Original scientific article

The organochlorine pesticides in food samples

Monika **MARIĆ**[1], Marina **VUČENOVIĆ**[2], Ingrid **ŠUTIĆ**[1], Aleksandar **BULOG**[2*]

ABSTRACT
Nowadays the concept of food is changed, because food is no longer considered as a source of energy alone, but must provide a sense of pleasure and satisfaction with eating meals. But the unwillingness to develop awareness and raise the concept of food to a higher level, we have to emphasize and warn of the food safety related to chemical hazards. The presence of pesticides in food has a detrimental impact on human health and we tested the presence of organochlorine pesticides in eleven food samples of plant origin, randomly selected in one supermarket in Rijeka. During the sample analysis, the methods of extraction, reextraction, degradation, purification, GPC and GC-ECD were used. Our data indicated the presence of pesticides in the tested food samples, but the level is below the maximum permissible concentration (MPC). According to the results it can be concluded that the analyzed food samples did not have harmful effect on human health.

Key words: food of plant origin, organochlorine pesticides, health care, GPC, GC – ECD

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[1] University of Rijeka, Faculty of Medicine, Braće Branchetta 20, 51000 Rijeka, Croatia

[2] Department of Public Health, University of Rijeka, Faculty of Medicine, Braće Branchetta 20, 51000 Rijeka, Croatia

[*] *Corresponding author*
Assoc. Prof. Aleksandar Bulog, PhD, Bsc Sanitary engineering
Department of Public Health, University of Rijeka, Faculty of Medicine
Braće Branhetta 20, 51000 Rijeka, Croatia
E-mail: aleksandar.bulog@zzjzpgz.hr

INTRODUCTION

The topic that nowadays occupies a great deal of attention in the everyday life of people is food safety. In many European countries food samples contain pesticide residues within legal limits, but continuous monitoring is necessary. Part of the health safety assessment of food is the remains of pesticides whose detection is more precise and accurate today, which allows us to constantly develop technology [1]. Food safety dimensions are: food availability, food access, utilization and stability [2]. Pesticide residues are the result of the use of pesticides in plant protection in veterinary medicine and biocidal products. They include active substances, degradation products or reactants of active substances or their metabolites previously used or currently used in plant protection products. The levels of pesticide residues depend on the amount of pesticide applied, the physicochemical properties of pesticides, the number of applications and the agricultural culture to which it applies [3]. Given that excessive use of pesticides results in their accumulation in the environment as well as in treated herbs, pesticide residue monitoring is carried out. Organochlorine pesticides, pesticides easily enter the food chain due to their lipophilic nature and accumulate in egg yolks, fatty tissue and liver of different animals [4]. Symptoms of acute poisoning with organochlorine pesticides include nausea, vomiting, tremor, dizziness, fatigue, headache, cramping and lethargy, while signs of chronic poisoning are anemia, anorexia, weight loss, skin rash, memory loss, vision problems, insomnia, nervousness , anxiety and muscular weakness [4]. On the other hand, pesticides are essential for food production and human survival, as the speed of life and growth of the world population results in the needs of larger quantities of food. That is why the use of pesticides must be under constant and strict control of science [5]. Since pesticides are applied in agriculture and livestock farming, food of plant and animal origin may contain pesticide residues. If food pesticides do not break down, there is a buildup of a toxic compound in food or a phenomenon of residues of pesticides which are often not destroyed by food preparation [6]. There are three major groups of organochlorine insecticides (Table 1.)

The levels of pesticide residues depend on the amount of pesticide applied, the physicochemical properties of pesticides, the number of applications and the agricultural culture to which it applies.

Table 1. Organochlorine insecticides

Groups of organochlorine insecticides	Types of organochlorine insecticides
Dichlorodiphenylethane	DDT, DDD, DDE, Rotary, Pertane, Methoxychlor, Methochlor, Dikofol
Chlorinated cyclodienes	Endosulfan, Clordan, Endrin, Dieldrin, Aldrin, Heptachlor
Chlorinated benzene and cyclohexanes	Klordekon, HCB, HCH, Mireks, Lindan, Toksafen

One of the ways to combat the excessive use of pesticides today is organic production where pesticide use is prohibited.

