UDC 637.127.6:632.95
ISSN 1330-9862

professional paper

(FTB-1015)

Evidence of Some Trace Elements, Organochlorine Pesticides and PCBs in Slovenian Cow's Milk

Vesna Cerkvenik[1], *Darinka Zdenka Doganoc*[1]* *and Jurij Jan*[2]

[1]Institute for Food Hygiene and Bromatology, Veterinary Faculty, Gerbičeva 60, SI-1000 Ljubljana, Slovenia

[2]Institute of Public Health of the Republic of Slovenia, Grablovičeva 44, SI-1000 Ljubljana, Slovenia

Received: January 19, 2000
Accepted: May 17, 2000

Summary

From year 1994 to 1998 samples of raw cow's milk from 19 dairy locations were examined for residues of trace elements. Pb contents in all 188 samples corresponded to tolerance level (0.1 mg/kg) and in 98 % of these samples were below 0.05 mg/kg. All samples examined for residues of Cd (133), As (67) and total Hg (48) were completely acceptable as well. Cd and As residues were under detectable levels, while the highest total Hg value was 0.007 mg/kg. Residue levels of organochlorine pesticides in all 174 analysed samples were far below tolerance levels, too. In 90 % of samples α-HCH and in 86 % of samples lindane was below the limit of detection (0.003 mg/kg of fat). The highest α-HCH and lindane content was 0.008 and 0.023 mg/kg of fat, respectively. The level of heptachlor was below the limit of detection (0.003 mg/kg of fat). In 93.1 % of samples total DDT residues were between <0.005 and 0.02 mg/kg of fat, while the median and highest values were 0.007 and 0.091 mg/kg of fat, respectively. 108 samples were examined for PCB residues with the highest residue level of 0.14 mg of total PCB/kg of fat content. The median residue level was 0.005 mg of total PCB/kg of fat, which is 200 times lower than tolerance. 87 % of samples contained PCB residues between 0.002 and 0.08 mg PCB /kg of fat.

Key words: trace elements (Pb, Cd, As, Hg), lindane, α-HCH, DDT, polychlorinated biphenils, cow's milk

Introduction

Environmental pollutants such as trace elements of Pb, Cd, As or Hg, organochlorine pesticides and polychlorinated biphenyls (PCBs) can also be present in raw milk, therefore permanent monitoring of milk for these residues is necessary.

Trace elements are fairly widespread in the environment. Their level with an anthropogenic origin is much higher than the level from natural sources. These elements are non-essential for almost all living organisms. The largest amount of trace elements found in humans has been absorbed through food.

Organochlorine pesticides and polychlorinated biphenyls are very toxic organic compounds of anthropogenic origin which pose a serious threat to the environment and human health. The main organochlorine pesticides and PCBs are considered to be persistent organic pollutants (POPs) *(1)*. These compounds are a special problem because they persist in the environment for a long time before they break down, travel over long

* Corresponding author; Phone: ++ 386 (0)1 1779 100

Table 1. Maximum residue levels (MRLs) for some trace elements, organochlorine pesticides and PCBs in cow's milk in Slovenia *(3,4);* EU tolerated levels of organochlorine pesticides and PCBs are also presented *(5,6)*

Trace elements	Slovenia (mg/kg of milk)		
Pb	0.1		
Cd	0.01		
As	0.1		
total Hg	0.03		
OC pesticides	Slovenia (mg/kg of milk fat)	EU *(5)* (mg/kg of raw milk)	MRL ratio Slovenia:EU[b]
α-HCH	0.05[a]	0.004	10:1
γ-HCH (lindane)	0.1	0.008	10:1
Heptachlor	0.05	0.004	10:1
Total DDT	0.5	0.04	10:1
PCBs	Slovenia (mg/kg of milk fat)	EU *(6)* (mg/kg of milk fat)	MRL ratio Slovenia:EU
Total PCB	1.0	0.1[c]	10:1

[a] – together with β-HCH and δ-HCH as a sum
[b] – proposed 4 % of fat in raw milk
[c] – sum of the following PCBs (IUPAC): 28, 52, 101, 118, 138, 153, 180

distances to all parts of the globe and poison humans and wildlife. They tend to concentrate in the fatty tissues of humans and animals that are at the top in the food chain. The only way that the organisms can excrete them is through milk *(2)*.

