose tissue were described as well as the ratio of means and medians and a regression model:

$$\text{BLOOD} = a + b\ \text{ADIPOSE TISSUE}$$

was fitted with constant *a* and slope *b* for each congener after a check of the assumption of the blood levels following a normal distribution. The model fit was also examined for potential outliers by a scatter plot.

3 Results

Table 1 shows the parameters of the empirical distribution of levels in blood and adipose tissue and of the corresponding ratios. In spite of the fact that the blood samples had been analysed about 7 years later by a different laboratory, there was a good agreement between both results. There were elevated concentrations for all dioxins and for most of the furans (2,3,4,7,8-PCDF, 1,2,3,4,7,8- and 1,2,3,6,7,8-HCDF, both HpCDFs). In particular, for 2,3,7,8-TCDD the mean level in blood was 96.7 while that in adipose tissue was 117.4.

For every person blood samples had been taken before tissue biopsy. Mean time distance was 3.8 months (standard deviation 1.0). Measurements in blood and fat were highly correlated. For the dioxins the correlation coefficient was at least 0.9, for the furans 0.7 except for 2,3,7,8-TCDF and OCDF (both 0.27). 2,3,7,8-TCDD's correlation was 0.91. Table 1 also presents the ratios of blood and fat levels' means and medians. The median ratio of 2,3,7,8-TCDD was 0.82. For the other dioxins the ratio increased with chlorination from 0.49 for 1,2,3,7,8-PCDD to 0.95 for OCDD. A similar trend could be observed for the furans except for 2,3,7,8-TCDF and 2,3,4,6,7,8-HCDF: an increase from 0.55 for 1,2,3,7,8-PCDF to 2.5 for OCDF.

Table 1: Concentration of PCDD/Fs in whole blood and adipose tissue (ng/kg fat) and corresponding ratios

	Whole Blood				Adipose Tissue				Blood/Fat		Corr.
	Median	Mean	Min.	Max.	Median	Mean	Min.	Max.	Median	Mean	Coeff.
2,3,7,8-TCDD	107.1	96.7	22.8	141.5	131	117.4	20	158	0.82	0.82	0.91
1,2,3,7,8-PCDD	60.3	74.1	12.3	141	122	135.4	13	328	0.49	0.55	0.93
1,2,3,4,7,8-HCDD	108	169.5	15.7	472.3	179	258	14	824	0.60	0.66	0.99
1,2,3,6,7,8-HCDD	412.3	679.7	67.4	2304.3	646	953.9	55	3404	0.64	0.71	0.99
1,2,3,7,8,9-HCDD	141.5	178.6	15.6	527	147	201.7	9	661	0.96	0.89	0.98
1,2,3,4,6,7,8-HpCDD	668.6	1069.4	171.7	3402.5	755	1064.4	83	4120	0.89	1.00	0.97
OCDD	2139	4667.6	650	16540	2260	4165.9	329	15892	0.95	1.12	0.97
2,3,7,8-TCDF	2.5	3.1	1.8	6.6	2	2	1	3	1.25	1.55	0.27
1,2,3,7,8-PCDF	1.1	1.5	0.6	4.3	2	1.7	0.8	2.8	0.55	0.87	0.71
2,3,4,7,8-PCDF	52.8	88	21.4	406.7	67	70.8	18	170	0.79	1.24	0.91
1,2,3,4,7,8-HCDF	160.8	218.4	21.7	705.1	174	209.7	11	750	0.92	1.04	0.98
1,2,3,6,7,8-HCDF	65.7	76.6	11.6	240	69	99.1	8.3	328	0.95	0.77	0.98
2,3,4,6,7,8-HCDF	9.6	9.5	3.5	17.5	13	11.7	2.5	28	0.74	0.81	0.91
1,2,3,4,6,7,8-HpCDF	161.6	227.5	25	758	154	215.3	11	752	1.05	1.06	0.98
1,2,3,4,7,8,9-HpCDF	5.1	6.4	0.9	16.3	5.5	5.6	0.4	15	0.93	1.14	0.77
OCDF	2.5	3.1	2.5	5	1	1.4	1	4.3	2.5	2.28	0.27

In the regression models the estimates for the constants *a* did not differ significantly from 0, so models were fitted without constant *a* and the estimates for *b* could be interpreted as relation of measurements of blood to adipose tissue. The results are shown in table 2. For the 4- to 6-fold chlorinated dioxins all parameter estimates were significantly (α=0.05) below 1. For 2,3,7,8-TCDD the estimate was 0.83. In contrast 1,2,3,4,6,7,8-HpCDD and OCDD did not differ significantly from 1. Less consistency was observed for the furans: the coefficients for 2,3,4,7,8-PCDF was significantly above 1 whereas 1,2,3,6,7,8- and 2,3,4,6,7,8-HCDF were below. The remaining parameters were either close to 1 or showed a great variation.

Table 2: Parameter estimates from regression model: BLOOD = *b* ADIPOSE TISSUE (based on 9 persons resp. 8 after exclusion of an outlier).

