CONFERENCE PAPER

ORGANOCHLORINE COMPOUNDS IN HUMAN MILK AND FOOD OF ANIMAL ORIGIN IN SAMPLES FROM CROATIA

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Human milk and food of animal origin (milk, meat, and fish) have been analysed over several decades for the DDT-complex, PCBs, HCB, and HCH-isomers. Human milk has also been analysed for PCDDs and PCDFs. Human milk samples were collected in six inland sites and one island, and food samples in marketplaces and individual households throughout Croatia. All human milk samples contained DDE and PCBs, and almost all food samples contained the DDT-complex. All analysed pools of human milk contained PCDDs and PCDFs. Levels of most analysed compounds show a decreasing trend over the past decade. The obtained data were the basis for the calculation of the daily intake of organochlorine compounds in breast-fed infants and adults.

Key words:
calculated daily intake, DDT-complex, dioxins, organochlorine pesticides, PCBs, PCDDs, PCDFs,

Organochlorine compounds listed in Table 1 are classified as persistent organic pollutants (POPs) due to their common characteristic, that is, longer persistence in the environment than their intended use (1). DDT, HCH-isomers, and HCB are pesticides; in many countries their use is restricted or even banned. PCBs are industrial chemicals. PCDDs and PCDFs, often referred to as dioxins, are unwanted by-products of various technological processes; they are not produced commercially and have no intended use.

Human exposure to these compounds is mainly attributed to the food chain, and contamination of food occurs through the pollution of the air, water, or soil. Human

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exposure to dioxins occurs mainly in industrialised settings and in areas where waste is inappropriately burned. The toxicity of the listed compounds varies greatly, with 2,3,7,8-TCDD being the most toxic.

Table 1 *List of organochlorine compounds and abbreviations used in this paper*

Compound	Abbreviation
Hexachlorobenzene	HCB
α-hexachlorocyclohexane	α-HCH
β-hexachlorocyclohexane	β-HCH
γ-hexachlorocyclohexane	γ-HCH, lindane
1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane	DDT
1,1-dichloro-2,2-di(4-chlorophenyl)ethane	DDD
1,1-dichloro-2,2-di(4-chlorophenyl)ethene	DDE
DDT + DDD + DDE	DDT-complex
Polychlorinated biphenyls	PCBs
2,4,4'-trichlorobiphenyl	PCB-28
2,2',5,5'-tetrachlorobiphenyl	PCB-52
2,2',4,5,5'-pentachlorobiphenyl	PCB-101
2,2',3,4,4',5'-hexachlorobiphenyl	PCB-138
2,2',4,4',5,5'-hexachlorobiphenyl	PCB-153
2,2',3,4,4',5,5'-heptachlorobiphenyl	PCB-180
Polychlorinated dibenzo-p-dioxins	PCDDs
2,3,7,8-tetrachlorodibenzo-p-dioxin	2,3,7,8-TCDD
1,2,3,7,8-pentachlorodibenzo-p-dioxin	1,2,3,7,8-PeCDD
1,2,3,4,7,8-hexachlorodibenzo-p-dioxin	1,2,3,4,7,8-HxCDD
1,2,3,6,7,8-hexachlorodibenzo-p-dioxin	1,2,3,6,7,8-HxCDD
1,2,3,7,8,9-hexachlorodibenzo-p-dioxin	1,2,3,7,8,9-HxCDD
1,2,3,4,6,7,8-heptachlorodibenzo-p-dioxin	1,2,3,4,6,7,8-HpCDD
Octachlorodibenzo-p-dioxin	OCDD
Polychlorinated dibenzofurans	PCDFs
2,3,7,8-tetrachlorodibenzofuran	2,3,7,8-TCDF
1,2,3,7,8-pentachlorodibenzofuran	1,2,3,7,8-PeCDF
2,3,4,7,8-pentachlorodibenzofuran	2,3,4,7,8-PeCDF
1,2,3,4,7,8-hexachlorodibenzofuran	1,2,3,4,7,8-HxCDF
1,2,3,6,7,8-hexachlorodibenzofuran	1,2,3,6,7,8-HxCDF
1,2,3,7,8,9-hexachlorodibenzofuran	1,2,3,7,8,9-HxCDF
2,3,4,6,7,8-hexachlorodibenzofuran	2,3,4,6,7,8-HxCDF
1,2,3,4,6,7,8-heptachlorodibenzofuran	1,2,3,4,6,7,8-HpCDF
1,2,3,4,7,8,9-heptachlorodibenzofuran	1,2,3,4,7,8,9-HpCDF
Octachlorodibenzofuran	OCDF

We started a monitoring programme of organochlorine compounds in human milk in 1977. Food has been systematically monitored by the Croatian National Institute of Public Health. This paper gives a selection of published data to document our general conclusions.

