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DETERMINATON OF PERSISTENT ORGANOCHLORNE PESTICIDES IN MILK BY GC/ECD

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Abstract

Organochlorine insecticides are among the most important organotoxins. Physicochemical properties of these toxins, especially their lipophilicity, facilitate the absorption and storage of these toxins in human and animal bodies. The existence of the residues of these toxins in milk which is one of the most widely used foodstuff containing lipids can be a quantitative and qualitative index for the presence of these toxins in animal bodies.

The goal of this research was to establish presence of seven important organochlorine insecticides in raw milk produced in farms located in three different regions of our country. This milk is used as raw material in dairy industry and it can be found in the msarket. Detection and measurement of toxic substances was achieved using a gas chromatograph with ECD detector. It was calculated dependence in % of OCPs from % fatty of milk, and the applied temperature which was adequate to the same used for milk homogenization and pasteurization.

The quantities of OCP's were determined in three regions of Republic of Macedonia: Pelagonia region, St. Nikole region and Skopje region.

In Pelagonia region, DDT is most present inseticide and its max value was 0.21% and the minimum value 0.17%. In the region of St. Nikole, most dominant inseticide was Heptachlor, in amount of 0.35%. In the Skopje region, it is the similar situation, meaning that is mainly present OCP is Heptachlor 0.17% followed by DDT with 0.16%.

Based on the resultsfrom our investigation we can conclude that the presence of these pesticides in milk and dairy products is minimal, which meet quality requirements.

Key words: *Pesticides, organochlorine pesticides, gas chromatography, dihlor-difenil-trichloroethane, residues, raw milk.*

1. Introduction

Pesticides are large group of chemical substances that are used in agriculture in order to prevent, destroy or control agricultural crops pests, and thus getting higher yields as in the form of food, etc [1]. Organochlorine pesticides (OCPs) are categorized as a group of persistent organic pollutants (POPs) found in soil, food and water, and most of these compounds have been prohibited from use due to their toxic effects (Watson H. [2]). OCPs are characterized by high reliability, low volatility, low polarity, low solubility in water and high solubility in fat (liposolubility) and consequently have the ability for bioaccumulation in fatty tissues (Julijana T. *et al.* [3]). OCPs are resistant to low temperature, chemical degradation and biotransformation in the environment. Decomposition is very slow and they can remain in the environment for a long time. Half-life of different OCPs is different, for ex. from one year for γ-HCH to ten years for toksafen (Willson R. [4]). Pesticides are entering into milk in two ways, directly and indirectly. The direct way is realized after the application of pesticides during plant protection operations. To indirect contamination of milk with pesticides comes through the rotation of persistent pesticides in the food chain. The later is of particular importance from a toxicological aspect, since some pesticides can be found in animal foods 10 or even 20 years after their application (Barlas E. [5]).

World Health Organization (WHO) and Environment Program of United Nations estimates that every year 3

million agricultural workers worldwide in developing countries are facing severe poisoning from pesticides, and about 18,000 of them resulting in death. Codex Alimentarius recommended international standards for maximum residue limits for individual pesticides in food (FAO/WHO [6]).

Pesticides may affect human health when they come into contact with humans by: contact through the skin during pesticides application; inhalation of dust from pesticides sprays; and ingestion - when pesticides are introduced through contaminated food (Bhatia R. *et al.* [7]) .

In this assay examined OCP group includes pesticides that contain chlorine: Lindane (γ -1, 2, 3,4,5,6 - hexachlorocyclohexane or γ-HCH) - $C_6H_6Cl_6$ - synthetically derived chlorinated pesticide, and 2 other stereoisomer (α-HCH, β-HCH) are lindane by- products, characterized by high presence in environment, (U.S.EPA [8]) and (UNEP, POPRC [9]). p, p – DDT [1,1,1-trichloro-2, 2-bis-(p-chlorophenyl) ethylene]- $C_{14}H_9Cl_5$; p, p′- DDE [1,1- dichloro-2,2-bis-(p-chlorophenyl) ethylene] - $C_{14}H_8Cl_4$; p, p′- DDD [1,1- dichloro-2, 2-bis-(p-chlorophenyl) ethane] - $C_{14}H_{10}Cl_4$; p, p′- DDE and p, p′- DDD occur in chemical transformations of p, p -DDT which was first synthetically produced pesticide (Alvarez M. *et al.* [10]), heptachlor - $C_{10}H_5Cl_7$, aldrin - $C_{12}H_8Cl_6$, dieldrin - $C_{12}H_8Cl_6O$, endrin - $C_{12}H_8Cl_6O$ and endosulfan - $C_9H_6Cl_6O_3S$. These OCP$_s$ commonly are used as insecticides, herbicides, etc. (Jan R., *et al.* [11]).

