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Chlorinated hydrocarbons residues in milk fat of selected farm animals from the north-eastern part of Poland

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Abstract

The aim of the study was to determine and compare the concentrations of chlorinated hydrocarbons residues (DDT, DDE, DDD, γ-HCH) in the milk fat of selected species of farm animals. The experiment was carried out on cow's, sheep's, goat's, and mare's milk samples originating from different parts of north-eastern Poland. The samples were prepared using Röse-Gottlieb's and Ludwicki's methods. The determination of the compounds was conducted with gas chromatography. All tested samples contained the residues of chlorinated hydrocarbons. The results varied depending on the animal species as well as the places of sample collections. The highest content of γ-HCH and ΣDDT was determined in cow's milk (22.75; 53.12 µg/kg of fat, respectively). The lowest level of γ-HCH and ΣDDT was observed in sheep's milk (0.25; 5.94 µg/kg of fat, respectively). The content of chlorinated hydrocarbons did not exceed the maximum acceptable levels of these compounds.

Key words: cow, sheep, goat, mare, milk, organochlorine compounds.

Introduction

The residues of chlorinated hydrocarbons like bis(4-chlorophenyl)-1,1,1-trichloroethane (DDT), and lindane (γ-HCH) in the natural environment still constitute a current problem. Despite the fact that pesticides containing synthetic chloroorganic compounds are no longer used in Europe, their presence is still found in food especially of animal origin (10, 17, 21). In Poland, preparations containing DDT were removed from production in 1976; however, they still have been in use in some countries as the most effective formula in combating flies spreading malaria, as well as in protecting crops of tobacco and cotton. The results of the research carried out confirm that even low contents of these persistent compounds lead to their accumulation in human and animal organisms (2, 22, 27). The use of lindane as an active substance of plant protection products has been banned in Europe in 1980/90. There are also well-known reports about the highly harmful influence of these compounds on health. Chlorinated hydrocarbons like γ-HCH and DDT, as well as their metabolites are considered to have oestrogenic, mutagenic, and cancerogenic activity (3, 7, 23, 24). These compounds, by penetrating the organism, stimulate the activity of oestrogens produced in the organism, or change hormone activity (9, 26). Chloroorganic hydrocarbons are a group of xenobiotics of particular toxicological importance, which due to their lipophilic character and persistency in the environment are dangerous to human and animal health (25, 28). Food is a source of harmful substances. Among many food products, there are products of animal origin, which are indicated to be the main source of chemical residues and contaminants including organochlorine compounds (4, 12, 13, 15). Due to the toxic effects of the organochlorine compounds, standards for maximum residue levels (MRL) in food were introduced. As far as cow's milk is concerned, MRL for γ-HCH is 0.001 mg/kg of milk and for ΣDDT - 0.04 mg/kg of milk (8). Nowadays, milk is a very popular component of human's everyday diet. It plays an important role in human nutrition all over the world. In Poland, like in other European countries, milk is obtained mainly from cows and in a lesser degree from sheep and goats (11). In turn, mare's milk begins to appear on the market in certain countries of Western Europe as food with pro-healthy properties, recommended especially for people with allergic problems (5).

The aim of the study was to determine and compare the concentrations of chlorinated hydrocarbons residues (DDT, DDE, DDD, γ-HCH) in the milk fat of selected species of farm animals, collected in 2010 from different parts of the north-eastern Poland.

Material and Methods

Material. The experiment was carried out on 30 samples of cow's (Holstein-Friesian breed), 12 – sheep's (Kamieniecka breed), 10 – goat's (White Goat breed), and 20 – mare's (Polish Cold-blooded breed) milk originating from different parts of North-Eastern Poland (Fig. 1). The samples were collected from individual producers: cow's milk from four (A, B, C, D); goat's milk from three (E, F, G); mare's milk from three (H, I, J), and sheep's milk from one (K) different places, from April to June 2010. The samples were collected and prepared according to Polish Norm PN-EN ISO 707:2009 (20). Before the analysis, the samples were stored in a freezer (-30°C).