Many countries have adopted regulations that state the maximum residue levels of environmental pollutants in food in order to protect consumers. In Slovenia, official tolerance of these compounds in food of animal origin is derived from the legislation of the former Yugoslavia *(3,4)*, although new regulations are under preparation. Current maximum residue levels for some environmental pollutants in cow's milk in Slovenia are presented in Table 1, where Slovenian levels of tolerance for organochlorine pesticides and PCBs are compared to European Union levels of tolerance *(5,6)*. Slovenian tolerance levels for HCH (α, β, γ and δ), heptachlor and total DDT are one time lower, but 10 times higher than the EU tolerance level for total PCB.

In Slovenia residue monitoring for organochlorine pesticides in food of animal origin, including cow's milk, began in 1974. Since 1980, it has been overseen by the Veterinary Administration of the Republic of Slovenia *(7)*. The first records of trace elements in raw cow's milk were made in 1981 *(8)*. Since then, regular monitoring of environmental pollutants in cow's milk has been carried out annually *(9–13)*. Analyses of Pb, Cd, As and organochlorine pesticides have been carried out at the Slovenian Institute for Food Hygiene and Bromatology at the Veterinary Faculty in Ljubljana. Analyses of Hg and PCBs have been carried out at the Institute of Public Health of the Republic of Slovenia in Ljubljana.

This paper presents the results of residue monitoring and environmental pollutant scanning of Slovenian raw cow's milk from 1994 to 1998. Samples from 19 dairies from all over Slovenia were brought to our Institute and were analysed for residues of trace elements, organochlorine pesticides and PCBs.

Materials and Methods

Sampling strategy

Samples of raw cow's milk were collected from 19 Slovenian dairies in accordance with the Monitoring Programme prescribed and organized by the Veterinary Directorate of the Ministry for Agriculture, Forestry and Food, Republic of Slovenia. The number of samples taken was based on the daily milk repurchase of an individual dairy. For every 15,000 liters one collective sample was taken annually.

The dairies were distributed over six geographical regions (Fig. 1).

The annual and total numbers of samples of raw cow's milk analysed for residues of trace elements, organochlorine pesticides and PCBs from 1994 to 1998 are shown in Table 2.

Table 2. Number of Slovenian raw cow's milk samples analysed for trace elements, organochlorine pesticides and PCBs content from 1994 to 1998

Year	Number of samples analysed on:					
	Pb	Cd	As	Hg	organochlorine pesticides	PCBs
1994	19	–	–	–	21	4
1995	34	–	–	–	33	11
1996	54	53	25	8	52	48
1997	53	53	32	31	49	26
1998	28	27	10	9	19	19
Total	188	133	67	48	174	108

The majority (about 40 %) of the samples analysed for trace elements originated from the last two regions in the eastern and north-eastern part of the country. About 30 % of the samples analysed for residues of organochlorine pesticides were from Štajerska region.

