

## Influence of the seasonal and compositional changes on 4,4'-dichlorodiphenyltrichloroethane (DDT) contamination in cow's milk

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The article presents the results of investigations on the determination of the content of organochlorine pesticides and DDT in the milk samples obtained in some settlements (village Movchanivka, Leninske, Mali Lisivtsi, Velikopolovetskye, and Pustovarivka), Skvirsky district, Kyiv oblast. The analysis of fat and protein content in milk in the winter and summer periods of the year. We registered that in the winter period with an increase of fat content in the milk, the concentration of DDT and its derivatives also increases. The content of organochlorine compounds in cow's milk did not exceed the maximum permissible level. In case of prolonged inflow of pesticide residues with food products into the human organism or feed into the animal organism, toxic substances gradually accumulate in them and have a negative influence on different functional systems of organisms, causing disturbances in their work. Purification from harmful substances is carried out by metabolic conversion of toxicants into more mobile in tissues compounds that can be more easily removed from the body. The process of removal of harmful substances, including metabolites of pesticides from the body warm-blooded with biological fluids and fecal masses is very long, and its intensity is mostly due to the degree of toxic load of xenobiotics on a person or animal. An indicator of the degree and quality of toxic exposure of humans and domestic animals to pesticides that live in a particular agricultural region may be the levels of these pesticides in human and warm-blooded milk. However, the most convenient and affordable indicator of pesticide exposure to organisms may be found in cattle milk, primarily cow milk. The great advantage of this object of toxic load indication is the unlimited possibility of biomaterial selection and deficiency of volumes, necessary for sanitary and hygienic researches because herds of cattle are almost in every farm of different agricultural regions.

**Keywords:** pesticides, DDT (4,4'-dichlorodiphenyltrichloroethane), cattle, cow's milk, accumulation.

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### Introduction

Milk and dairy products take an important place in the human diet. The quality of cow's milk, and products made from it, are the important component of the human diet. Therefore, the content of harmful substances in milk (e.g., dichlorodiphenyltrichloromethyl methane (DDT) and its derivatives and hexachlorocyclohexane (HCG)) in concentrations above the maximum residues level (MRL) can cause health deterioration. Sanitary and hygienic control over compliance with the MRL of pesticides in foodstuffs allows reducing the impact of organochlorine pesticides (OCPs) on the population.

The peculiarity of CHPs is extreme stability in environmental objects: they practically do not decompose under the influence of temperature, moisture, and other environmental factors. This group of pesticides has a pronounced cumulative ability in the fatty tissues of living organisms, cytogenetic activity. Besides, it is known that DDT decomposes with the formation of dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD) metabolites, which are also toxic and even more stable substances in the environment and are classified as "possible" carcinogens for humans (Agroecological, 2005; Moklyachuk, 2008). The use of pesticides as chemical means of plant protection from pests in field crop rotation plots, as a rule, leads to accumulation of toxic residues in soil, further migration to environmental objects, and their second entry to plants, determines the content of residual pesticides in finished agricultural products (Moklyachuk LI, 2008; State sanitary, 2001; Lunev, 1992).

Some plant products are used by humans as food products or are raw materials for their production, and the other - as feed for farm animals. Animals' consumption of fodder containing OCP residues and human consumption of contaminated food products of plant and animal origin is the main source of toxic substances in their organisms. When pesticide residues from food or animal feed enter the human body over a long period, toxic substances gradually accumulate in them and cause adverse effects on various functional systems of the body, causing disruption (Toxicology, 1986; Klisenko et al., 1994).

The studies aimed to study the chemical composition of milk from cows kept in the Svirsky district and to establish the content of DDT and its derivatives.

## Materials and methods

The research was conducted in the Laboratory of Quality and Safety of Agricultural Products of the National University of Bioresources and Nature Management of Ukraine. Milk samples were taken in villages Movchanivka, Leninske, Mali Lisivtsi, Velikopolovetske, and Pustovarivka (Kiyv oblast, Ukraine).

