Dioxins (PCDDs and PCDFs) in human milk from Flanders, Belgium: concentration levels and congener profile

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ABSTRACT

Nine human milk samples, collected on a voluntary basis from nursing mothers living in different Flemish provinces, have been analysed for polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). The average TEQ content, on milk fat basis, amounts to 34.4 pg/g, with individual values between 27.3 and 43.2 pg/g; this dioxin burden agrees well with that documented from other highly industrialised West European countries. Dioxins and furans contribute almost equally to the TEQ content, of which about 45% is made up by 2,3,4,7,8-P_5CDF. A tendency towards somewhat higher TEQ values at urban locations is observed; the limited size of the study, however, does not allow to demonstrate the statistical significance or prove other correlations with personal data or dietary habits.

INTRODUCTION

Some three years ago we set up a research programme aimed at assessing the dioxin contamination of the Flemish environment and food supply. A further objective was to evaluate the impact of the PCDD and PCDF levels detected, on the basis of the often comprehensive studies that already have been undertaken in surrounding countries. So far our screening mainly focused at sampling and analysis of air[1], traffic[2] and waste incinerator emissions, atmospheric deposition, soil[3] and cow's milk[4].

The relatively elevated levels of PCDDs and PCDFs in two pooled samples of Belgian human milk[5], reported in 1989 from a study set up by the WHO, have caused considerable debate and public concern in Belgium about the safety of breast feeding. Moreover the levels in human milk reflect the body burden of the lactating individual, which has its origin in the spread of these substances in the total environment and food chain. In view of the lack of further analytical evidence, it was felt appropriate to provide some additional and more recent data on the dioxin contamination of human milk in Flanders, Belgium.

PROCEDURE FOR SAMPLING AND ANALYSIS

In collaboration with a public service supervising the newborns' health and evolution, in each of the five Flemish provinces one or two nursing mothers were invited to participate in the study on a voluntary basis. The donors were chosen randomly. They were all provided with pre-cleaned glass vials, a breast pump and instructions on how to collect and store samples, and asked to supply approximately 100-200 ml of breast milk. In addition a questionnaire had to be completed concerning personal data, living conditions and food consumption. All samples were collected in the spring and autumn of 1992, and kept frozen at -20 °C until analysis.

The samples were analysed individually for the 17 2,3,7,8- PCDD and PCDF congeners, allowing calculation of the toxicity equivalents (TEQ) for each sample using the I-TEF scheme[6]. Details on the analytical method and its validation have been published earlier[4] and will not be repeated here. Briefly, the fat fraction is isolated by repeated liquid-liquid extraction with diethyl ether and petroleum ether after addition of sodium oxalate and methanol. After evaporation of the extracting solvent, the remaining fat is weighed and, after redissolving, spiked with an internal standard mixture containing the 17 ^{13}C-labelled isomers corresponding to the analytes. The extract is purified by, consecutively, gel permeation chromatography using a Bio-beads S-X3 column, high pressure liquid chromatography using a Hypercarb S porous graphitised carbon column, and adsorption chromatography on basic alumina. After further concentration of the purified extract, a recovery standard (^{13}C-1,2,3,4-T_4CDD) is added, the final volume of the extract amounting to about 50 µl. Quantification of the analytes is carried out using gas chromatography - high resolution mass spectrometry; separation takes place on a 60 m x 0.25 mm x 0.25 µm DB-5ms fused silica capillary column, and detection by selected ion recording on a VG Autospec Q instrument in the electron impact mode, tuned to resolution 10000.

RESULTS AND DISCUSSION

The results of the nine human milk analyses have been summarised in Table 1, both as absolute concentrations and as toxicity equivalents. The table contains the mean, median value and range for each of the individual congeners as well as for the sum of PCDDs, the sum of PCDFs and the total PCDD and PCDF content.