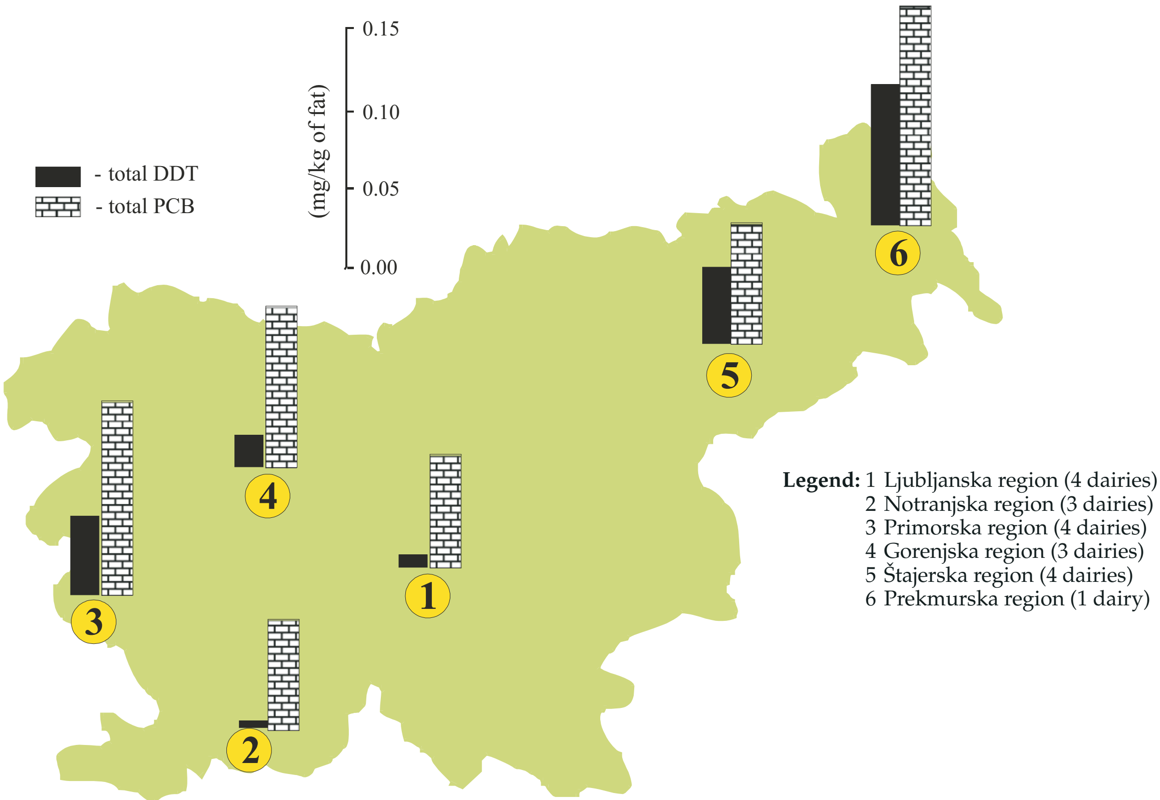

Fig 1. Highest content levels of total DDT and total PCB in raw cow's milk in six separate Slovenian regions, period 1994–1998

Analytical procedures

The concentrations of Pb and Cd in whole cow's milk samples were determined by flame atomic absorption spectrophotometry (FAAS). Samples were dry-ashed at 450 °C. Lead and cadmium were determined as diethylammonium diethyldithiocarbamate complex in methyl isobutyl ketone. The method of standard addition was employed for calculations. Details of the sample digestion procedure, a preconcentration step and the instrumental technique are described in references *(14,15)*. A spectrophotometrical method was used to determine As content *(14)*. The total Hg was determined by the cold vapor AAS technique *(14)*. Limits of detection were 0.05 mg/kg for Pb and As, 0.003 mg/kg for Cd and 0.001 mg/kg for total Hg.

Analytical quality assurance included periodical analyses of reference materials, including milk powder No. 151 from the Community Bureau of Reference, participation in interlaboratory control with FAPAS from Great Britain and recovery tests. Recovery for Pb was 94.6 % (mean value of annual recoveries in period from 1994 to 1998; n = 6/year) and 98.8 % for Cd, obtained analysing reference materials and spiked samples. Recovery for As over the entire period was 91.6 % (n = 39 spiked samples).

Fat was used as target matrix for analysis of organochlorine pesticides and PCBs due to the high lipophilicity of these pollutants. The fat was extracted from milk using sodium sulphate and petroleum ether to detect organochlorine pesticides. After solvent evaporation, the fat was refrigerated at 4 °C until clean-up.

The following organochlorine pesticides were analysed: α-HCH, lindane, heptachlor, 4,4'-DDE, 2,4'-DDD, 4,4'-DDD, 2,4'-DDT and 4,4'-DDT (the sum of the last 5 compounds was expressed as total DDT). The detectable limit in fat for the first three compounds was 0.003 mg/kg, and 0.005 mg/kg for the other five. Samples were cleaned-up with partly deactivated Al_2O_3 *(16,17)* and analysed using gas chromatography with ^{63}Ni electron capture detection (ECD). Before May 1996, the chromatographic process was performed with a packed glass column after a manual injection. Two packings were used to fill a 1.9 m × 3 mm ID column. The first mix was QF_1 2.5 % with DC 200 2.5 % on varaport 30, and the second mix was OV-17 2.5 % with QF_1 2.5 % on varaport 30. The temperature of the injector and detector was 205 °C and oven temperature was 200 °C. After May 1996, the chromatographic process was performed with a Hewlett Packard fused silica capillary HP-5 35 m × 0.2 mm × 0.33 µm column with crosslinked phenyl methyl silicone as stationary phase. The results obtained using both packed and capillary stationary phases were comparable. Nitrogen was used as the carrier and make-up gas. Injection was automatic and in split mode with a ratio of 30:1. The operating temperatures were as follows: injector 250 °C, detector 310 °C, oven during the gradient programme from 150 °C to 290 °C.