In the Republic of Croatia, pesticides are controlled by the Pesticide Sustainable Use Policy (OG 014/2014). It has defined the distribution and sales system, handling and sustainable use of pesticides. It also wants to raise levels of pesticide awareness to reduce the negative effects and risk of pesticide use [7]. One of the ways to combat the excessive use of pesticides today is organic production where pesticide use is prohibited. However, given the fact that pesticides are overwhelmed and found in the air, soil and water, the question is if we really have the possibility of such production [8].

The aim of this paper was to investigate the amount of pesticide by reference methods that provide reliable results in randomly selected foods and to determine whether there is presence of pesticides in quantities greater than MPC as well as their harmfulness to human health.

METHODS

All the samples were collected by the same trained professional person. The food samples were taken from 11 different owners, but every sample has also been taken in three independent pattern. This investigation was a part of regular/state monitoring in the Department of Public Health of Primorsko-Goranska County. All samples were transported by special transport car with refrigerator in which there is a system of constant measurement of the temperature of the cooling chamber. Within this study 11 samples of food of plant origin were analyzed.

They are marked with numbers from 1 to 11 in the following order (Figures 1 and 2).

1. Choco Muesli (K plus)
2. Apple Chips (Velis)
3. Slow chips (K plus)
4. Rice (K plus)
5. Sweet paprika – minced spice pepper (K plus)
6. Wheat white flour – smooth (millet)
7. Swamp Juice Soup (Knorr)
8. Apples and concentrated fruit juice nectar (TO)
9. Pineapple Compounds (K plus)
10. Apple Golden Delis (Moslavina fruit d.o.o.)
11. Pepperoni (Pepperoni Rossi)

These 11 food samples were analyzed for the following pesticides (Table 2).

Figure 1. Mix dry samples.
Sample 1. – Choco Muesli; sample 2. – Apple chips; sample 3. – Slow chips; sample 4. – Rice; sample 5. – Sweet pepper – ground spice pepper; sample 6. – Wheat white flour – smooth; sample 7. – Mushroom soup with raspberries.

Figure 2. Mix wet samples.
Sample 8. – Apples and concentrated fruit juice nectar; sample 9. – Pineapple compote; sample 10. – Apple Gold Delis; sample 11 – Paprika red.

Table 2. Pesticides analyzed in the samples

Organochlorine pesticides				Other pesticides containing chlorine	
No.	Name of pesticide	No.	Name of pesticide	No.	Name of pesticide
1.	alpha – HCH	12.	4,4` – DDE	1.	PCIB
2.	beta – HCH	13.	2,4` – DDT	2.	HCB
3.	linden	14.	endrin	3.	pentachlorophenol
4.	delta – HCH	15.	endosulfanII	4.	vinclozolin
5.	heptachlor	16.	4,4` – DDD	5.	alachlor
6.	aldrin	17.	endrin aldehyde	6.	isodrin
7.	heptachlorepoxide	18.	endosulfan sulfate	7.	captan
8.	gamma – chlordane	19.	4,4` – DDT	8.	tolyfluanid
9.	endosulfan I	20.	endrin ketone	9.	iprodione
10.	alpha – chlordane	21.	methoxychlor		
11.	dieldrin				

The methods used during sample analysis are: extraction, reextraction, degreasing, purification, GPC and GC – ECD. Pesticides were determined according to the norms:

1. HRN EN 12393-1: 2013 (EN 12393-1: 2013) "Food of plant origin – Multi-lingering methods for determination of residues of pesticides by gas chromatography – Part 1: General considerations".

2: Methods for extraction and purification "(EN 12393-1: 2013)" Food of plant origin – Multi-lingering methods for the determination of residues of pesticides using GC or LC-MS / MS".

3. HRN EN 12393-1: 2013 (EN 12393-3: 2013) "Plant origin foods – Multi-lingual methods for the determination of residues of pesticides using GC or LC-MS / MS – Part 3: Determination and Certification" [9].

RESULTS

This paper examines the presence of organochlorine pesticides in 11 randomly selected samples. 21 organochlorine pesticides have been determined and 9 are not organoclorinate but have chlorine. The quantification limit is a parameter in quantitative analysis. It represents the smallest amount of an analyte in a sample that is quantified with precision and accuracy and is determined when the level of analyte concentration is low [10].