The content of DDT and its derivatives were determined by gas-liquid chromatography with Crystal Lux 4000, using a Zebron ZB-1 capillary GC column (Phenomenex, Inc., USA). We used method described in Antonov (1989) for sample preparation and analysis. The external standard was DZSU (manufacturer of SKTB from OP FHY NANU, Odessa, Ukraine). The content of mass fraction of fat was determined by acid method (GOST 5867-90), the content of protein mass fraction - by refractometric method (GOST 25179-90) using protein analyzer ABM-1 (Milk and dairy products, 1991; Milk, 2009).

## Results

DDT and its derivatives get into the body of cows with feed or during the treatment of their skin are removed from milk for a long time. We determined the mass fraction of fat and protein in milk from cows kept in the villages of Svirsky district, considered that organochlorine pesticides accumulate more intensively in adipose tissue and fatty milk balls (Table 1).

It was found out that in summer the lowest fat contamination in milk was in cows from Leninskoe village, Svirskiy district (Table 1). The highest rate of fat in the milk was in cows from the village of Pustovarivka. The fat content in milk of cows from the village Movchanivka and Mali Lisivtsi was almost at the same level.

In winter (December) the mass fat content in milk increased by 2.2 -3.9% in comparison with summer period. The explanation for this is the decrease in milk yield per cow in winter and the change of diets (replacement of green mass with hay, haylage, and silage).

**Table 1.** Fat and protein content in cow milk,  $M \pm m$ ,  $n = 25$

Place and date of sampling	Fat, %	Protein, %
village Movchanivka (June)	3.41±0.43	3.31±0.21
village Movchanivka (November)	3.52±0.13	3.33±0.30
village Leninske (June)	3.32±0.47	3.08±0.10
village Leninske (November)	3.34±0.29	3.23±0.22
village Mali Lisivtsi (June)	3.42±0.38	3.28±0.19
village Mali Lisivtsi (November)	3.55±0.27	3.29±0.28
village Velikopolovetske (June)	3.38±0.39	3.32±0.30
village Velikopolovetske (November)	3.47±0.17	3.34±0.31
village Pustovarivka (June)	3.51±0.39	3.38±0.22
village Pustovarivka (November)	3.59±0.29	3.40±0.18

The highest mass fraction of protein was in the milk of cows from Pustovarivka village. In other variants, the index was at the level of 3.23-3.32%. Milk obtained in the winter period had insignificant protein content increase in comparison with paired summer milk.

The content of DDT and its derivatives in the milk of cows varies depending on the place of production and time of year. The highest content of DDT in the milk was found in the cows from Movchanivka village. This index was the lowest in milk from Pustovarivka village (Table 2). The content of organochlorine pesticides in milk from village Pustovarivka was 46,8% less than in milk from village Movchanivka.

**Table 2.** Content of DDT and its derivatives in milk of cows (mg/kg,  $M \pm m$ ,  $n = 25$ )

Place and data of sampling)	4,4'-DDE	4,4'-DDT	4,4'-DDT
village Movchanivka (June)	0.020±0.001	0.016±0.0004	0.032±0.001
village Movchanivka (November)	0.023±0.003	0.017±0.001	0.037±0.002
village Leninske (June)	0.032±0.002	0.017±0.001	0.029±0.003
village Leninske (November)	0.034±0.003	0.018±0.001	0.028±0.003
village Mali Lisivtsi (June)	0.021±0.002	0.015±0.001	0.023±0.002
village Mali Lisivtsi (November)	0.029±0.001	0.017±0.001	0.030±0.004
village Velikopolovetske (June)	0.020±0.002	0.010±0.0003	0.018±0.001
village Velikopolovetske (November)	0.31±0.003	0.012±0.001	0.02±0.003
village Pustovarivka (June)	0.019±0.003	0.012±0.001	0.017±0.001
village Pustovarivka (November)	0.021±0.002	0.016±0.002	0.021±0.003

Milk from Velikopolovetske village was also characterized by low DDT content. This indicator was at the level of 0.018 mg/kg. In milk from Svirsky region settlements, DDE and DDT were 0.02-0.034 and 0.01-0.018 mg/kg, respectively.

It should be noted that during the winter period the content of DDT and its derivatives in milk increased. Analyzing the increase of fat content in milk and the increase of pesticide concentration, it was found that in milk received from cows from Leninske village, where the fat content in winter increased by a small difference in the concentration of DDE, DDD increased only by 6.2% and 5.8%, respectively. In the village of Pustovarivka, the fat content of milk in the winter period increased significantly. Concentrations in milk received in December DDE, DDD and DDT increased by 10.5%, 33.3, and 23.5% respectively compared to milk received in July. A similar pattern was found in villages where an increase in fat content was found in milk during the winter period. This can be explained by the fact that DDT and its derivatives are concentrated in lipids, which are excreted with milk in milk fat globules.