Analytical methods. Milk fat was extracted by Röse-Gottlieb's method (19). The method involves mixing the milk sample with ammonia, alcohol, diethyl ether, and petroleum ether. After separation and distillation of the ether fractions, the fat was obtained. In order to determine chlorinated hydrocarbons residues, the samples of extracted fat were prepared by the Ludwicki's method, which involves decomposition of lipids by concentrated sulphuric acid, and releasing organic insecticides to the hexane layer (14).

Gas-chromatographic analysis. The separation and quantitative determination of DDT, DDE, DDD, and γ-HCH were conducted with gas chromatography using an electron capture detector – ECD, under the following conditions: a 6890N Agilent Technologies gas chromatograph equipped in a capilar column (25 m × 0.32 mm) filled with a liquid phase: DB-1701 P. Film thickness was 0.25 µm, separation temperatures were: 280°C for the detector, 250°C for the injector, and 200°C for the column. Helium was used as the carrier gas supplied at a flow 2.5 cm^3/min.

The content of chlorinated hydrocarbons residues was calculated by comparing the peak areas of the samples examined with that of the applied standard using an Unicam 4880 computer software. The separation was conducted on the basis of reference material (Supelco – Pesticide Std. Mix A-1; B-1, cat. no. 47977; 47978), which was previously prepared in n-hexane in a concentration of 0.1 µg/cm^3. The obtained results were mean values of three parallel determinations and were expressed as µg/kg fat.

Statistical analysis. Statistical analyses including calculation of the mean value (X) and standard deviations (SD) were conducted using Microsoft Excel software. The significance of difference of the mean values between the samples of milk collected from different parts of the region and originating from selected species of animals was determined with non-parametric Kruskal-Wallis test (analysis at the level of significance of $P \leq 0.05$) using Statistica 9.0 PL.

Results

The content of chlorinated hydrocarbons residues in tested milk samples expressed as a mean value and standard deviation is presented in Table 1.

Chlorinated hydrocarbons were found in all samples of milk. It was determined that the content of γ-HCH, DD, DDE, DDD varied within one kind of milk originating from different regions, and milk of different species of animals.

The average content of γ-HCH was statistically different among all species of the animals. The highest content of this compound was determined in cow's milk (9.15 µg/kg of fat) and the lowest- in sheep's milk (0.25 µg/kg of fat).

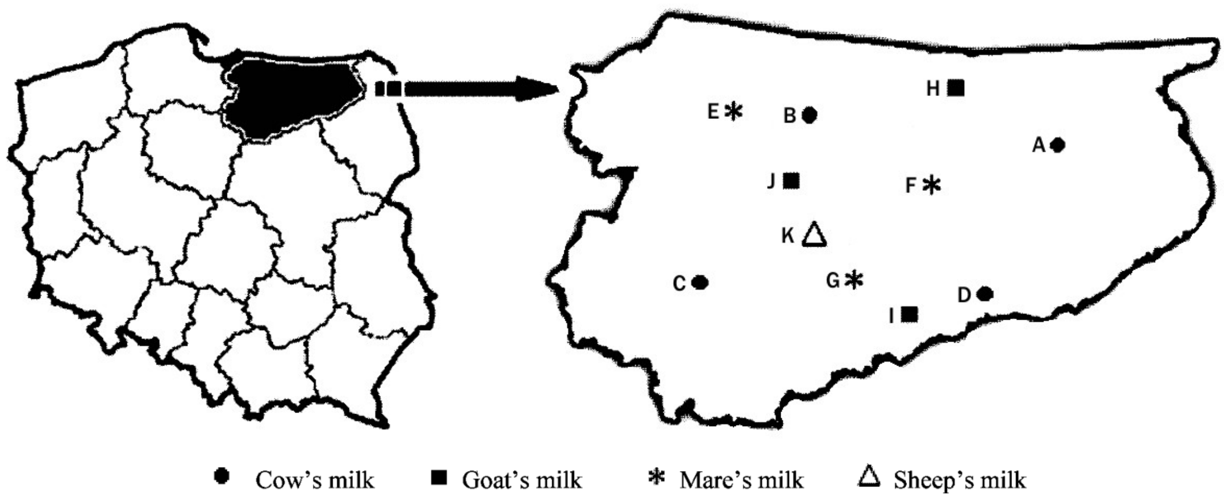

Fig. 1. Areas of collecting milk samples

Table 1. The content of chlorinated hydrocarbons in milk fat of selected farm animals from the north-eastern Poland (μg/kg of fat) (X ± SD)