Objectives of this research were:

- Development of methods for determination of OCPs in milk and dairy products by GC, which can be used for routine analysis in laboratory work.
- Determination of the OCPs amounts in raw cow milk from three different regions of our country.
- The impact of present fatt on OCPs in raw milk.
- The influence of temperature on the amounts (w) in % OCPs in raw milk.
- Examination of the relationship between solubility, fatt and temperature on the presence of OCPs in the milk.

2. Materials and Methods

This research has been conducted in order to determine the quantitative and qualitative values of unionized organochlorine pesticides (OCP's) that can be accumulated in milk. The research has been conducted on raw milk from different dairy farms in three regions in Macedonia. Because of the OCP's solubility in the fat, and because one of the milk quality parameters is milk fat, it was necessary to determine the fat content of the milk according to the Gerber method.

The OCP's quantity was determined in milk kept at t = 4 °C for 24 hours, as well as in milk treated at three different temperature intervals at predetermined retention time, which temperatures in the same time are the technological criteria for homogenizing and pasteurizing the milk in the dairy industry:

a) At temperature of 63 - 65 °C for 30 minutes;
b) At temperature of 71 - 74 °C for 15 seconds;
c) At temperature of 89 - 100 °C for 1 second.

Also the determination of OCP's has been conducted in frozen milk kept at -18 °C for 24 hours.

Determination of organochlorine pesticides (OCP's)

The apparatus used for determination of the organochlorine pesticides concentration in milk was a gas chromatograph manufactured by "Agilent technologies", model GC 7890N equipped with adequate software, or more precisely:

1. Gas chromatograph with ECD (electron capture detector)
2. Chromatographic capillary column J&W Scientific High Resolution Chromatography Coiumn DB 1701 30 m (lenth) x 0.32 mm (id) x 0.25 mm (film); Column temperature limit: -20 °C to 280 °C (300 °C)
3. Auto injector, model 7683 B with eight (8) work spaces in a rotor
4. Centrifuge IEC Centra CL2
5. Rota evaporator, model Haildorf Loborota efficient, equipped with suitable vacuum pump.

Working conditions of the gas chromatograph

Injector: 250 °C temperature, injection volume 1µl; carrier gas: helium (He), 65 mL/min, pressure in the injector: 10.341 psi, column: DB-1701, 30 m x 320 µm x 0.25 µm, detector: µECD, 300 °C; temperature program: 70 °C min^{-1} → 20 °C min^{-1} to 180 °C → 10°C min^{-1} to 230 °C maintained 3 min → 5 °C min^{-1} to 270 °C; duration of the analysis: 22.5 min.

Reagents and solvents

Magnesium sulfate puriss. p.a., anhydrous, - Acetonitrile, 99.9% HPLC, Sodium citrate tribasic dehydrate, puriss; - Sodium hydrogencitrate sesquihydrate, 99% (these reagents were purchased fromSigma-Aldrich). Sodium chloride purum p.a.; ≥ 99.5 %; and Sodium sulfate, puriss. p.a.; ≥ 99.9%, anhydrous; - Hexane, ≥ 99.0%, (GC reagents purchased from Fluka), as well as PSA Bonded Silica Supelclean LC-18 (purchesed from Supelco).

Standard substances

Mixture of OCP's, 200 µg/mL in hexane from Sigma-Aldrich contains Lindane, Heptachlor, Aldrin, Dieldrin, Endosulfan, Endrin, and DDT. Working solutions was prepared of OCP's in concentrations of 6µg/mL, 2µg/mL, and 0.8µg/mL.

Method of Gas chromatography

The research was conducted according to the standardized European method EN 15662QuEChERS, U.S.EPA, Multiresidual method for analysis of traces of pesticides using from (Anastassiades M. *et al.* [12]).