ANALYSIS OF COMPOUNDS

Compounds in human milk were analysed by gas chromatography and were identified and quantified by comparison with known standards (2). Concentrations of total PCBs were based on Aroclor 1260 as the standard. As the dioxins in human milk could not be analysed in our country due to the lack of equipment, the analysis of pooled samples for seven PCDD congeners and ten PCDF congeners was performed by collaborating laboratories in the USA, the Netherlands, and Sweden. The analysis of milk samples was verified through the Analytical Quality Assurance programmes of the World Health Organization (WHO) in Geneva, the WHO European Office in Copenhagen, or the US Environmental Protection Agency.

Compounds in food were analysed by gas chromatography on capillary columns (3).

SAMPLING SITES

Human milk samples were collected in six inland sites and one island: Zagreb and Sisak (industrialised towns in the north-west of the country), Osijek (town in the Panonian plane on the river Drava close to the Danube), Jastrebarsko and Karlovac (smaller towns south from Zagreb), Labin (small town in the centre of the Istrian peninsula), and the island of Krk in the northern Adriatic. Samples were collected from mothers who were not occupationally exposed to organochlorine compounds, who were resident in the respective towns/sites for at least five years, and who were nursing one child only.

Food samples were collected in marketplaces in various towns throughout Croatia and the fish samples were also collected from individual households.

COMPOUNDS IN HUMAN MILK

All analysed samples contained DDE. Other pesticides or their metabolites (DDT, DDD, HCH-isomers, and HCB) were not found in all samples. DDD and a-HCH were the rarest. All samples also contained PCBs. Of the PCB congeners listed in Table 1, congeners PCB-153, PCB-138 and PCB-180 were found in all samples (2, 4–13).

Most data on the analysed pesticides and PCBs in human milk were obtained from samples collected in Zagreb. Table 2 illustrates the changes in concentrations over years, One can observe a decrease in the levels of DDE, PCBs, HCB, and b-HCH. A comparison of data from Zagreb with those from other sampling sites revealed no significant difference in concentrations of any analysed compound, as illus-

Table 2 *Median concentrations (μg/kg milk fat or *μg/kg milk) of organochlorine compounds in human milk. N = number of analysed samples*

	Zagreb										
Compound	**1977/79* N=34**	**1977/79* N=37**	**1981/82 N=50**	**1985 N=18**	**1986/87 N=41**	**1987/89 N=22**	**1987/90 N=40**	**1990/91 N=30**	**1991/93 N=54**	**1994/95 N=54**	**1996/98 N=43**
HCB	–	–	210	210	120	60	31	20	15	11	15
α-HCH	0	0	–	0	0	0	0	0	0	0	0
β-HCH	–	–	280	230	170	40	39	40	24	35	30
γ-HCH	0	0	–	0	60	0	0	0	2	5	16
DDE	30	63	1900	1060	1480	620	491	450	282	247	409
DDD	0	3	–	0	0	0	0	0	0	0	0
DDT	0	0	180	130	70	0	0	0	0	9	9
PCBs	–	–	620	440	450	290	243	230	213	212	145
Ref.	4	4	5, 6	7	7	8	9	7	9	9	Unp.

Table 3 *Median concentrations (µg/kg milk fat) of organochlorine compounds in human milk. N = number of analysed samples (4, 14)*

Compound	Karlovac 1987, N=9	Sisak 1988, N=9	Krk 1986/87, N=33	Labin 1989, N=10
HCB	75	36	100	0
α-HCH	0	0	0	0
β-HCH	45	59	100	50
γ-HCH	0	0	0	0
DDE	600	633	1080	550
DDD	0	0	–	0
DDT	31	0	160	0
PCBs	300	431	500	270

trated in Table 3 for organochlorine pesticides and PCBs in samples from Karlovac, Sisak, Krk and Labin (4, 14).

All pooled milk samples contained PCDDs and PCDFs (15–19). Of the congeners listed in Table 1, OCDD had the highest concentration (between 53 and 116 ng/kg milk fat). In seven pooled samples the concentrations of 2,3,7,8-TCDD ranged from 0.8 to 1.9 ng/kg milk fat. In five samples 2,3,7,8-TCDD was not detected, but the 2,3,7,8-TCDD detection limits in these analyses ranged between 1.0 and 9.6 ng/kg milk fat (18, 19).