Taking into account that protein concentration in milk has not changed much, there is no clear correlation between its content and DDT content in milk. Analyzing the content of DDT and its derivatives in the milk of cows from villages of Svirsky district, it should be noted that the pesticide concentration did not exceed the maximum permissible level.

It is well known that OCP's are extremely stable in the environment and practically is not destroyed by any environmental factors, especially moisture and temperature. Chloro-organic pesticides have a high cumulative capacity in human and animal fatty tissues. The pesticide DDT is known to break down to form the metabolites Dichlorodiphenyldichloroethylene (DDE) and Dichlorodiphenyldichloroethane (DDD), which are more toxic and stable in the environment. When animals consume contaminated feed containing residues of organochlorine pesticides, there is an accumulation of toxicants in the fatty tissues, with prolonged consumption of contaminated products there is a gradual accumulation of toxicants in the human body, which leads to negative effects on various functional systems of the body and disruption of their work. DDT and its derivatives, once in the body of cows with feed, are removed for a long time, including with milk.

Gross violation of sanitary norms is the grazing of cattle near the sources of negative anthropogenic pressure, such as poisonous chemicals warehouses. During the period of the research, the facts of cattle grazing of private farms by the residents of Torchitsa village were repeatedly recorded near a half-destroyed warehouse of toxic chemicals. To determine possible contamination of cow milk with residues of organochlorine pesticides, samples were taken in 5 private farms of the village Torchitsa during 2014 in summer (July) and winter (December) periods. Taking into account the fact that organochlorine pesticides accumulate more intensively in adipose tissue and fatty milk balls, we conducted a study to determine the mass fraction of fat and protein in milk from cows kept in Torchitsa village of Stavishensky region (Fig. 1). Milk samples were taken in 5 private farms in the village of Torchitsa (Fig. 1), where the grazing of cows was recorded near the toxic chemicals warehouse. Previous studies identified the significant levels of soil contamination in the sanitary protection zone of the pesticide depot and found contamination of wild and crops with organochlorine pesticide residues. We found that in all milk samples, a low mass fraction of fat was found in cow milk in summer (July). The fat content varied between 3.7 and 5.2%.

In winter (December), the fat mass fraction in milk increased as compared to summer and reached 4.8-6.0%. This is explained by reduced milk yield in winter and change of diets (replacement of green mass with hay, haylage, and silage) (Fig. 1).