Extraction treatment of the sample for analysis

Take 5mL from the sample for analysis and place in a centrifuge cuvette from 50 mL. Add 10 mL acetonitrile and the mixture is well stirred. To this mixture, add 4 g $MgSO_4$, 1g NaCl, 0.5 g Sodium citrate tribasic dehydrate and 0.5g Sodium hydrogencitrate sesquihydrate, stir vigorously and place the cuvette an a centrifuge at 3000 rpm^{-1} for 5 minutes. This results in forming two layers, and from the top layer (acetonitrile extract) take 8 mL and transfer in a 15 mL micro cuvette for centrifugation. To this volume in the micro cuvette add 1.2 g $MgSO_4$, 0.2 g PSA and 0.2 g C18. The content of the micro cuvette is vigorously stirred and is placed in centrifuge at 3000 rpm^{-1} for 5 minutes. After the centrifugation the content of the micro cuvette is filtered through anhydrous Na_2SO_4 and the filtrate is evaporated in a rot evaporator until dry. The dry residue is than reconstituted with 1mL hexane. The prepared solution is further used for chromatographic analysis.

OCP's determination in raw cow's milk from: Pelagonia, St. Nicole and Skopje region

OCP's residues were analyzed during three months. Their quantities were followed and calculated in percent. The OCP's quantities (*w/w %*) in the milk were measured depending of the milk fat content. Also the quantities (*w/w %*) were determined in a thermally treated milk. The regimes of temperature treatment of the milk were as follows: 4 °C for 24 hours, temperature of 63-65 °C for 30 minutes, 71-74 °C for 15 seconds, then temperature of 89-100 °C for 1 second, and milk kept at -18 °C for 24 hours.

The values in Table 1, Table 2 and Table 3 present the average values of three consecutive measurements in sample of milk.

Table 1: Quantity (*w/w%*) of OCP's dependent of the temperature treatment of the milk and the milk fat content for the Pelagonia region for three consecutive months

Name of the pesticide	Month	Milk fat content/%	w/w % at t = 4 °C T = 24 h.	w/w % at t = 63-65 °C T = 30 min.	w/w % at t = 71-74 °C T = 15 sec.	w/w % at t = 89-100 °C T = 1sec.	w/w % at t = - 18 °C T = 24 h.
Lindane	December 2009	3.75	0.09155	0.067374	0.069068	0.065368	0.077814
	January 2010	3.9	0.129818	0.10936	0.07872	0.072886	0.065798
	February 2010	3.9	0.01819	0.082604	0.077726	0.076888	0.074094
	$\bar{x}$	3.85	0.07985267	0.086446	0.07517133	0.071714	0.07256867
	SD	0.07071068	0.04631647	0.01735467	0.00433474	0.00477548	0.00502268
	VAR	2.28828444	0.00146708	0.00094449	0.00081413	0.00073061	0.00074513
Heptachlor	December 2009	3.75	0.10484	0.085778	0.137448	0.084772	0.095396
	January 2010	3.9	0.124228	0.148548	0.194298	0.194506	0.05767
	February 2010	3.9	0.05483	0.299744	0.191186	0.074478	0.080604
	$\bar{x}$	3.85	0.09463267	0.17802333	0.17431067	0.11791867	0.07789
	SD	0.07071068	0.02923654	0.08980334	0.02609679	0.05431824	0.01552068
	VAR	2.28828444	0.00170295	0.00604849	0.00460445	0.00247293	0.00100899