Table 4 shows concentrations of dioxins expressed in 2,3,7,8-TCDD toxic equivalents (TEQ) for all analysed pools of human milk. Most data are from samples collected in Zagreb. The dioxin concentrations in samples from Zagreb are about the same as in samples collected in Labin, Jastrebarsko, Osijek, and the island of Krk. We believe that so far no general conclusion can be made concerning changes in levels over the observed period. However, a WHO comparative study of dioxin levels in different European countries drew from our data on milk samples collected in Zagreb and Krk a conclusion that the trend was slightly decreasing in Croatia (17).

A comparison of dioxin concentrations in samples from Croatia with data from other European countries indicates that our concentrations are in the lower half of the European concentration range (Figure 1).

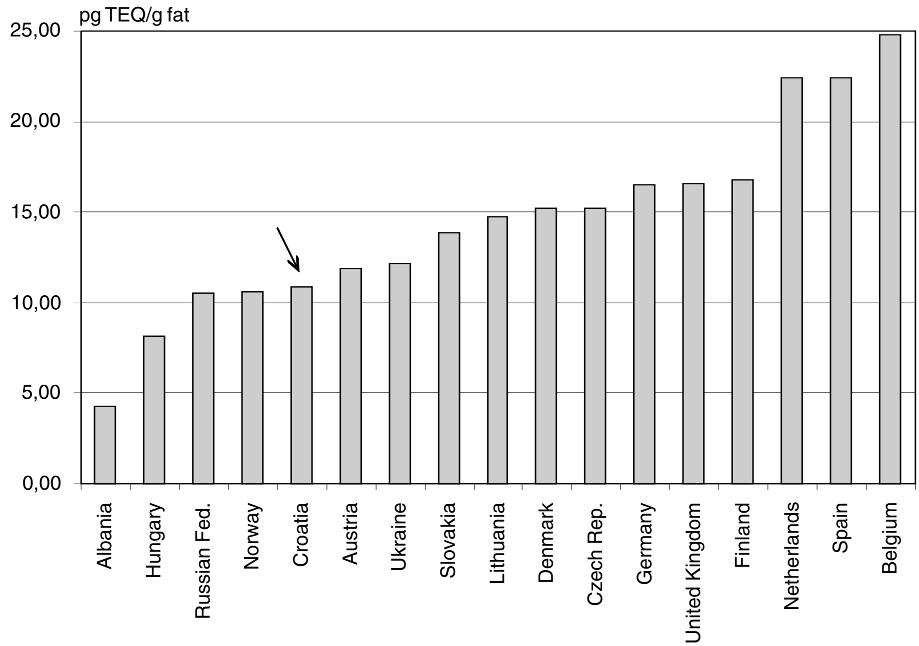

Figure 1 *Dioxin concentrations expressed in TEQ in human milk samples from different European countries collected in 1993 (17)*

COMPOUNDS IN FOOD OF ANIMAL ORIGIN

Food of animal origin has been systematically analysed for organochlorine pesticides for more than forty years. A detailed account was recently published by the Croatian National Institute of Public Health (3) on the analysis of food samples during 1986/89, and the data compared with results obtained from the analysis of food samples during 1999. Samples were analysed for DDT and its metabolites, HCB, and a- and g-HCH. No samples contained all compounds. The frequency was the highest for DDT and its metabolites (89–98% in 1999), followed by g-HCH (70–89%). Ten years earlier, those frequencies were about the same. The frequencies of HCB and a-HCH were between 5 and 39% in 1999, which was considerably lower than in 1986/89 (26–70%). Table 5 shows a summary of concentrations found in these samples, from which follows that levels of all compounds were invariably lower in 1999 than in 1986/89.

Table 5 *Mean concentrations (µg/kg fat and for fish µg/kg wet weight) of organochlorine compounds in food samples; number of analysed samples is given in brackets (3)*

Food and year	HCB	α-HCH	γ-HCH	DDT-complex
Fish and fish products				
1986/89 (153)	5	2	25	127
1999 (46)	0.1	0.1	0.5	4.7
Meat and meat products				
1986/89 (733)	3	2	25	75
1999 (80)	0	1	6	62
Milk and milk products				
1986/89 (438)	7	3	24	83
1999 (52)	1	1	6	35

The analysis of PCBs in food has not been very systematical (7, 20–22). Table 6 shows some data on PCBs in fish (20–22). The river Kupa south of Zagreb became polluted with PCBs due to improper storage of industrial chemicals in 1986, and this reflected in increased PCB levels in fish (21). Seawater fish contained lower PCB levels during that period (20).