Sample 1 – Choco Muesli (K plus) in Table 3.

Table 3. Display results obtained by GC-ECD method in sample 1. organochlorine pesticides (ND-non detected)

Name	Time [Min]	Quantity [mg/kg]	Area [μV. Sec]	Area [μV. SMin]	Height [μV]	Ret. time Offset [Min]	Quantification limits [mg/kg]
alfa-HCH	7.97	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
beta-HCH	8.91	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
lindan	9.20	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
delta-HCH	10.11	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
heptaklor	12.28	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
aldrin	13.89	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
heptaklorepoksid	15.89	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
gama-klordan	17.11	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
endosulfan I	17.71	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
alfa-klordan	17.92	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
dieldrin	19.09	0.0017	241533.5	4025.6	32658.7	0.01	0,004
4,4'-DDE	19.32	0.0001	15892.8	0264.9	2969.4	0.01	0,004
2,4'-DDT	19.71	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
endrin	20.15	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
endosulfan II	20.68	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
4,4'-DDD	21.49	0.0006	63350.5	1055.8	9803.1	-0.03	0,004
endrin aldehid	21.69	0.0006	52765.5	879.4	8730.0	-0.04	0,004
endosulfan sulfat	23.03	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
4,4'-DDT	23.32	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
endrin keton	25.41	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
metoksiklor	26.94	N.D.	N.D.	N.D.	N.D.	N.D.	0,020
Total	-	**0.0031**	**61867367.7**	**1031122.8**	**2770873.8**		

Table 4. Display results obtained by GC-ECD method in sample 1. Other pesticides containing chlorine (ND-non detected)

Name	Time [Min]	Quantity [mg/kg]	Area [μV. Sec]	Area [μV. SMin]	Height [μV]	Ret. time Offset [Min]	Quantification limits [mg/kg]
PCIB	5.28	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
HCB	8.26	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
pentaklorfenol	9.13	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
vinelozolin	12.15	0.0003	18324.8	305.4	3929.0	0.05	0,002
alaklor	12.46	N.D.	N.D.	N.D.	N.D.	N.D.	0,005
izodrin	15.25	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
captan	16.28	0.0012	8108.0	135.1	2539.0	0.02	0,020
tolilfluanid	16.34	N.D.	N.D.	N.D.	N.D.	N.D.	0,020
iprodion	26.14	N.D.	N.D.	N.D.	N.D.	N.D.	0,010
Total	-	**0.0016**	**61222567.3**	**1020376.1**	**2505895.0**		

Sample 2 – Apple Chips (Velis)

Table 5. Display results obtained by GC-ECD method in sample 2. Organochlorine pesticides (ND-non detected)

Name	Time [Min]	Quantity [mg/kg]	Area [μV. Sec]	Area [μV. SMin]	Height [μV]	Ret. time Offset [Min]	Quantification limits [mg/kg]
alfa-HCH	7.96	0.0001	13294.5	221.6	1517.8	-0.01	0,002
beta-HCH	8.91	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
lindan	9.20	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
delta-HCH	10.11	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
heptaklor	12.28	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
aldrin	13.92	0.0014	213494.8	3558.2	19941.9	0.03	0,002
heptaklorepoksid	15.89	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
gama-klordan	17.11	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
endosulfan I	17.71	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
alfa-klordan	17.92	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
dieldrin	19.08	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
4,4'-DDE	19.31	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
2,4'-DDT	19.71	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
endrin	20.15	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
endosulfan II	20.72	0.0024	0.0024	255938.4	4265.6	50953.5	0,004
4,4'-DDD	21.52	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
endrin aldehid	21.73	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
endosulfan sulfat	23.03	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
4,4'-DDT	23.32	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
endrin keton	25.41	0.0002	14584.8	243.1	3677.1	0.00	0,004
metoksiklor	26.94	N.D.	N.D.	N.D.	N.D.	N.D.	0,020
Total	-	**0.0040**	**61867367.7**	**1031122.8**	**2770873.8**		