Aldrin	December 2009	3.75	0.022954	0.017792	0.017376	0.014852	0.0205
	January 2010	3.9	0.02813	0.033966	0.021652	0.020318	0.02987
	February 2010	3.9	0.00477	0.028288	0.024054	0.017156	0.022808
	$\bar{x}$	3.85	0.018618	0.026682	0.02102733	0.017442	0.02439267
	SD	0.07071068	0.01001742	0.00669995	0.00276183	0.00224063	0.00398603
	VAR	2.28828444	0.00185279	0.00564641	0.00440507	0.00249623	0.00105082
Dieldrin	December 2009	3.75	0.016802	0.02509	0.022778	0.022936	0.02634
	January 2010	3.9	0.034594	0.03645	0.03281	0.026066	0.026576
	February 2010	3.9	0	0.026812	0.022164	0.025252	0.01857
	$\bar{x}$	3.85	0.017132	0.02945067	0.02591733	0.02475133	0.02382867
	SD	0.07071068	0.01412487	0.00499895	0.00488029	0.00132595	0.00371969
	VAR	2.28828444	0.00166149	0.00477703	0.00371292	0.00211693	0.00093572
Endosulfan	December 2009	3.75	0.015328	0.041292	0.041929	0.035134	0.034319
	January 2010	3.9	0.054846	0.055984	0.041154	0.040151	0.035836
	February 2010	3.9	0.007352	0.038825	0.037814	0.034301	0.031124
	$\bar{x}$	3.85	0.025842	0.045367	0.040299	0.03652867	0.03375967
	SD	0.07071068	0.02076581	0.00757461	0.00178542	0.00258385	0.0019639
	VAR	2.28828444	0.00144509	0.004005	0.00311962	0.00178742	0.0008179
Endrin	December 2009	3.75	0.033378	0.072056	0.064076	0.048014	0.06342
	January 2010	3.9	0.035944	0.06514	0.037968	0.033354	0.052148
	February 2010	3.9	0.0054	0.053034	0.04801	0.043242	0.052724
	$\bar{x}$	3.85	0.02490733	0.06341	0.050018	0.04153667	0.05609733
	SD	0.07071068	0.01383349	0.00786146	0.0107527	0.00610519	0.00518324
	VAR	2.28828444	0.0012723	0.00346756	0.00268893	0.00155404	0.00079146
DDT	December 2009	3.75	0.028754	0.170536	0.183788	0.162452	0.197496
	January 2010	3.9	0.248928	0.2417	0.275446	0.20647	0.304922
	February 2010	3.9	0.04583	0.197758	0.12838	0.1236472	0.135866
	$\bar{x}$	3.85	0.10783733	0.20333133	0.19587133	0.16418973	0.21276133
	SD	0.07071068	0.10000943	0.02931865	0.06064436	0.03383459	0.06985583
	VAR	2.28828444	0.00241943	0.00512403	0.00461016	0.00287384	0.00389866

Table 2: Quantity (*w/w %*) of OCP's dependent of the temperature treatment of the milk and the milk fat content for the St. Nikole region for three consecutive months

Name of the pesticide	Month	Milk fat content/%	w/w % at t = 4 °C T = 24 h.	w/w % at t = 63-65 °C T = 30 min.	w/w % at t = 71-74 °C T = 15 sec.	w/w % at t = 89-100 °C T = 1sec.	w/w % at t = - 18 °C T = 24 h.
Lindane	December 2009	3.8	0.064682	0.087508	0.080874	0.076414	0.075168
	January 2010	4.1	0.084298	0.180498	0.100634	0.086076	0.080132
	February 2010	3.8	0.07017	0.080232	0.06391	0.06438	0.065804
	$\bar{x}$	3.9	0.07305	0.11607933	0.081806	0.07562333	0.07370133
	SD	0.14142136	0.00826308	0.04564763	0.01500699	0.00887498	0.00594061
	VAR	2.27230615	0.00071254	0.00204392	0.00084906	0.00076011	0.00075582
Heptachlor	December 2009	3.8	0.20813	0.511002	0.35604	0.33232	0.27378
	January 2010	4.1	0.166954	0.452758	0.287688	0.194646	0.076452
	February 2010	3.8	0.068766	0.072176	0.079136	0.072562	0.172796
	$\bar{x}$	3.9	0.14795	0.345312	0.24095467	0.19984267	0.17434267
	SD	0.14142136	0.0584605	0.19459453	0.11777651	0.1061094	0.08056624
	VAR	2.27230615	0.00368659	0.02676203	0.01189765	0.00847248	0.00577166
Aldrin	December 2009	3.8	0.016134	0.022652	0.02235	0.019374	0.016672
	January 2010	4.1	0.020114	0.053904	0.043096	0.02432	0.039068
	February 2010	3.8	0.023248	0.029548	0.0207	0.020238	0.019014
	$\bar{x}$	3.9	0.019832	0.035368	0.02871533	0.02131067	0.024918
	SD	0.14142136	0.00291112	0.01340587	0.01019095	0.00215696	0.01005114
	VAR	2.27230615	0.00359798	0.02318406	0.01034095	0.00755289	0.00519667
Dieldrin	December 2009	3.8	0.020264	0.036268	0.02778	0.023892	0.018848
	January 2010	4.1	0.027328	0.043962	0.066956	0.03129	0.041784
	February 2010	3.8	0	0	0	0	0
	$\bar{x}$	3.9	0.015864	0.02674333	0.03157867	0.018394	0.02021067
	SD	0.14142136	0.01158231	0.01916949	0.02746633	0.01335258	0.01708544
	VAR	2.27230615	0.00312491	0.01946068	0.00855973	0.00633989	0.00443066
Endosulfan	December 2009	3.8	0.044324	0.076956	0.046754	0.039636	0.028314
	January 2010	4.1	0.053102	0.098301	0.065244	0.054448	0.034431
	February 2010	3.8	0.027566	0.033337	0.028774	0.028965	0.030015
	$\bar{x}$	3.9	0.041664	0.06953133	0.046924	0.04101633	0.03092
	SD	0.14142136	0.01059335	0.02703608	0.0148893	0.01044908	0.00257794
	VAR	2.27230615	0.00260256	0.01603727	0.00712981	0.00524714	0.00374635