Table 6 *Median concentrations or concentration ranges of PCBs in food samples*

Food and sampling year	PCBs	Reference
	µg/kg wet weight	
Fish 1987/88		
Seawater (Rijeka, Adriatic sea)	16–120	20
Freshwater (Petrinja, river Kupa)	70–1233	21
Fish 1992/96		
Seawater (Adriatic sea)	46	22
	µg/kg fat	
Pork 1992		
Fat	19	7
Fat tissue	12	7
Poultry 1992		
Fat tissue	14	7
Cow 1992		
Milk	73	7
Butter	20	7

CALCULATED DAILY INTAKE OF ORGANOCHLORINE COMPOUNDS

The calculation of the daily intake of organochlorine compounds by selected population groups relied on data obtained from the analysis of food and human milk and on questionnaires concerning food intake (Table 7).

Table 7 *Calculated daily intake (ng/kg bw) of organochlorine compounds. Individual numbers were calculated from mean or median concentrations in food, while ranges were calculated from the minimum and maximum concentrations*

Food source and population group	HCB	γ-HCH	DDT–complex	PCBs	Reference
Fish (river Kupa)					
Fishermen 1985/88	–	–	0–158	8–3550	21
Fish (from the market)					
Adults 1992	–	–	–	12	22
Adults 1992/96	0.5	5.7	10.0	–	22
Mixed diet 1987/89					
Adult women	2–700	20–600	200–1200	–	7
Nursing mothers	2–1100	30–1200	500–2400	–	7
Human milk					
Breast-fed infants					
1981/82	0–2400	0–200	700–25100	3000	7
1987/95	–	–	1620	1120	23

The intake of PCBs from fish spanned over a broad range, which was attributed to different eating habits of fishermen *vs.* other adults, and also to contamination of the river Kupa (21, 22).

The intake of organochlorine pesticides from mixed food was calculated for two groups of women of the same age: nursing and non-nursing women (7). As expected from their eating habits, nursing women had a higher intake of organochlorine pesticides. The calculated daily intake of organochlorine pesticides for breast-fed infants approached or were even higher than for the nursing women, and the intake of PCBs approached that of the fishermen eating contaminated fish from the river Kupa (7, 21). The daily intake of dioxins for beast-fed infants calculated from the data shown in Table 4 amounts to an average of 65 pg/kg body weight (bw) expressed in TEQ. Expressed in the same unit, the WHO reported in 1988 and 1989 that infants in the European countries consumed on average 70 pg/kg bw (16).

At present, guidelines concerning acceptable daily intakes (ADI) of organochlorine compounds exist only for a very limited number of compounds. Of the compounds discussed in this paper the ADI for the DDT-complex is 20 μg/kg bw (1). The ADI for HCB has recently been withdrawn (1) and there are no ADIs for PCBs (1). However, WHO experts agreed on a tolerable daily intake for dioxins to be between 1 and 4 pg/kg bw expressed in TCDD toxic equivalents (24) and for lindane (g-HCH) 5 μg/kg bw (25). ADIs and suggested tolerable daily intakes apply to adults for a life-long consumption, but so far there are no guidelines concerning the intake of any organochlorine compound for infants.

CONCLUSIONS

Studies conducted so far in Croatia have shown that residues of organochlorine compounds discussed in this paper are widely distributed in human milk and food of animal origin. No pronounced difference in levels was observed amongst different parts of Croatia such as rural v. urban or inland v. coastal areas. The levels of the studied compounds are comparable to levels reported in the industrialised European countries.

Having in mind that organochlorine compounds are widely distributed in the biosphere and are very stable, it seems to us that ADIs or tolerable daily intakes should be evaluated for a broad range of organochlorine compounds, including PCBs and dioxins. Some guidelines should also exist for infants whose only food is milk.

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Sažetak

ORGANOKLOROVI SPOJEVI U UZORCIMA HUMANOG MLIJEKA I HRANE ŽIVOTINJSKOG PODRIJETLA SKUPLJENIM U HRVATSKOJ

Humano mlijeko i hrana životinjskog podrijetla (mlijeko, meso i riba) analiziraju se već nekoliko desetljeća na prisutnost DDT-kompleksa, PCB, HCB i HCH-izomere. Humano se mlijeko također analizira na prisutnost PCDD-a i PCDF-a. Uzorci humanog mlijeka prikupljaju se na šest kontinentalnih lokacija i na jednom otoku, a uzorci hrane na tržnicama diljem Hrvatske i u domaćinstvima. Svi analizirani uzorci humanog mlijeka sadržavali su DDE i PCB, a gotovo svi uzorci hrane DDT-kompleks. Svi skupni uzorci humanog mlijeka sadržavali su PCDD i PCDF. Koncentracije gotovo svih analiziranih spojeva postupno su se smanjivale tijekom prošlog desetljeća. Na osnovi rezultata analiza izračunani su dnevni unosi organoklorovih spojeva u dojenčad i u odrasle osobe.

Ključne riječi:
DDT-kompleks, dioksini, izračunani dnevni unos, organoklorovi pesticidi, PCB, PCDD, PCDF