Table 6. Display results obtained by GC-ECD method in sample 2. Other pesticides containing chlorine (ND-non detected)

Name	Time [Min]	Quantity [mg/kg]	Area [µV. Sec]	Area [µV. SMin]	Height [µV]	Ret. time Offset [Min]	Quantification limits [mg/kg]
PCIB	5.28	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
HCB	8.26	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
pentaklorfenol	9.13	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
vinelozolin	12.10	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
alaklor	12.46	N.D.	N.D.	N.D.	N.D.	N.D.	0,005
izodrin	15.25	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
captan	16.26	N.D.	N.D.	N.D.	N.D.	N.D.	0,020
tolilfluanid	16.34	N.D.	N.D.	N.D.	N.D.	N.D.	0,020
iprodion	26.14	N.D.	N.D.	N.D.	N.D.	N.D.	0,010
Total	-	**0.0000**	**11172514.5**	**186208.6**	**1913786.0**		

Sample 3 – Slow Chips (K plus)

Table 7. Display results obtained by GC-ECD method in sample 3. Organochlorine pesticides (ND-non detected)

Name	Time [Min]	Quantity [mg/kg]	Area [µV. Sec]	Area [µV. SMin]	Height [µV]	Ret. time Offset [Min]	Quantification limits [mg/kg]
alfa-HCH	7.97	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
beta-HCH	8.91	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
lindan	9.20	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
delta-HCH	10.11	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
heptaklor	12.28	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
aldrin	13.89	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
heptaklorepoksid	15.89	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
gama-klordan	17.11	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
endosulfan I	17.71	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
alfa-klordan	17.92	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
dieldrin	19.09	0.0002	9753.7	162.6	3727.2	0.01	0,004
4,4'-DDE	19.34	0.0004	22587.3	376.5	4810.2	0.03	0,004
2,4'-DDT	19.71	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
endrin	20.15	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
endosulfan II	20.68	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
4,4'-DDD	21.52	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
endrin aldehid	21.73	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
endosulfan sulfat	23.03	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
4,4'-DDT	23.32	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
endrin keton	25.41	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
metoksiklor	26.94	N.D.	N.D.	N.D.	N.D.	N.D.	0,020
Total	-	**0.0006**	**58137688.9**	**968961.5**	**1690362.6**		

Table 8. Display results obtained by GC-ECD method in sample 3. Other pesticides containing chlorine (ND-non detected)

Name	Time [Min]	Quantity [mg/kg]	Area [µV. Sec]	Area [µV. SMin]	Height [µV]	Ret. time Offset [Min]	Quantification limits [mg/kg]
PCIB	5.28	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
HCB	8.26	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
pentaklorfenol	9.13	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
vinelozolin	12.10	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
alaklor	12.46	N.D.	N.D.	N.D.	N.D.	N.D.	0,005
izodrin	15.25	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
captan	16.26	N.D.	N.D.	N.D.	N.D.	N.D.	0,020
tolilfluanid	16.34	N.D.	N.D.	N.D.	N.D.	N.D.	0,020
iprodion	26.14	N.D.	N.D.	N.D.	N.D.	N.D.	0,010
Total	-	0.0000	58985425.2	983090.4	1445137.7		

The values that deviate but do not exceed MPC are 2, 4'-DDT, 4, 4'-DDT and vinclozolin. Derived values (2, 4'-DDT and 4, 4`-DDT) were found in samples 4 and 6 (sample 4 – Rice, sample 6 – Wheat white flour – smooth) while the vinclozoline elevated value was found in the sample 8 (Apple nectar and concentrated fruit juice). Therefore, the calibration directions (Figures 3, 4) and their values of computer curves are shown.