Material		n	γ-HCH	DDE	DDD	DDT	ΣDDT
Cow's milk	A	6	22.75 ± 6.33	43.32 ± 14.28	2.43 ± 0.83	7.37 ± 3.25	53.12 ± 17.07
n = 30	B	8	8.91 ± 1.21	7.55 ± 0.55	0.81 ± 0.13	0.00 ± 0.00	8.36 ± 0.57
	C	10	8.25 ± 1.72	20.18 ± 3.99	0.74 ± 0.21	0.00 ± 0.00	20.92 ± 4.12
	D	6	2.92 ± 1.01	5.42 ± 1.64	0.67 ± 0.16	0.00 ± 0.00	6.09 ± 1.67
mean			9.15 ± 7.91 a	16.38 ± 14.01a	1.06 ± 0.79 a	1.47 ± 3.29 b	18.92 ± 19.7 a
Goat's milk	E	4	9.51 ± 0.73	9.45 ± 1.27	2.36 ± 0.92	6.37 ± 2.22	18.18 ± 4.08
n = 10	F	3	14.51 ± 4.15	11.16 ± 2.30	3.14 ± 0.71	3.09 ± 0.44	17.39 ± 2.81
	G	3	1.67 ± 0.28	3.29 ± 0.21	1.52 ± 0.48	5.79 ± 1.19	10.61 ± 0.97
mean			8.80 ± 5.14 a	8.34 ± 3.39 a	2.34 ± 0.93 a	5.41 ± 2.13 b	16.09 ± 4.08 a
Mare's milk	H	5	2.90 ± 0.55	11.78 ± 2.52	0.55 ± 0.12	18.04 ± 2.83	30.37 ± 5.47
n = 20	I	8	4.22 ± 1.01	1.96 ± 0.57	0.00 ± 0.00	11.14 ± 1.74	13.10 ± 1.87
	J	7	5.57 ± 1.09	5.24 ± 1.15	0.45 ± 0.12	11.64 ± 2.57	17.33 ± 3.52
mean			4.69 ± 1.33 a	4.42 ± 3.15 b	0.26 ± 0.25 b	12.06 ± 2.96 a	16.72 ± 5.86 a
Sheep's milk n = 12	K	12	0.25 ± 0.11 b	1.71 ± 0.27 b	0.92 ± 0.18 a	3.31 ± 0.63 b	5.94 ± 0.85 b

a, b, c - the significance of difference of the mean values between the samples; P ≤ 0.05;
A, B, C – places of collecting samples; n – number of samples

Table 2. Percentage contribution of DDE, DDD, and DDT to the total sum of DDT in milk fat of selected farm animals (%)

Material		DDE	DDD	DDT
Cow's milk	A	81.56	4.57	13.87
	B	90.31	9.69	0.00
	C	96.46	3.54	0.00
	D	89.00	11.00	0.00
mean		89.33	7.20	3.48
Goat's milk	E	51.98	12.98	35.04
	F	64.18	18.06	17.76
	G	31.04	14.34	54.62
mean		49.06	15.13	35.81
Mare's milk	H	38.79	1.81	59.40
	I	14.96	0.00	85.04
	J	30.24	2.59	67.17
mean		27.99	1.45	70.54
Sheep's milk	K	28.79	15.49	55.79

A, B, C …..K – places of collecting samples

A similar tendency was observed in DDE concentration. Cow's milk was characterised by the highest (16.38 μg/kg of fat) and sheep's milk by the lowest (1.71 μg/kg of fat) mean value of residues of these compounds.

The average value of DDD metabolite was the lowest in mare's milk (0.26 μg/kg of fat) and the highest in goat's milk (2.34 μg/kg of fat); however, as far as DDT is concerned, the trend was reversed (12.06; 3.31 μg/kg of fat).

Sheep's milk was distinguished by the lowest content of ΣDDT (5.94 μg/kg of fat), which differed statistically from other milk samples.