The mean and median value of the TEQ content amount to 34.4 and 32.5 pg/g milk fat, respectively, with individual values ranging between 27.3 and 43.2 pg/g. On average, dioxins and furans contribute almost equally (47% vs. 53%) to the TEQ content; 85% of the TEQ are made up by four congeners, being 2,3,4,7,8-P_5CDF (45%), 1,2,3,7,8-P_5CDD (17%), 2,3,7,8-T_4CDD (12%) and 1,2,3,6,7,8-H_6CDD (10%). In absolute concentration, dioxins are by far the most abundant; on average they represent 85% of the total PCDD and PCDF concentration. The congener profile is clearly dominated by O_8CDD and , to a lesser extent, by 1,2,3,4,6,7,8-H_7CDD, which make up about 55% and 16%, respectively, of the total PCDD and PCDF content.

Typical toxicity equivalent levels in human milk from Belgium and other countries have been compiled graphically in Figure 1. It follows that the dioxin burden for Belgium, as assessed in this study, agrees well with that documented for other highly industrialised West European countries. Interestingly, the average TEQ in this study is 10-15% lower than the values reported earlier for Belgium, which were based on two pooled human milk samples (together composed from 64 individual samples) analysed in the framework of a field study co-ordinated by the WHO[5]. This may indicate that the dioxin burden of human milk somewhat decreased between 1988 and 1992; similarly, extensive monitoring of the TEQ content in human milk in Germany (North Rhine-Westphalia)[7] during the period 1987-1991 has demonstrated a clear tendency to lower levels, the decline amounting to up to 30%. Generally, the average TEQ levels in the Netherlands, Belgium, Germany and the United Kingdom, which appear to be the highest world-wide, range between 25 and 40 pg/g fat. On an individual basis, however, the variation of the TEQ content may be much larger, e.g. 5.6 - 87.1 pg/g fat for Germany[7] and 17.5 - 92.9 pg/g fat for the Netherlands[8,9]. The average TEQ levels in other countries, such as Japan, Canada, the USA, New Zealand and the Scandinavian countries, typically are situated between 13 and 21 pg/g fat, thus appearing considerably lower than in the heavily industrialised and densely populated part of Western Europe. It remains as yet unclear, however, to what extent these differences are statistically significant.

Looking at the procentual contribution of individual PCDD and PCDF congeners to the TEQ content of Flemish human milk, as plotted in Figure 2 (upper) together with that for selected other countries, obviously the congener profile closely agrees with that obtained in the German monitoring

programme mentioned earlier. Surprisingly, however, both congener profiles differ considerably from the British and Dutch ones; the latter are characterised by a less important share of 2,3,4,7,8-P_5CDF (-13%) which is mainly compensated for by 2,3,7,8-T_4CDD (+6%) and 1,2,3,6,7,8-H_6CDD (+6%). This unexpected observation remains as yet unexplained; it must be noted, however, that the trend in Germany (1987-1991) also points to a gradually increasing share of 2,3,7,8-T_4CDD in the TEQ content, whereas the share of 2,3,4,7,8-P_5CDF is gradually diminishing. For Flanders, this relative abundance of 2,3,4,7,8-P_5CDF is also observed in the TEQ emitted by elder-type incinerators, which are (still) densely spread. The difference of the congener profile in the German vs. the less polluted American human milk has been highlighted earlier and suggested to be related to different exposure sources[10].