Endrin	December 2009	3.8	0.059088	0.09351	0.07606	0.073714	0.060684
	January 2010	4.1	0.035896	0.08938	0.079886	0.075338	0.041784
	February 2010	3.8	0.04195	0.046546	0.03953	0.04313	0.072512
	$\bar{x}$	3.9	0.04564467	0.07647867	0.06515867	0.06406067	0.05832667
	SD	0.14142136	0.00982192	0.02123264	0.01818939	0.01481506	0.01265491
	VAR	2.27230615	0.0022458	0.01365324	0.00609464	0.0044898	0.00322301
DDT	December 2009	3.8	0.112532	0.081582	0.154618	0.149178	0.193572
	January 2010	4.1	0.226058	0.12171	0.245806	0.245928	0.062244
	February 2010	3.8	0.202264	0.095088	0.192778	0.240722	0.049852
	$\bar{x}$	3.9	0.18028467	0.09946	0.197734	0.21194267	0.10188933
	SD	0.14142136	0.04888324	0.01667133	0.03739193	0.04443218	0.06502653
	VAR	2.27230615	0.00382311	0.01194212	0.00703749	0.00629488	0.00337941

Table 3: Quantity (*w/w%*) of OCP's dependent of the temperature treatment of the milk and the milk fat content for the Skopje region for three consecutive months

Name of the pesticide	Month	Milk fat content/%	w/w % at t = 4 °C 24 hours	w/w % at t = 63-65 °C 30 min.	w/w % at t = 71-74 °C 15 sec.	w/w % at t = 89-100 °C 1sec.	w/w % at t = - 18 °C 24 hours
Lindane	December 2009	4	0.049966	0.056424	0.061324	0.05693	0.068788
	January 2010	3.95	0.05344	0.048684	0.052274	0.06259	0.073086
	February 2010	3.8	0.084854	0.098126	0.098264	0.062024	0.088048
	$\bar{x}$	3.91666667	0.06275333	0.06774467	0.07062067	0.06051467	0.07664067
	SD	0.08498366	0.01569176	0.02171399	0.0198929	0.00254525	0.00825484
	VAR	2.35342048	0.00050211	0.00062191	0.00064917	0.00054156	0.00078914
Heptachlor	December 2009	4	0.119922	0.130748	0.136814	0.124086	0.083128
	January 2010	3.95	0.087312	0.075418	0.078708	0.07296	0.308266
	February 2010	3.8	0.21924	0.114836	0.254618	0.096716	0.11929
	$\bar{x}$	3.91666667	0.142158	0.10700067	0.15671333	0.09792067	0.170228
	SD	0.08498366	0.05610751	0.02325792	0.07318046	0.02088948	0.09871774
	VAR	2.19195787	0.00344004	0.00161861	0.00452038	0.00140428	0.00632819
Aldrin	December 2009	4	0.011456	0.015556	0.015104	0.015302	0.01539
	January 2010	3.95	0.022142	0.012206	0.0218	0.013058	0.019132
	February 2010	3.8	0.031782	0.02843	0.01692	0.017438	0.031756
	$\bar{x}$	3.91666667	0.02179333	0.01873067	0.01794133	0.015266	0.02209267
	SD	0.08498366	0.00830172	0.00699349	0.00282742	0.00178831	0.0070017
	VAR	2.21538038	0.0032117	0.00173332	0.0043319	0.00147406	0.00568082