	Parameter	95%-Confidence Limits	
	Estimate for *b*	Lower	Upper
2,3,7,8-TCDD	0.83	0.74	0.91
1,2,3,7,8-PCDD	0.51	0.44	0.58
1,2,3,4,7,8-HCDD	0.61	0.56	0.66
1,2,3,6,7,8-HCDD	0.69	0.65	0.74
1,2,3,7,8,9-HCDD	0.84	0.76	0.91
1,2,3,4,6,7,8-HpCDD	0.89	0.76	1.02
OCDD	1.08	0.95	1.2
2,3,7,8-TCDF	1.42	0.91	1.93
2,3,7,8-TCDF (without one outlier)	1.2	0.87	1.54
1,2,3,7,8-PCDF	0.9	0.62	1.18
1,2,3,7,8-PCDF (without one outlier)	0.68	0.54	0.83
2,3,4,7,8-PCDF	1.57	1.01	2.12
2,3,4,7,8-PCDF (without one outlier)	0.8	0.69	0.9
1,2,3,4,7,8-HCDF	0.98	0.89	1.08
1,2,3,6,7,8-HCDF	0.75	0.68	0.82
2,3,4,6,7,8-HCDF	0.75	0.64	0.87
1,2,3,4,6,7,8-HpCDF	1.03	0.94	1.13
1,2,3,4,7,8,9-HpCDF	1.03	0.72	1.34
OCDF	1.53	0.77	2.29

Scatter plots of the observed measurements combined with the respective estimated regression line were used to examine the models dependence on outliers. A good fit was observed for all dioxins, the hexa-furans and the 1,2,3,4,6,7,8-HpCDF. In the contrast 2,3,7,8-TCDF and both the penta-furans were strongly influenced by one outlier with rather high blood levels. In the case of 2,3,7,8-TCDF and 1,2,3,7,8-PCDF the respective person did

not have any striking values at the examined factors (see 2.1), in the case of 2,3,4,7,8-PCDF the influential observation was from the man who had suffered from liver cirrhosis since end of exposure.

Excluding the respective cases led to distinctly lower coefficients also presented in table 2. With these estimates for the penta-furans a course similar to that of the dioxins was observed: a decreasing overweight in adipose tissue related to an increasing chlorination. For 2,3,7,8-TCDF a great variation remained. No conclusions could be drawn on the blood/adipose tissue relation for OCDF, because there were only two different values observed in fat.

4 Discussion

The partitioning of 2,3,7,8-substituted PCDD/Fs between blood and adipose tissue was analyzed on the basis of 9 men's lipid based determinations in whole blood and adipose tissue. High correlations were in agreement with results from Schecter et al.[1] who had examined 20 Vietnam veterans and for example yielded a correlation coefficient of 0.94 for 2,3,7,8-TCDD, with Patterson et al.[8] who found a correlation of 0.98 for the same congener among 50 persons, with Kahn et al.[9] who reported a coefficient of 0.89 among 22 persons with at least 1 ppt 2,3,7,8-TCDD in blood and fat and finally with Gochfeld et al.[10] with the same coefficient among Vietnam veterans. For the other congeners the latter observed less pronounced associations with coefficients between -0.27 and 0.7. But in contrast to this study those authors used determinations in serum or plasma.

On the one hand we estimated the relation between PCDD/Fs in blood lipids and in extracted lipids from adipose tissue by the ratio of median levels in both matrices, on the other hand by the slope of a regression model. In general, both methods produced similar results. For 2,3,7,8-TCDD the model estimate 0.83 differed significantly from 1 (α=0.05). This is in contrast to Patterson et al.[8] with a ratio of mean levels of 1.09 (not significant), but equal to the ratio reported by Schecter et al.[1]. In agreement with the latter authors we observed a trend of increasing coefficient with increasing chlorination, but in contrast to them this tended towards a balance between blood and fat, whereas Schecter et al.[1] found a ratio of 2 for OCDD.

For 2,3,7,8-TCDF and OCDF no conclusions could be drawn from the available data. This might be due to the low levels observed and possibly relatively less exact measurements. Schecter et al.[1] reported a ratio of 0.81 for 2,3,7,8-TCDF, but did not determine OCDF. Results for the other furans suggest a course similar to those of the dioxins, and again this agrees with the analysis of these authors according to the trend, but not to the explicit values as they yielded ratios up to 1.5 for 1,2,3,6,7,8-HCDF and 1,2,3,4,6,7,8-HpCDF.

These differences might be due to the time between the two periods of analyses and to the two seperate laboratories involved. Besides Schecter et al.[1] measured in serum. They compared their results in a simultaneous study with whole blood/adipose tissue ratios in 4 persons and also found lower values: between 0.78 for 2,3,7,8-TCDD and 1.21 for 1,2,3,4,6,7,8-HpCDD among the dioxins and up to 1.55 for 2,3,4,6,7,8-HCDF among the furans[11]. In addition a problem of this study's data is the time distance between both samples. According to the half life of each congener blood levels might have been further decreased until time of tissue biopsy. We tried to minimize this bias by excluding persons with distances longer than 6 months.

Finally, the observed levels of higher chlorinated dioxins and furans in general were elevated and far above background concentrations (German background values[12][13]) in contrast to

those from other studies. Scatter plots showed that the models fitted well over considerable ranges, but it might be assumed that measurements in background regions are less exact, especially in blood, because of the lower absolute PCDD/F amount available.

In summary, the presented results suggest to correct lipid based determinations in adipose tissue concerning 4- to 6-fold chlorinated dioxins as well as 1,2,3,6,7,8-HCDF and 2,3,4,6,7,8-HCDF by the factors shown in table 2, supposed a simultaneous analysis with measurements in blood is intended. With regard to the remaining 2,3,7,8-substituted PCDD/Fs this paper supports taking the levels determined in fat as those measured in blood.

5. References

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