	Concentration (pg/g fat)			Toxicity equivalents (pg/g fat, as TEQ)		
CONGENER	MEAN	MEDIAN	RANGE	MEAN	MEDIAN	RANGE
2,3,7,8-T_4CDD	4.2	4.5	2.9 - 5.1	4.21	4.53	2.92 - 5.06
1,2,3,7,8-P_5CDD	11.9	11.6	8.4 - 16.6	5.94	5.80	4.18 - 8.30
1,2,3,4,7,8-H_6CDD	7.1	6.4	5.0 - 11.0	0.71	0.64	0.50 - 1.10
1,2,3,6,7,8-H_6CDD	35.3	35.6	27.8 - 45.5	3.53	3.56	2.78 - 4.55
1,2,3,7,8,9-H_6CDD	8.0	8.1	6.5 - 11.1	0.80	0.81	0.65 - 1.11
1,2,3,4,6,7,8-H_7CDD	81.4	68.6	40.8 - 142	0.81	0.69	0.41 - 1.42
O_8CDD	272	232	154 - 455	0.27	0.23	0.15 - 0.46
Total PCDDs	**420**	**375**	**266 - 644**	**16.3**	**16.0**	**12.3 - 19.7**
2,3,7,8-T_4CDF	1.3	1.2	0.7 - 1.9	0.13	0.12	0.07 - 0.19
1,2,3,7,8-P_5CDF	0.9	0.7	0.5 - 1.8	0.04	0.03	0.02 - 0.09
2,3,4,7,8-P_5CDF	31.1	27.7	24.7 - 42.6	15.56	13.85	12.35 - 21.30
1,2,3,4,7,8-H_6CDF	8.6	8.8	6.8 - 11.0	0.86	0.88	0.68 - 1.10
1,2,3,6,7,8-H_6CDF	7.8	7.6	6.3 - 10.4	0.77	0.76	0.63 - 1.04
1,2,3,7,8,9-H_6CDF	0.5	0.4	<0.1 - 1.0	0.05	0.04	<0.01 - 0.10
2,3,4,6,7,8-H_6CDF	4.9	4.6	2.0 - 7.0	0.49	0.46	0.20 - 0.70
1,2,3,4,6,7,8-H_7CDF	13.4	10.4	5.4 - 30.1	0.13	0.10	0.05 - 0.30
1,2,3,4,7,8,9-H_7CDF	5.0	3.6	2.5 - 15.0	0.05	0.04	0.03 - 0.15
O_8CDF	3.4	2.9	1.6 - 7.0	0.00	0.00	0.00 - 0.01
Total PCDFs	**77**	**71**	**55 - 123**	**18.1**	**16.0**	**14.5 - 24.1**
Total PCDDs/PCDFs	**497**	**458**	**333 - 715**	**34.4**	**32.5**	**27.3 - 43.2**

Table 1: Summary of the PCDD and PCDF content of the nine human milk samples from Flanders, Belgium, which were analysed in this study