Dieldrin	December 2009	4	0.035064	0.043774	0.041972	0.059598	0.012558
	January 2010	3.95	0.040096	0.03923	0.014986	0.042868	0.045696
	February 2010	3.8	0.022978	0.03223	0.026138	0.030132	0.029688
	$\bar{x}$	3.91666667	0.03271267	0.03841133	0.02769867	0.04419933	0.029314
	SD	0.08498366	0.00718346	0.00474824	0.01107212	0.01206622	0.01353112
	VAR	2.21171539	0.0026576	0.00144953	0.00359231	0.00121443	0.00468464
Endosulfan	December 2009	4	0.021365	0.022576	0.036138	0.035961	0.067147
	January 2010	3.95	0.019539	0.021406	0.022192	0.023668	0.02998
	February 2010	3.8	0.040736	0.048062	0.036519	0.055278	0.043431
	$\bar{x}$	3.91666667	0.02721333	0.03068133	0.03161633	0.03830233	0.04685267
	SD	0.08498366	0.00959098	0.01229927	0.00666582	0.01301049	0.01536505
	VAR	2.21228288	0.00227691	0.00126046	0.00303132	0.0010505	0.00389398
Endrin	December 2009	4	0.024488	0.027094	0.024184	0.05798	0.031458
	January 2010	3.95	0.03439	0.037858	0.032058	0.037894	0.18762
	February 2010	3.8	0.065048	0.055094	0.067476	0.05135	0.06125
	$\bar{x}$	3.91666667	0.04130867	0.04001533	0.04123933	0.04907467	0.09344267
	SD	0.08498366	0.01726614	0.01153229	0.01882856	0.00835642	0.06769499
	VAR	2.21219486	0.00197514	0.00111447	0.00261363	0.0009534	0.00386719
DDT	December 2009	4	0.03962	0.113258	0.113586	0.046294	0.118056
	January 2010	3.95	0.101254	0.095686	0.108562	0.116554	0.18762
	February 2010	3.8	0.138662	0.098956	0.250782	0.094538	0.089306
	$\bar{x}$	3.91666667	0.09317867	0.10263333	0.15764333	0.08579533	0.13166067
	SD	0.08498366	0.04083493	0.00763046	0.06589091	0.02934215	0.04127328
	VAR	2.21220851	0.00209317	0.0014046	0.00375386	0.00113037	0.00401562

3. Results and Discussion

The data presented graphically in Fig. 1, Fig. 3 and Fig. 5 are showing that solubility of OCP's in fat from milk is different. Fat in all milk samples from three different regions have similarly value range of 3.7 - 4.0%. Also, from all OCP's, Heptachlor, DDT than Lindane are most dissolved in fat, in the samples of treated milk on temperatures of 63 - 65 ºC for 30 minutes, and 71-74 ºC for 15 seconds, while other 4 types of OCP's are minimaly soluble in the milk fat. From Fig. 2, Fig. 4, and Fig. 6 can be seen that presence of OCP's independent of applied temperature. It can be said that sterilization and pasteurization have good influence on OCP's reduction in the milk.

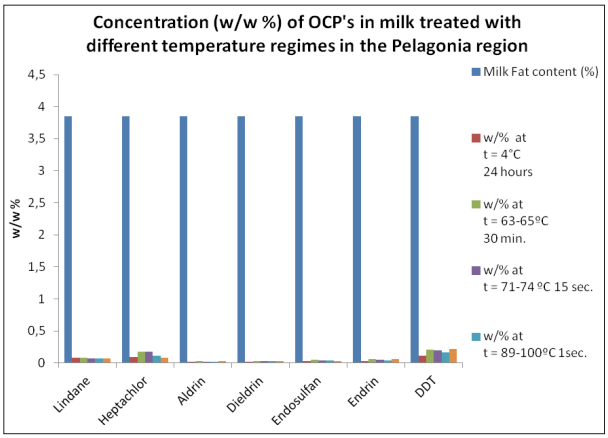

Figure 1. Dependence of OCP's solubility in milk fat from Pelagonia region

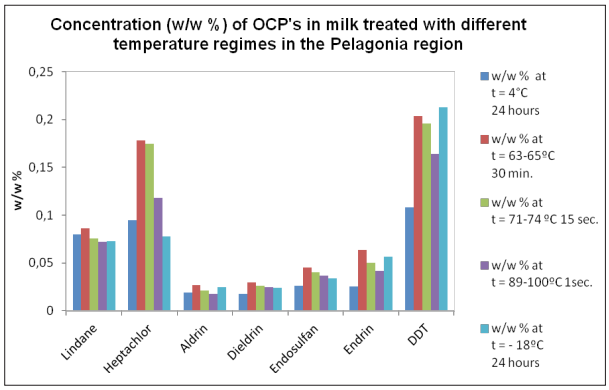

Figure 2. Solubility of OCP's in raw cow's milk from Pelagonia region treated at different temperatures