For sample extraction and clean-up of PCBs, standard methods were used *(18,19)*. The hexane extract of the sample was digested with concentrated sulphuric acid, followed by alkaline hydrolysis and a Florisil clean-up. PCBs were analysed using capillary gas chromatography with ECD, using a Supelco fused silica SPB-5 30 m × 0.25 mm and an SGE Varian OV-1701 50 m × 0.2 mm capillary column. Nitrogen was used as the make-up gas and beside helium also as the carrier gas. Injection was in splitless mode. The operating temperatures were as follows: injector 240 °C, detector 280 °C, oven during the gradient programme from 50 °C to 200 °C *(20)*.

Total PCB level was expressed as a sum of the 7 following congeners numbered by IUPAC as: PCB 28, 52, 101, 118, 138, 153 and 180. The limit of detection of individual congeners was 0.0002 mg/kg of fat and 0.002 mg/kg of fat for total PCB content.

Results and Discussion

Residues of Pb, Cd and As were found only in certain samples of milk. Lead concentration, in the majority of samples (98 %), was under detectable limit. The highest concentration of Pb was 0.07 mg/kg. The same was established for Cd and As content, as no samples exceeded detectable limits. The highest concentration of total Hg was 0.007 mg/kg, which represented only a quarter of tolerance. The minimum concentration of total Hg was under detectable limit.

Residues of α-HCH and lindane in all 174 analysed samples of raw cow's milk were far below the maximum residue levels and heptachlor was under the limit of detection (0.003 mg/kg of fat) for all of those samples. 90 % of samples of α-HCH and 86 % of samples of lindane were below the limit of detection (0.003 mg/kg of fat) as well. The highest contents of α-HCH and lindane were 0.008 mg/kg of fat in 1994 and 0.023 mg/kg of fat in 1997, respectively. The situation for total DDT residues was more heterogeneous, and as such it deserves a more detailed analysis. The 5-year results were distributed chronologically into concentration classes (Table 3). In 32.8 % of the samples, total DDT levels were below the limit of detection (0.005 mg/kg of fat), but the majority of the samples (47.1 %) fell in the next concentration class with a concentration of up to 0.01 mg/kg of fat. Only in 3 samples (1.7 %) total DDT did exceed 0.05 mg/kg of fat. The median level over the whole period was 0.007 mg/kg of fat which is only 1.4 % of the level tolerated by law (*3,4*). The maximum level was 0.091 mg/kg of fat, found in 1998 in one sample from a Murska Sobota dairy (region No. 6 in Fig. 1). The main member of the DDT »family« in almost all samples examined was the 4,4'-DDE metabolite.

Total PCB residues in 108 analysed samples of raw cow's milk ranged from <0.002 to 0.14 mg/kg of fat. A maximum was found in 1996 in one sample from the Murska Sobota dairy. The median level over the whole period was 0.005 mg/kg of fat which is only 0.5 % of the level tolerated by law *(3,4)*. A distribution of analysed samples into concentration classes of total PCB residues is presented chronologically in Table 4. The majority of the samples (95.3 %) fell into the lowest concentration class of up to 0.08 mg PCB/kg of fat which is only 8 % of the level tolerated by law.