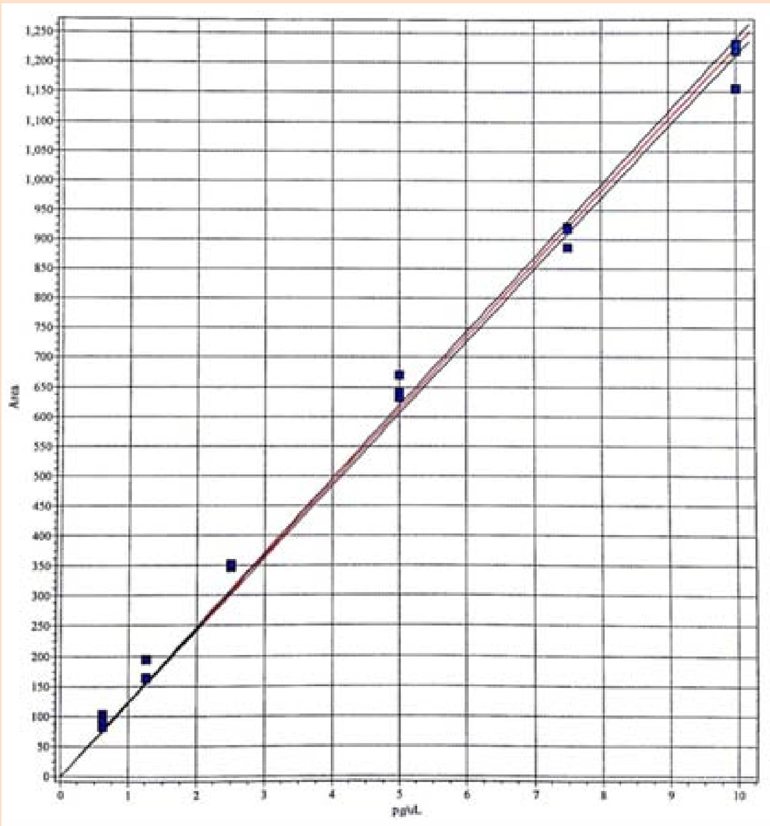


Figure 3.
The dependence of the concentration c (pg µL) on the surface of the sample point 2.4 & gt; DDT.

Figure 4.
Graphic representation of the concentration dependence of c (pg/μL) on the surface of the sample point 4,4-DDT.

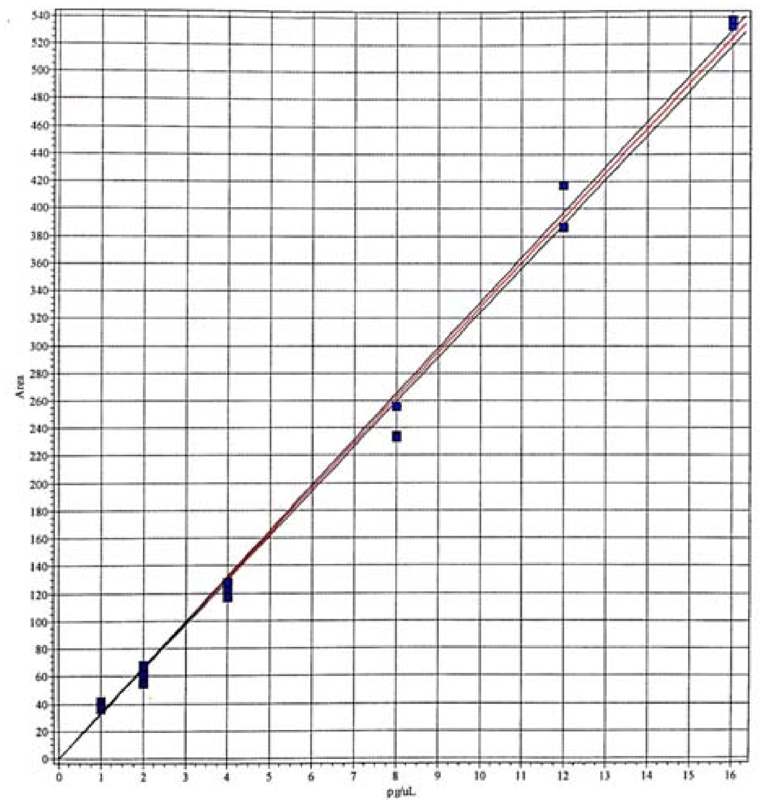
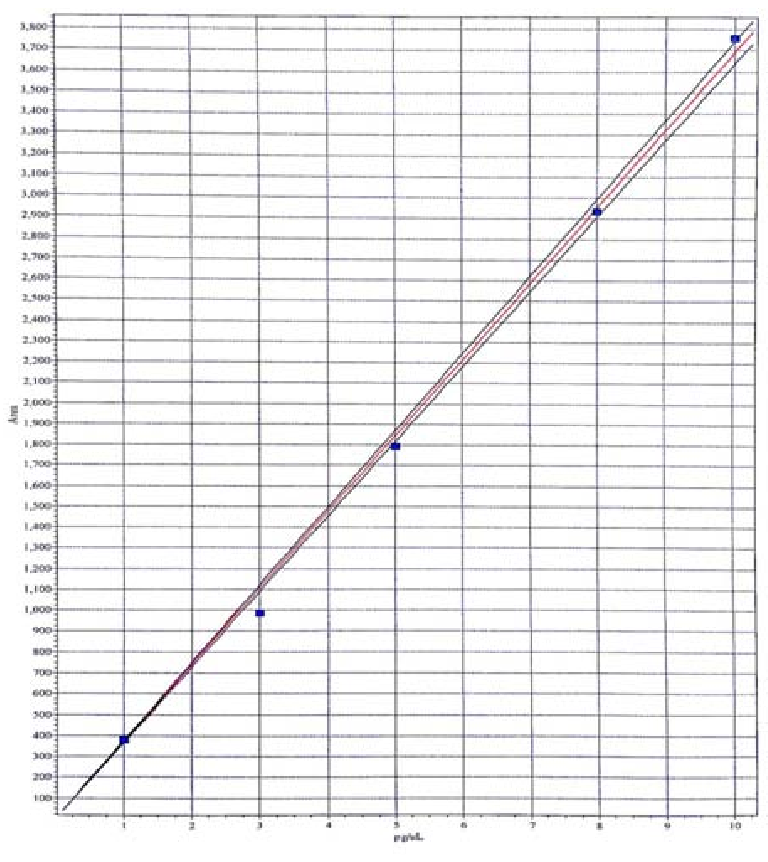