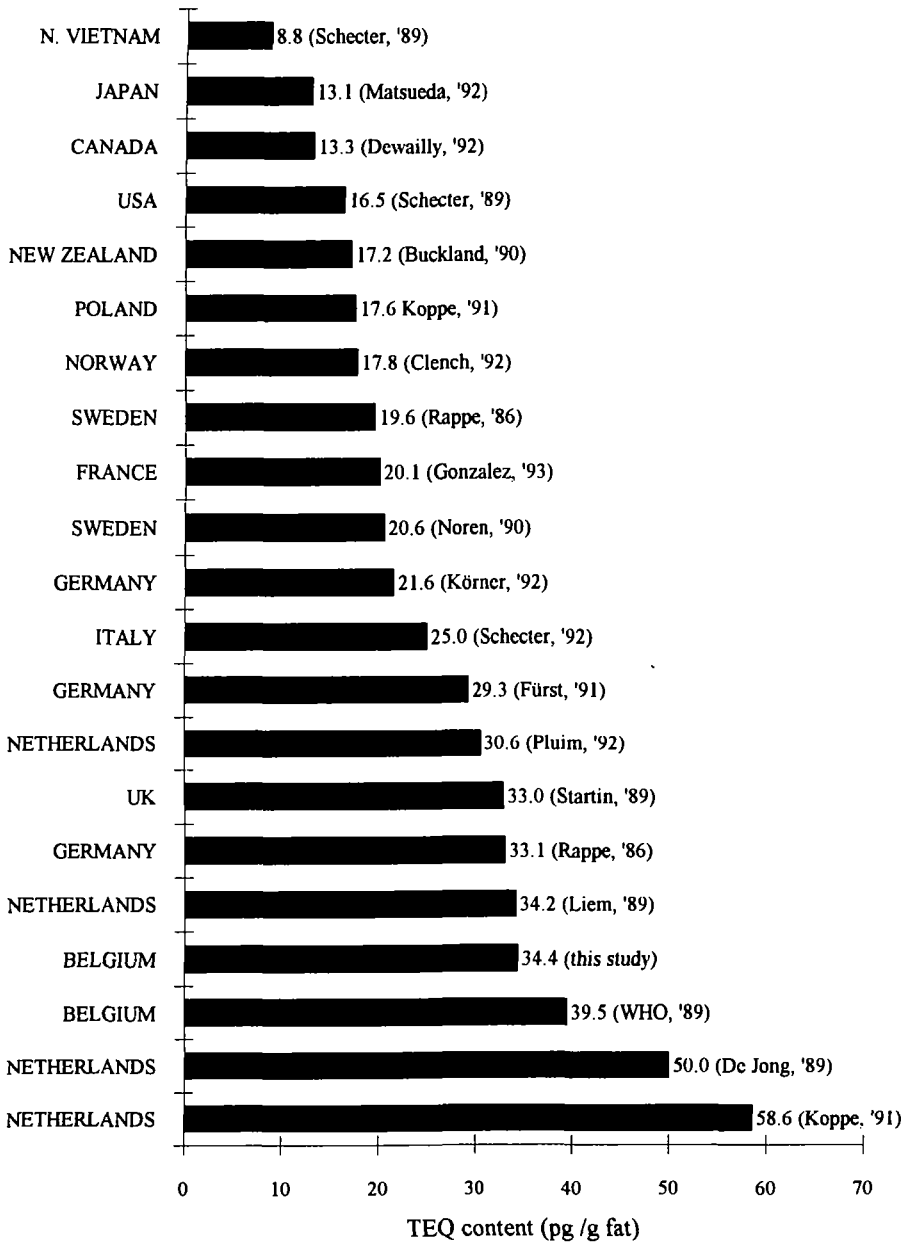

Figure 1: Survey of typical PCDD and PCDF levels (as I-TEQ) in human milk from various countries, as documented in the literature

