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RESIDUES OF POLYCHLORINATED-DIBENZODIOXINS, -DIBENZOFURANS AND -BIPHENYLS IN HUMAN MILK SAMPLES COLLECTED IN A CONTINENTAL TOWN IN CROATIA, YUGOSLAVIA

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The levels of several polychlorinated-dibenzodioxins (PCDDs), polychlorinated-dibenzofurans (PCDFs) and total polychlorinated-biphenyls (PCBs) were determined in pooled human milk samples. The samples were collected in a continental town of Croatia, Yugoslavia, during the years 1981/82 and 1985. The concentrations of seven PCDDs and nine PCDFs were within the range < 0.2 – 116 ng/kg milk fat; the total PCBs were 0.47 and 0.32 mg/kg milk fat. The levels determined were about the same as those reported for the general population in other countries.

Human milk is known to be contaminated with organochlorine compounds, and residues of different organochlorine compounds have been found in milk samples collected at different places in Yugoslavia (1). No data, however, have been available for polychlorinated-dibenzodioxins (PCDDs) and polychlorinated-dibenzofurans (PCDFs). Several compounds from these two groups are extremely toxic and residues have recently been found in human milk from various countries. The major biological effect of these polychlorinated aromatic compounds is a long-lasting inductive effect on hepatic microsomal mixed function oxidases which catalyse the metabolism of a variety of xenobiotics (drugs, food additives, pesticides); these enzymes are also involved in the metabolism of different physiologically important substances (2-4).

In this paper we report levels of several PCDDs and PCDFs in human milk samples collected in a continental town of Croatia, Yugoslavia, in the years 1981/82 and 1985. The samples were also analysed for their content of polychlorinated-biphenyls (PCBs). The PCDDs and PCDFs analyses in two pooled samples were made in The Department of Organic Chemistry, University of Umea, Umea, Sweden, and the PCBs analysis (in individual and pooled samples) was made in The Department of

Biochemistry, Institute for Medical Research and Occupational Health University of Zagreb, Zagreb, Yugoslavia.

MATERIAL AND METHODS

In 1981/82 fifty samples of human milk were collected and individually analysed for their content of PCBs (1). The pool of these samples (Pool A) was now analysed for PCDDs and PCDFs. In 1985 seventeen samples were collected and individually analysed for PCBs; the pool of these samples (Pool B) was analysed for PCDDs and PCDFs. Milk samples were collected between one and 25 weeks after delivery. The mother's age was between 18 and 38 years. Each mother was breast feeding only one child, who was her first or second. All mothers were on a mixed diet and had not been exposed to organochlorine compounds more than the general population. The pooling of milk samples was done on the basis of equal volumes. The PCBs were extracted from milk samples (2.5 g) with chloroform/methanol. The extract was purified with H₂SO₄.

The residues were dissolved in hexane and the PCBs determined by gas chromatographic analysis. All extractions were made in duplicates and each extract was chromatographed on two different columns. Column (a): 1.3% SF -96 + 5.3% QF -1on Supelcoport 100/2000 mesh. Column (b): 5% OV-101 on Chromosorb W DMCS/TAW 80/100 mesh. The Aroclor 1260 was used as the standard. Quantification was done by comparing the peak heights in the sample with the peak heights in the standard. The total PCBs concentration was calculated by summation of seven major peak heights from column (a) and 12 major peak heights from column (b). The mean recovery of PCBs was 74% and all the results were corrected accordingly. Details of the procedure were described earlier (1). The PCDDs and the PCDFs were extracted from the milk (pool A 196 g, pool B 154 g) with diethylether-hexane (5). The fat extracts (pool A 7.4 g, pool B 4.6 g) were cleaned up by liquid chromatography including enrichment on a PX-21 carbon column (6). The identification and quantification of the PCDDs and PCDFs were performed by HRGC/MS using a Finnigan 4500 quadrupole mass spectrometer, operating in NCI (CH₄) mode. A 60 m SP 2330 fusedsilica column (i.d. 0.32 mm) was used for the isomer specific separation (7).

Table 1

Median concentrations of total PCBs in human milk

Year of sampling	n	PCBs mg/kg fat	Fat content % (w/w)
1981/2	50	0.62 (0.30 - 1.7)	3.7 (1.5 - 7.4)
1985	17	$\begin{array}{c} 0.42 \\ (0.21 - 3.0) \end{array}$	3.0 $(1.1 - 7.6)$

n is the number of samples;

Concentration ranges are given in brackets

Quantification was done by comparing the peak heights for the different isomers with the corresponding isomers in a quantitative standard and by correcting for the ¹³C-recoveries using a set of eight ¹³C-isomers which had been added to the milk before extraction.

RESULTS AND DISCUSSION

The PCBs concentrations in the milk samples are shown in Table 1. The PCBs were present in all samples. The median concentrations and concentration ranges were similar in the two sampling years. The PCBs concentrations in the pooled samples were

^{*}from ref. 1

0.47 (Pool A) and 0.32 (Pool B) mg/kg fat. The concentrations of seven PCDDs and nine PCDFs in the pooled samples are given in Table 2. In both pools the concentrations were similar. The concentrations were in the range of nanograms per kilogram of fat as compared to PCBs which were present in milligrams per kilogram of fat. PCDDs and PCDFs contain each between one and eight chlorine atoms giving rise to 75 PCDD and 135 PCDF congeners. The toxicity of the congeners is very different;

