

PCDDs/PCDFs AND PCBs IN MOTHERS MILK, ORIGINATING FROM SEVESO, MILAN AND A RURAL LOMBARDI AREA IN ITALY. A PILOT STUDY.

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Introduction

In 1976, an accident of the chemical plant ICMESA in Seveso, Italy exposed the surrounding population to levels of TCDD, which were among the highest ever recorded. The catastrophe has given a unique opportunity to evaluate the potential dose-response relationship between TCDD exposure and reproductive health in humans. Thus, several studies, chiefly on blood serum and plasma have been performed. In the first reproductive study that used serum TCDD as a means of exposure, a significant excess of female births (sex ratio = 0.54) was found from 1977-84 in the most heavily contaminated area (zone A)¹. Elevated parental serum TCDD concentration was found to be associated with a reduced proportion of male births whereas the maternal TCDD concentrations were not a significant predictor of the probability of male birth².

Other reproductive discrepancies that have been reported are an elevated rate of miscarriages from 1976 to 1978³ and an increased frequency of aberrant cytogenetic findings in aborted fetuses⁴. However the individual serum TCDD levels vary considerably between individuals. For example, in 298 males and females living in zone A, the TCDD level in serum collected 1976 ranged from non-detectable (<10ppt) to 56 000 ppt⁵.

A study of PCB's, DDT's and some other pesticides in milk from 12 mothers from each of three sampling sites, i.e. Seveso, (region 1), Milan (region 2), and an area outside Milan (region 3), which was collected at birth (colostrum), one month and three months after birth has recently been conducted at the Special Analytical Laboratory at the Museum of Natural History in Stockholm, Sweden⁶.

As a follow up to this study, a pilot study of dioxins has been performed using the same samples that were extracted for the previous study⁶. The purpose of this new study was to test the hypothesis that the concentrations of PCDDs/PCDFs, and non-*ortho*-PCBs in human milk from the town of Seveso, may still be elevated in comparison to a control material from the center of Milan, and a rural area outside this city, 25 years after the catastrophe.

Materials and methods

The samples were collected at local hospitals where they were frozen and stored in cleaned and heat-treated glass bottles. The previously cleaned samples of all 12 mothers from each area were pooled. Each of them contained 360-390 mg fat. The results can be recalculated on fresh weight basis.

In the samples, which had been cleaned-up for pesticides and PCBs, a separation from the non-

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planar molecules and a further clean-up step from non-planar molecules were performed. The analyses were carried out at Ergo Forschungsgesellschaft in Hamburg, Germany.

Coplanar molecules (PCDD/PCDF and non-*ortho*-PCB) were separated from the lipids and non-planar molecules with an active carbon column. The lipid fraction was first eluted with Hx:DCM (1:1), then the coplanar compounds were eluted in the reverse mode with toluene. The PCDD/PCDF and non-*ortho*-PCB fraction was further purified by a combined column, consisting of a H₂SO₄ column and an Al₂O₃ column. The samples were transferred to the combined column with Hx. The H₂SO₄ column was removed and the Al₂O₃ column was flushed with Hx, DCM:Hx (2:98) and finally eluted with DCM:Hx (1:1). After the separation were the samples evaporated to dryness in inserts and spiked with 10 µl (10 pg/µl) standard consisting of ¹³C-labeled 1,2,3,4-TCDD and ¹³C-labeled 1,2,3,4,6,7,8-HpCDF.

Analysis was performed using HRGC/HRMS of type Autospec. A DB 5 column, 60m long, with a thickness of 0.25mm and with a 0.1mm film, was used. The analysis was made in splitless mode and with an injector temperature at 270 °C and MS source temperature was 280 °C. The injection volume was 2 µl. The temperature program was 90 °C/min (3min), 25 °C/min until 210 °C, 3 °C/min until 275 °C and finally 275 °C (12.5 min). The mass spectrometer was operated in EI mode at a resolution of 10000. The limit of detection was set to three times the background noise.

The Ergo laboratory has successfully participated in a recent international quality assurance exercise performed by the National Institute of Public health, Norway, 2000⁶, which confirms the reproducibility of the analytical method. All calculations presented in this study are based on the most recent revised version of WHO-TEF values used⁷.

Results

The concentrations of all congeners are given in table 2. On a lipid weight basis, the total PCDD/PCDF concentrations decrease between month 0 and 3 mainly due to the amount of PCDD. The concentration of non-*ortho*-PCB does not change significantly between month 0 to 3. Except for TCDD, which was more than twice as high on lipid weight basis as in the other samples (fig 2), the overall concentration is lower in the samples from Seveso (fig 1). The non-*ortho*-PCB TEQ values are also lower in the Seveso samples and do not change significantly between month 0 to 3. CB 126 accounts for the main part of the PCB TEQ value.

Table 1. The mean fat content in the mother's milk samples (%)

	Seveso	Milan	Outside Milan
Month 0	2.7	1.4	1.6
Month 3	4.2	4.5	4.3

The fat content in the mother's colostrual milk was lower than after 3 months (se table 1). In colostrual milk from Seveso the mean value was higher than in the other 2 samples.

In this report are the results from the former analyses, performed at RSL, Sweden, not discussed, but worth mentioning is that almost all substances analysed there (DDE, HCH, HCB, PCBs among others) have a lower concentration in the Seveso samples than in the samples from Milan and outside Milan.