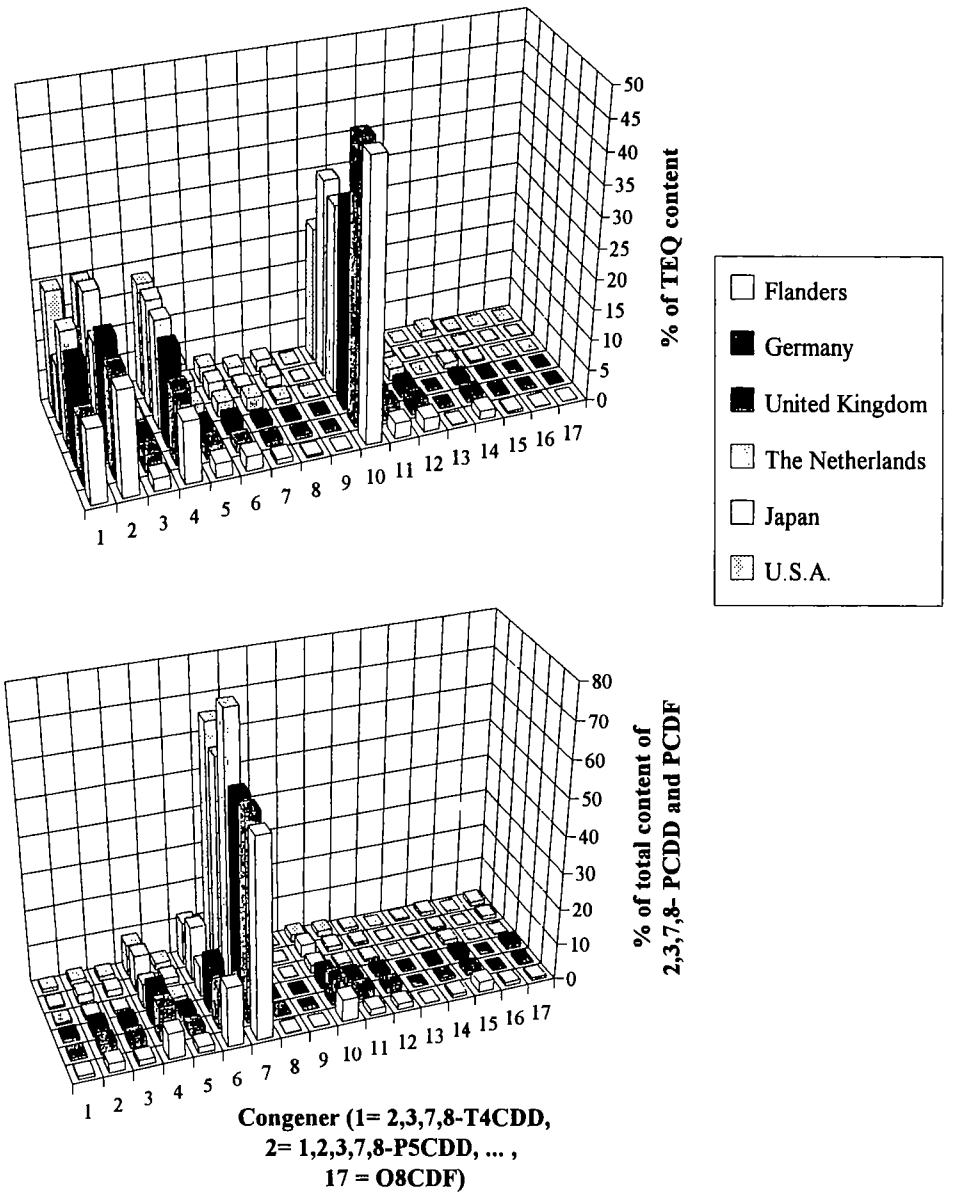

Figure 2: Procentual contribution of individual PCDD and PCDF congeners to the I-TEQ content *(upper)* and total 2,3,7,8-PCDD and PCDF content *(lower)* of human milk, as observed in this study for Flanders, Belgium, and documented in the literature for selected other countries

On the basis of a questionnaire completed by the nursing mothers, we attempted to correlate the analytical results to personal data, the living area and dietary habits. The North Rhine - Westphalian monitoring programme[7], with a sufficiently large and representative number of samples to allow a detailed statistical evaluation, has pointed out that the levels of PCDDs and PCDFs are mainly influenced by personal data. More specifically, the levels were found to decrease with increasing number of breast-fed children and with increasing total length of nursing periods, so that breast feeding may be regarded as a kind of detoxification for the mother. On the other hand, the relatively long half-life of the lipophilic contaminants resulted in an increase of the levels in human milk with the age of women nursing their first child. Probably due to the limited size of the screening that we report in this paper, we could not prove any such correlation with personal data. The same holds for possible correlations with food consumption habits.

From said North Rhine - Westphalian monitoring programme it was concluded that living in a rural or urban area does not affect the body burden, confirming the opinion that diet represents the main route for exposure. The limited data set which we report here for Flanders, Belgium, shows a tendency towards somewhat elevated TEQ levels in human milk of mothers who are living in urban areas since several years. For the latter sub-group, the TEQ content of human milk amounts to 37.6 $\pm$ 6.1 pg/g (n=5); for rural areas a value of 30.3 $\pm$ 2.4 pg/g (n=4) or 28.4 $\pm$ 1.6 pg/g (n=2) is obtained, depending on whether two mothers living in a rural area surrounded by (petro-)chemical industry are classified in the rural sub-group or not. On the basis of the first option, the difference between urban and rural TEQ values is not significant at the p=0.05 level.

In summary, the concentration levels of PCDDs and PCDFs in the Flemish human milk samples analysed in this study confirm that the degree of contamination in Flanders is quite similar to that in surrounding highly industrialised countries. There are some indications that the dioxin levels in human milk in this part of Western Europe, which tend to be the highest world-wide, are gradually decreasing, but further investigations remain necessary to evaluate the significance and continuation of this time trend. It will be worth re-investigating the PCDD/PCDF concentration levels and congener profile in Flemish human milk after the elder-type incinerators have been closed down or adapted with a suitable flue gas cleaning system.

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