We were interested in total DDT and total PCB pollution levels of milk according to geographical region

Table 3. Distribution of samples of raw cow's milk analyzed from 1994 to 1998 into concentration classes based on total DDT content

Year	n	Concentration classes of total DDT (mg/kg of fat)				
		<0.005	0.005–0.010	0.011–0.020	0.021–0.050	0.051–0.100
1994	21	12	8	1		
1995	33	15	18			
1996	52	16	20	9	6	1
1997	49	7	28	11	3	
1998	19	7	8	2		2
Total	174	57	82	23	9	3
%		32.8	47.1	13.2	5.2	1.7

Level of total DDT in milk and milk products tolerated = 0.5 mg/kg of fat

Table 4. Distribution of samples of raw cow's milk analyzed from 1994 to 1998 into concentration classes based on total PCB content

Year	n	Concentration classes of total PCB (mg/kg of fat)					
		<0.002	0.002–0.005	0.006–0.010	0.011–0.080	0.081–0.100	0.101–0.300
1994	4	1		3			
1995	11		7		4		
1996	48	1	8	4	30	2	3
1997	26	2	14	6	4		
1998	19	5	11	2	1		
Total	108	9	40	15	39	2	3
%		8.3	37	13.9	36.1	1.9	2.8

Level of total PCB in milk and milk products tolerated = 1.0 mg/kg of fat

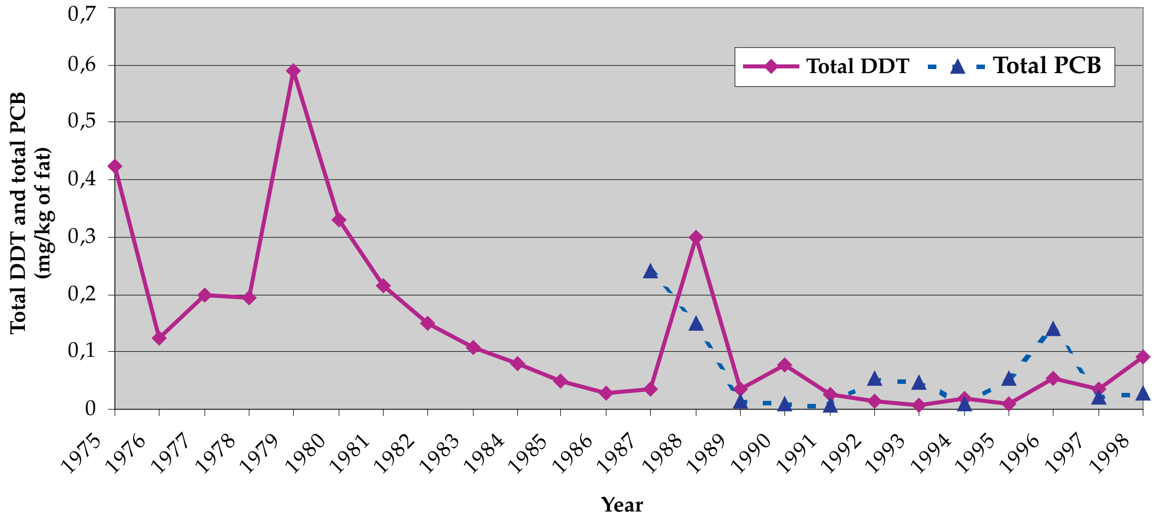

Fig. 2. Maximum annual total DDT and total PCB content in Slovenian raw cow's milk from 1975 to 1998 and from 1987 to 1998, respectively

over the entire period 1994–1998. Three criterion levels were set: samples below detectable limit, median levels and maximum levels.

Primorska (No. 3 in Fig. 1) was the region with the highest percentage of samples (47.8 %) with the level of total DDT below detectable limit, but only one such sample (6.3 %) from the Prekmurska region (No. 6 in Fig. 1) was identified. These two regions had the lowest (0.005 mg/kg of fat) and the highest (0.014 mg/kg of fat) median levels for the entire period, as well. Maximum residue contents ranged from 0.008 mg/kg of fat in the Notranjska region (No. 2 in Fig. 1) to 0.091 mg/kg of fat in the Prekmurska region. Prekmurska was the most polluted region according to all three criteria, which is not surprising because agriculture is carried out most intensively in this part of Slovenia. All levels of total DDT were far below those tolerated by both Slovenia and the EU (Table 1).