Discussion and Conclusion

The concentrations of PCDD/PCDF on a lipid weight basis were found to decline from month 0 to 3 (fig 2). However, the decline will be compensated when the concentrations are calculated on a fresh

Table 2. Concentration results (pg/g lipid) of PCDD, PCDF and non-ortho-PCB congener in pooled samples originating from 3 locations in Italy (Seveso, Milan and a rural Lombardian area). The samples are collected during the first week and 3 weeks after delivery.

Congener	Seveso	Seveso	Milan	Milan	Outside Milan	Outside Milan
	Month 0	Month 3	Month 0	Month 3	Month 0	Month 3
2,3,7,8-TCDD	4.45	3.70	1.63	1.55	1.58	1.44
1,2,3,7,8-PeCDD	2.76	2.38	4.06	3.61	4.28	3.32
1,2,3,4,7,8-HxCDD	1.39	1.26	2.21	1.76	2.79	1.71
1,2,3,6,7,8-HxCDD	7.15	7.25	12.80	9.52	13.07	8.61
1,2,3,7,8,9-HxCDD	1.37	0.95	1.78	1.37	2.11	1.32
1,2,3,4,6,7,8-HpCDD	9.13	7.26	20.45	11.46	20.49	10.17
OCDD	72.95	38.60	169.12	49.45	190.39	50.16
2,3,7,8-TCDF	n.d.(0.82)	n.d.(0.82)	n.d.(0.76)	n.d.(0.76)	n.d.(0.82)	n.d.(0.82)
1,2,3,7,8-PeCDF	0.20	n.d.(0.16)	0.21	0.20	0.19	n.d.(0.16)
2,3,4,7,8-PeCDF	5.81	5.93	9.71	8.87	9.00	7.27
1,2,3,4,7,8-HxCDF	2.59	2.71	3.30	3.27	3.44	2.75
1,2,3,6,7,8-HxCDF	1.58	1.71	2.44	2.40	2.47	1.87
1,2,3,7,8,9-HxCDF	n.d.(0.12)	n.d.(0.07)	n.d.(0.05)	n.d.(0.07)	n.d.(0.04)	n.d.(0.03)
2,3,4,6,7,8-HxCDF	0.64	0.58	0.85	0.84	0.88	0.67
1,2,3,4,6,7,8-HpCDF	1.50	0.67	1.44	1.19	1.41	0.73
1,2,3,4,7,8,9-HpCDF	n.d.(0.13)	n.d.(0.13)	n.d.(0.12)	n.d.(0.12)	n.d.(0.13)	n.d.(0.13)
OCDF	n.d.(1.54)	n.d.(1.54)	n.d.(1.42)	n.d.(1.42)	n.d.(1.54)	n.d.(1.54)
Total PCDD	99.19	61.40	212.05	78.72	234.70	76.73
Total PCDF	12.32	11.59	17.95	16.78	17.40	13.30
Total concentration	111.51	72.99	230.00	95.50	252.10	90.03
CB 81	3	4	5	5	3	3
CB 77	n.d.(22.2)	n.d.(22.2)	25	26	27	n.d.(22.2)
CB 126	53	57	75	75	66	57
CB 169	31	28	56	49	52	37
Total concentration	87.00	89.00	161.00	155.00	148.00	97.00

n.d. not detectable, () detection limit

weight basis as the fat content is higher in mature than in colostral milk (tab 1).

When comparing our TEQ values with the congener profile from the mean TEQ value from 20 Swedish samples (fig 2) from 1997⁸, it is evident that the Italian samples have the same profile as the Swedish samples, except that from Seveso. The same congener profile as found in the Swedish samples has also been reported from other countries^{8,9}.

As is shown in table 2 and figure 2 the TCDD level is higher in human milk in Seveso than in the other regions even 25 years after the accident although the total TEQ value is within the same level in all 3 Italian regions.

After the accident in 1976, many blood samples from Seveso have been analysed. The Seveso accident has been found to cause adverse effects on reproductive health, but more extensive studies are needed to elucidate the dose-response mechanism in humans. Particular attention should then be given to the consequences of the vertical transmission of the highly toxic dioxin TCDD during pregnancy and lactation on the cognitive function of children who have been exposed to high amounts *in utero* and during infancy.

Further studies may elucidate whether the elevated TCDD level in milk from mothers living in Seveso is due to the fact that their milk contains TCDD fat, which has been stored in their fat for a long period of time or if their food still is contaminated with this compound.

Acknowledgement

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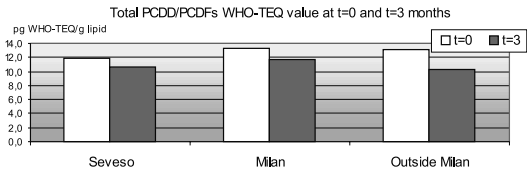


Figure 1. Mean total PCDD and PCDF WHO-TEQ value (pg WHO-TEQ/g lipids) in the pooled sample analysed

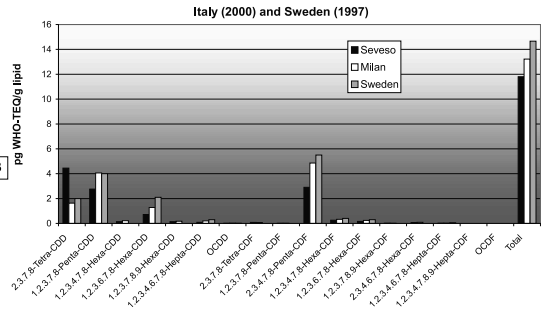


Figure 2. Total PCDD and PCDF congener WHO-TEQ value (pg WHO-TEQ/g lipids) profile in the samples originating from Seveso, Milan (2000) and Sweden (1997).

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