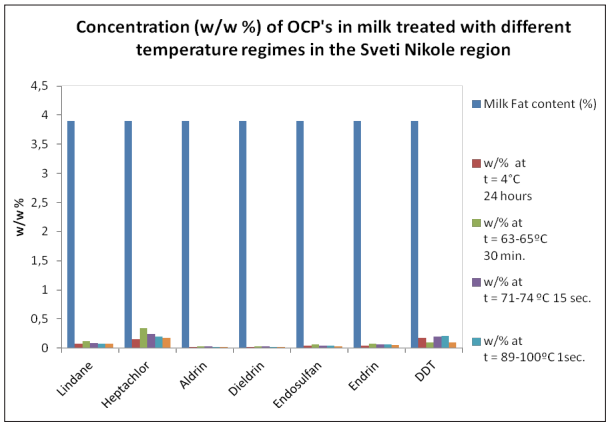

Figure 3. Dependence of OCP's solubility in milk fat from St. Nikole region

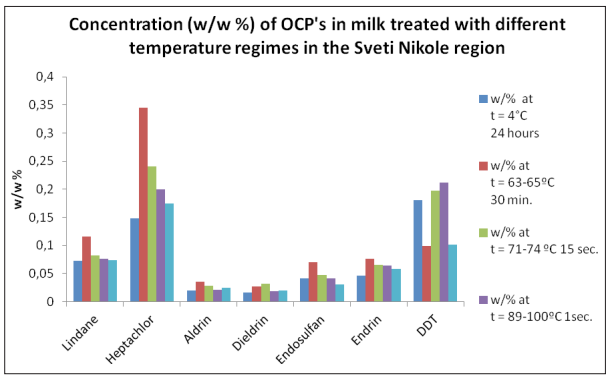

Figure 4. Solubility of OCP's in raw cow's milk from St. Nikole region treated at different temperatures

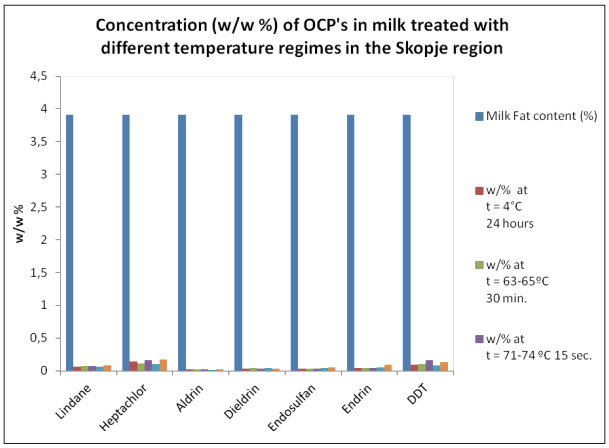

Figure 5. Dependence of OCP's solubility in milk fat from Skopje region

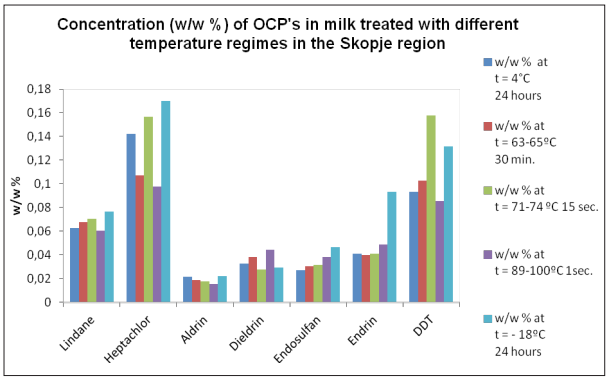

Figure 6. Solubility of OCP's in raw cow's milk from Skopje region treated at different temperatures

In Pelagonia region, DDT is most present OCP in relation with milk fat and all applied temperatures in milk. DDT in the sample of milk which was stored 24 hours at t^0 of 4 ^{0}C has max value 0.21%, and the minimum value 0.17% is found in the sample heated on t^0 from 89-100 ^{0}C for 1 sec. Heptachlor was found in ammount of 0.018% at t^0 of 63-65 ^{0}C and comes second (after DDT). Group of pesticides: Aldrin, Dieldrin, Endosulfan and Endrin, were present in quantities to 0.05%.