In comparing the highest annual residue contents from 1994 to 1998 with earlier results found since 1975 when recording began, a rapid decline can be observed for total DDT and other organochlorine pesticides in food of animal origin (Fig. 2) as a consequence of their prohibition or restriction in 1972 *(21)*. These findings agree with the results from Austria *(22)* and Italy *(23)*, two neighbouring countries where significant decreases in most organochlorine pesticide residues were observed as well. However, because of the longevity of these compounds in the environment, their residues are still found in food. As it can be seen in Fig. 2, in the last several years maximum levels of total DDT content in raw cow's milk have been increasing slightly again, although the median level for individual years has remained <0.010 mg/kg of fat. Such slightly elevated annual maximum levels in recent years need to be followed up carefully to determine if there are any possible residue problems. Residue monitoring programms in European countries have already shown residue problems related to the presence of organochlorine pesticides. For example, in the year 1995, unexpectedly high levels of lindane in milk were detected in the United Kingdom *(24)*. Recently we have also observed highly elevated levels of 4,4'-DDE in food of animal origin which has mostly been imported from the east European countries *(25)*.

Prekmurska and Notranjska regions (No. 6 and 2 in Fig. 1) had the lowest (0.004 mg/kg of fat) and the highest (0.017 mg/kg of fat) median levels, respectively of PCBs for the entire five-year period. The range of maximum levels of total PCB residues was from the same regions as the levels of total DDT. They ranged from 0.074 mg/kg of fat in Notranjska region to 0.140 mg/kg of fat in the Prekmurska region (Fig. 1). Maximum annual levels of total PCB content in Slovenian raw cow's milk from 1987 to 1998 are presented in Fig. 2.

Conclusions

Residues of environmental pollutants examined in Slovenian raw cow's milk were not problematic from 1994 to 1998. Amounts of the trace elements, such as Pb in 188 samples, Cd in 133 samples, As in 67 samples and total Hg in 48 samples, were mostly below the detectable limits or found only in particular samples similar to what was found previously. Residues of organochlorine pesticides in 174 samples and PCBs in 108 samples were all below tolerated levels, too. The median and the highest content levels for the entire period were for α-HCH <0.003 and 0.008 mg/kg of fat, respectively, for lindane <0.003 and 0.023 mg/kg of fat, respectively, for total DDT 0.007 and 0.091 mg/kg of fat, respectively and for total PCB 0.005 and 0.14 mg/kg of fat, respectively. Prekmurska, the north-eastern part of Slovenia, had the highest median and maximum levels of total DDT content in the milk samples analyzed because of its intensive agriculture.

Acknowledgements

The authors thank prof. dr. Marina Komar, univ. dipl. chem. and Blaž Repše, univ. dipl. ing. chem. techn. for performing part of organochlorine pesticide and PCB analyses, respectively. We also thank Ester Rotter, univ. dipl. chem. for performing analyses of total Hg.

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Utvrđivanje elemenata u tragovima, organokloriranih pesticida i polikloriranih bifenila u mlijeku krava s područja Slovenije

Sažetak

U razdoblju od 1994. do 1998. godine ispitani su elementi u tragovima u uzorcima svježeg kravljeg mlijeka iz 19 mljekara. Udjel olova u svih 188 uzoraka odgovarao je razini tolerancije (0,1 mg/kg), a u 98 % uzoraka bio je ispod 0,05 mg/kg. Isto tako su bili prihvatljivi svi uzorci u kojima su ispitani ostaci Cd (133), As (67) i ukupni Hg (48). Ostaci Cd i As bili su ispod razine tolerancije, dok je najviša vrijednost za ukupni Hg iznosila 0,007 mg/kg. Količina organokloriranih pesticida u sva 174 analizirana uzorka bila je kudikamo ispod granice tolerancije. α-HCH (u 90 % uzoraka) i lindan (u 86 % uzoraka) bili su ispod granice detekcije (0,003 mg/kg masti). Najveća količina α-HCH iznosila je 0,008 mg/kg masti, a lindana 0,023 mg/kg masti. Udjel heptaklora bio je ispod granice detekcije (0,003 mg/kg masti). U 93,1 % uzoraka ukupna količina ostataka DDT-ija iznosila je od $<0,005$ do 0,02 mg/kg masti, dok su srednje i najviše vrijednosti bile 0,007 i 0,091 mg/kg masti. U 108 uzoraka ispitani su ostaci PCB, a najveća količina iznosila je 0,14 mg ukupnog PCB/kg masti. Srednja vrijednost iznosila je 0,005 mg ukupnog PCB/kg masti, što je 200 puta manje od granice tolerancije. U 87 % uzoraka nađeno je od 0,002 do 0,08 mg PCB/kg masti.