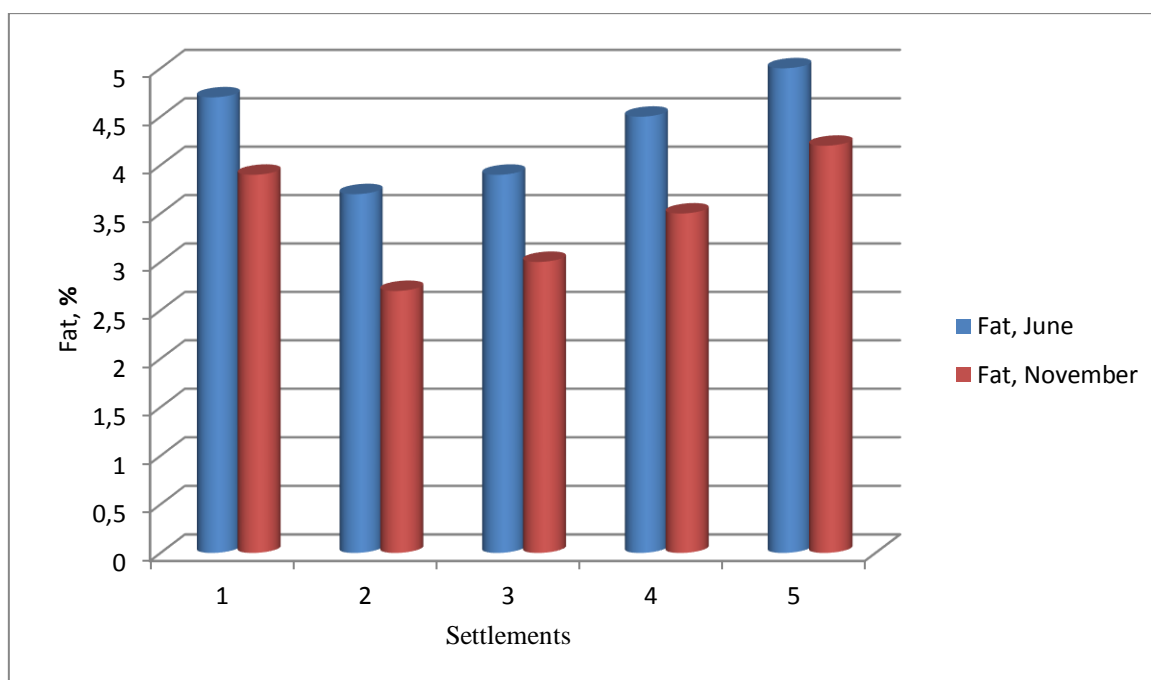


Fig. 1. Fat content in cow's milk, village Torchitsa, Stavishchensky district

We determined the content of the DDT isomers and metabolites in selected milk samples and found out the correlation between fat content in milk and its contamination with residues of organochlorine pesticides (Table 3).

**Table 3.** Content of DDT and its derivatives in cow's milk, village Torchitsa, Stavishensky rayon (July)

Special farms	Contamination				$\Sigma$ DDT, mg/kg
	Fat, %	DDE, mg/kg	DDT, mg/kg	DDD, mg/kg	
1	3.9	0.030	0.021	0.015	0.066±0.007
2	2.7	0.021	0.017	0.013	0.051±0.004
3	3.0	0.025	0.018	0.014	0.057±0.005
4	3.5	0.028	0.020	0.015	0.063±0.006
5	4.2	0.031	0.024	0.020	0.075±0.005

MRL<sub>adult</sub> 0.05 mg/kg, MRL<sub>children</sub> 0.01 mg/kg.

Such a high value of the sum of isomers and metabolites of DDT (0.075 mg/kg), which exceeds MRL by 1.5 times for adults and by 7.5 times for children, was found in the milk sample of private peasant farm No 5 (fat content 4.2%). The lowest content of the sum of isomers and metabolites of DDT (0.051 mg/kg), which corresponds to the level of MRL for adults, but exceeds the established norms for children by 5 times, was found in sample 2 with a fat content of 2.7%.

**Table 4.** Content of DDT and its derivatives in cow's milk, village Torchitsa, Stavishensky Raion (December)

Special farms	Contamination				$\Sigma$ DDT, mg/kg
	Fat, %	DDE, mg/kg	DDT, mg/kg	DDD, mg/kg	
1	4.7	0.0020	0.0016	0.0012	0.0048±0.0004
2	3.7	0.0011	0.0009	0.0004	0.0024±0.0003
3	3.9	0.0024	0.0016	0.0012	0.0052±0.0006
4	4.5	0.0014	0.0010	0.0007	0.0031±0.0003
5	5.0	0.0029	0.0018	0.0016	0.0063±0.0007

MRL<sub>adults</sub> 0.05 gm/kg - adults, MRL<sub>children</sub> - 0.01 mg/kg

Milk samples were taken during the winter period, despite the increase in the percentage of fat, contained residues of organochlorine pesticides in amounts of 0.0024 - 0.0063 mg/kg, did not exceed the established standards (Table 4). This is explained by the stabling conditions of animals and feeding them with OCP-free food. However, in all milk samples were found residual amounts of isomers and metabolites of DDT, which can be explained by the slow removal of toxicants with fatty milk balls accumulated in cows during the summer period. The direct correlation between fat content and organochlorine pesticide content can also be seen in winter milk samples.

## Conclusions

The content of DDT and its derivatives in milk from cows kept in the Svirsky region does not exceed the MRL. With increasing fat content in milk in winter, the concentration of dichlorodiphenyltrichloromethyl methane in it also increases, which confirms the accumulation of the pesticides in milk fat globules. A promising research direction is to reduce the intake of DDT and its derivatives into cow's milk.

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