Table 2

Concentrations of PCDDs and PCDFs in human milk

Compound	Concentration ng/kg fat		
	A	В	
PCDD:			
2,3,7,8-TCDD	< 1.0	< 1.0	
1,2,3,7,8-PeCDD	4.9	5.8	
1,2,3,4,7,8-HxCDD	3.6	3.5	
1,2,3,6,7,8-HxCDD	14	16	
1,2,3,7,8,9-HxCDD	< 0.5	< 0.8	
1,2,3,4,6,7,8-HpCDD	28	29	
OCDD	95	116	
PCDF:			
2,3,7,8-TCDF	< 1.2	< 1.9	
2,3,4,7,8-PeCDF	27	23	
1,2,3,4,7,8-HxCDF	3.4	4.0	
1,2,3,6,7,8-HxCDF	3.1	4.1	
1,2,3,7,8,9-HxCDF	< 0.2	< 0.3	
2,3,4,6,7,8-HxCDF	1.4	1.2	
1,2,3,4,6,7,8-HpCDF	3.4	3.5	
1,2,3,4,7,8,9-HpCDF	< 0.5	< 0.8	
OCDF	< 4.7	< 8.0	

A: pool of 50 samples. B: pool of 17 samples. Fat content (w/w): 3.8% in A and 3.0% in B.

the most toxic congeners contain 4-6 chlorine atoms in lateral positions. A health risk assessment of these compounds should therefore by based upon isomer-specific analysis, but information on the levels and pattern of individual isomers is still very fragmentary. The most toxic compounds in the two groups have been analysed in this study and their levels in the two pooled samples from Yugoslavia are about the same as those found in milk samples from Sweden, Netherlands, Germany and Vietnam (8). PCBs contain between one and ten chlorine atoms, which give rise to 209 congeners.

PCBs are considerably less toxic than the PCDDs and PCDFs, but in human milk they are present at higher concentrations than the latter compounds. No isomer-specific data on PCBs are available for human milk samples from Yugoslavia, but we showed earlier that the total PCBs (in a group of 50 samples) consisted of 30% heptachlorobiphenyls and 70% penta- and hexa-chlorobiphenyls (1). So far no adverse health effects have been reported which might be attributable to the presence of PCBs, PCDDs and PCDFs at levels found in human milk from the general population. However, more information is needed on exposure levels of infants and mothers before making assessments on possible individual and populational risks.

In conclusion, the results of this study show no difference in concentrations of the studied compounds between the samples collected within a time interval of five years. The levels of PCBs, PCDDs and PCDFs in human milk samples collected in the continental part of Yugoslavia are very similar to those from mothers living in other countries with a different climate, dietary habits and cultural pattern. Lack of data on the content of these compounds in the Yugoslav population necessitates a continuing investigation particularly because of an increasing number of possible sources of pollution.

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REFERENCES

- Krauthacker B, Kralj M, Tkalčević B, Reiner E. Levels of β-HCH, HCB, p,p'-DDE, p,p'-DDT and PCBs in human milk from a continental town in Croatia, Yugoslavia. Int Arch Occup Environ Health 1986;58:69.
- Yoshibara S, Nagata K, Wada I, Yoshimura H, Huroki H, Masuda Y. A Unique Change of Steroid Metabolism in Rat Liver Microsomes Induced with Highly Toxic Polychlorinated Biphenyl (PCB) and Polychlorinated Dibenzofuran (PCDF). J Pharmacobiodyn 1982;5:994.
- Yoshibara S, Nagata K, Yoshimura H. Different Responsiveness of Hepatic and Pulmonary Microsomal Mixed Function Oxidases to Phenobarbital-Type and 3-Methylcholantrene-Type Polychlorinated Biphenyls in Rats. J Pharmacobiodyn 1983;6:954.
- 4. Yushimura H, Yoshihara S, Koga N. et al. Inductive Effect on Hepatic Enzymes and Toxicity of Congeners of PCBs and PCDFs. Environ Health Perspect 1985;59:113.
- 5. P. O'Keefe, P, Neselson MS, Baughman RW. Neutral cleanup procedure for 2,3,7,8-tetrachlorodibenzodioxin residues in bovinc fat and milk. J Assoc Off Anal Chem 1978:61:621.
- Smith LH, Stalling DL, Johnson JL. Determination of part-per-trillion levels of polychlorinated dibenzofurans and dioxins in environmental samples. Anal Chem 1984;56:1830.
- Buser HR, Rappe C. Isomer-specific separation of 2,3,7,8-substituted polychlorinated dibenzo-p-dioxins by high-resolution gas chromatography/mass spectrometry. Anal Chem 1984;56:442.
- 8. Rappe C. Solving Hazardous Waste Problem. In: Exner J, ed. ACS Symposium Series. In press.

Sažetak

OSTACI POLIKLORIRANIH-DIBENZODIOKSINA, -DIBENZOFURANA I -BIFENILA U UZORCIMA HUMANOG MLIJEKA SAKUPLJENIM U KONTINENTALNOM GRADU U HRVATSKOJ, JUGOSLAVIJA

Određene su koncentracije nekoliko PCDD i PCDF spojeva te ukupnih PCB spojeva u zajedničkim uzorcima humanog mlijeka. Uzorci su sakupljeni u kontinentalnom gradu Hrvatske, Jugoslavija, tokom 1981/82 i 1985. Koncentracije sedam PCDD spojeva bile su u rasponu < 0,2-116 ng/kg mliječne masti; ukupni PCB spojevi bili su 0,47 i 0,32 mg/kg mliječne masti. Koncentracije određivanih spojeva slične su koncentracijama istih spojeva objavljenih za opće populacije ostalih zemalja.

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