In the region St. Nikole, Heptachlor is represented by the largest amount 0.35% in the treatment of milk t^0 from 63-65 ^{0}C during 30 minutes, than in same t^0 comes Linden, and 0.21% of DDT on heated samples t^0 from 89-100 ^{0}C for 1 sec. The presence of pesticides from group of Endosulfan and Endrin are in range of 0.05-0.08%. Aldrin and Dieldrin have presence under 0.05%.

In the region Skopje, from Fig. 6 can be seen that the situation is similar, or that Heptachlor is mainly present 0.17% in milk sample storaed 24 hours at t^0 of 4 ^{0}C. Then comes DDT with 0.16% at t^0 of 71-74 ^{0}C for 15 sec. Group of pesticides: Dieldrin, Endosulfan and Endrin in

this region were present in quantities to 0.054%, and Aldrin in amount of under 0.02%.

4. Conclusions

The results clearly are showing that the amount of applied insecticides should be adjusted to the levels which are not allowing their presence in feed and thus in raw cow's milk.

1. From these tests, we can conclude that in the raw cow's milk from all three regions various different pesticides are present, but it is characteristic that the highest amount of DDT is present in the milk from Pelagonia region, while Heptachlor is present in the milk from Skopje and St. Nikole, and least present is Aldrin.
2. Pesticides from the group Lindane in milk from Pelagonia region in terms of its presence in the fat and all applied temperatures is found in ammount of 2%, except for the milk that was treated with heating on t^0 of 89-100 ^{0}C where their presence is reduced to 1%.
3. The obtained values for the analytical yield R, which is determining the accuracy of Chromatography method within the limits of 98.113599% to 101.83674%, which indicates that the method is quantitative and accurate and can be applied for quantitative purposes.
4. This method gives measurable amounts of OCPs which are present in milk, and their values are not dangerous to human health.

5. References

[1] Food and Agriculture Organization of the United Nations (2002). *International Code of Conduct on the Distribution and Use of Pesticides*. Retrieved on 2007-10-25.

[2] Watson D. H. (ed.) (2004). *Pesticide, veterinary and other residues in food*. Woodhead publishing. ISBN 1-85573-734-5.

[3] Julijana T., Marta N., Katerina S., Stefce P. (2011). *Determination of persistent organochlorine pesticides in drinking water using LLE and GC/ECD*. Journal of Hygienic Engineering and Design, Vol. 1, 109-112.

[4] Willson H. R. (1996). *Pesticide Regulations*. University of Minnesota. Retrieved on 2007, 10-15.

[5] Barlas N. E. (2003). *Determination of organochlorine pesticide residues in agriculture food*. In: Inner Anatolia in Turkey. Bull. Environ. Contam. Toxicology: 69(2) 236-242.

[6] Joint FAO/WHO food standards programme, Codex Alimentarius Commission. (2010). *Report of the twenty-sixth session of the committee on general principles*, Paris, France.

[7] Bhatia R, Shiau R, Petreas M, Weintraub J. M., Farhang L., Eskenazi B. (2005). *Organochlorine pesticides and male genital anomalies in the child and development studies*. Environmental Health Perspectives, 113: 220-4.

[8] U.S.EPA. (2006). Assisment of lindane and other hexachlorocyclohexane isomers. February 8,2006

[9] United Nations Environmental Programme (UNEP) POPRC of the Stockholm Convention (2006). *Draft risk profile: Lindane*.

[10] Alvarez-Pedrerol M., Ribas-Fito N., Torrent M, Carrizo D., Grimalt J. O., Sunyer J. (2008). *Effects of PCBs, p,p´-DDT, p,p´-DDE, HCB and beta-HCH on thyroid function in preschool Children*. Occup Environ Med 65: 452-7.

[11] Jan M.R., Shah J., Khawaja M.A., Gui K. (2008). *DDT residue in soil and water in and around abandoned DDT manufacturing factory*. Environ Monit Assess.

[12] Anastassiades M., Lehotay S. J., Stajnbaher D., Schenck F.J. (2003). *Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce*. J AOAC Int.;86 (2):412-31.