The amount of γ-HCH detected in cow's milk from region A (22.75 μg/kg of fat) was many times higher in comparison with other milk samples. This sample was also characterised by the highest content of DDE (43.32 μg/kg of fat), which was at least twice as high as that in the rest samples. This value influenced the amount of ΣDDT, which also substantially exceeded that in other milks.

Percentage contribution of DDE, DDD, and DDT to the total sum of DDT in milk varied between particular animal species (Table 2).

The highest average percentage contribution of DDE metabolite, compared to milk samples of other species, was found in cow's milk (89.33%). The lowest percentage of a DDE metabolite was found in mare's and sheep's milk (27.99; and 28.79%, respectively). The highest percentage of DDT, compared to the other species of milk samples, was found in mare's milk (70.54%), while the lowest was determined in cow's milk (3.48%).

Discussion

Sheep's and mare's milk displayed a high concentration of DDT in relation to other metabolites included in the ΣDDT. Due to the fact that this compound is a primary form of dichlorodiphenyl-trichloroethane, which degrades over a long period, it can be assumed that the animals might have been kept in an area where pesticide burial sites occurred (1). Furthermore, a number of factors specific to the particular species (including breed, age, individual animal metabolism, lactation period) determining the selectivity of the organism in relation to a particular compound may also be responsible for the high content of DDT. In goat's, and especially in cow's milk, DDT apparently degraded to DDE and DDD metabolites. In our study, cow's milk originating from region A presented interesting case, since the content of detected chlorinated hydrocarbons was much higher than that in cows from other regions. It may suggest that this area has been persistently contaminated with organochlorine compounds.

There were stated statistical differences between the content of ΣDDT in sheep's milk and its concentration in the other species. Significant variations in the obtained values may be the result of diversity in animal species, as well as can be caused by external factors.

Comparing the concentrations of chlorinated hydrocarbons in the same region in previous years (17, 18) a decreasing trend of ΣDDT content in milk of farm animals over the years can be observed. On the basis of the results of research conducted between 1993 and1999, the presence of ΣDDT at the level of 141 µg/kg of fat in mare's and 140 µg/kg of fat in cow's milk samples was determined. The analysis performed between 2000 and 2001 showed the occurrence of ΣDDT at 36.7-96.3 µg/kg of fat of mare's milk (18). Between 1994 and 1998 in Slovenia, 93.1% of cow's milk samples were characterised by the levels of DDT at 5.0–20.0 µg/kg of fat (6). The content of DDT in cow's milk samples collected in 2003 from tropical regions of Mexico, where the chemicals are commonly used in animals breeding, ranged from 89.0 to 146.0 µg/kg of fat. The average level of DDE in the same milk samples depending on the region was: 39.0 µg/kg, 18.0 µg/kg, 24.0 µg/kg of fat (16).

In the study conducted by Flores *et al.* (11) in Mexico in goat's milk, ΣDDT at 66.14 µg/kg of fat was found. At the same milk, γ-HCH was at the level of 37.7 µg/kg of fat. In our study, the content of this compound was many times lower. Comparing the results of γ-HCH content in the milk of mares received between n 2000 and 2001 (6.7-15.1 µg/kg of fat) (18) with those from our study (2.9-5.57 µg/kg of fat) a downward trend was also observed.

Although chlorinated hydrocarbons have been removed from the production and use in the 70's, they are still detected in the ecosystem and food mainly of animal origin. It is observed that the content of organochlorine insecticides in the environment has been decreasing over time; however, people are still exposed to the absorption of these compounds from food and their accumulation in the body. Therefore, it is important to monitor and control food quality.

To conclude, the presence of chlorinated hydrocarbons was detected in all tested milk samples, which confirms their common existence in the environment and foods of animal origin. Nevertheless, the content of these compounds has shown a decreasing trend over the years. All samples of sheep's milk were characterised by the lowest content of chlorinated hydrocarbons, which may increase the popularity of this milk on the market. The content of ΣDDT and γ-HCH in cow's, goat's, sheep's, and mare's milk did not exceed the maximum acceptable levels of residues of these compounds in milk set up by Commission Regulation (8).

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