Figure 5.
A graph of the concentration dependence of c (pg/μL) on the surface of the sample point vinclozoline.



Next to the line is the equation of the line and the coefficient of correlation for each direction. The tracks are created in the Galaxy Chromatography Data system version 1.9.3.2. For all types of DDT (p, p – DDT, o, p – DDT, p, p – DDE and p, p – TDE) MDK for flour is 0.05. The same applies to rice. MPC vinklozolina with apples is 0.01.

When sample 4 and 6 were analyzed (sample 4. – Rice, sample 6. – Wheat white flour – smooth), the 2.4'-DDT was elevated as shown in the diagram (Figure 3), whose y is 122.52573 x and the value R is 0.9939.

When we analyzed samples 4 and 6 (sample 4 – Rice, pattern 6 – Wheat white flour – smooth), an elevated value of 4,4 μ-DDT was obtained as shown in diagram (Figure 4) whose y is 32.76046 x and the value R is 0.9944.

When sample 8 was analyzed (apple nectar and concentrated fruit juice), a vinclozoline elevated value was obtained as shown in the diagram (Figure 5) whose y is 368.65825 x and the value R is 0.9969.

For all types of DDT (p, p – DDT, o, p – DDT, p, p – DDE and p, p – TDE) MDK for flour is 0.05. The same applies to rice. MDK vinklozolina with apples is 0.01 [11].

DISCUSSION

Only 3 samples of the 11 analyzed, had a mild deviation (mild increase 27.27%), but below MPC. In samples 4 and 6 (sample 4. – Rice, sample 6. – Wheat white flour – smooth) there can be seen a slight increase of 2.4`-DDT and 4.4`-DDT. Since both pesticides have been increased in both samples, it is assumed that this is a possible impurity or an error in the analysis. Of course, because of the longtime of DDT's dissolution, it is possible that it is a real result. In the results in sample 8 (apple nectar and concentrated fruit juice), vinclozolin pesticides increased. MPC values were not exceeded. A more comprehensive picture of pesticide diffusion and their control can be given to us "Annual reports on the implementation of monitoring programs for pesticide residues in and on plant-based products" [12].

The 2013, report states that out of 335 analyzed samples, none of them contained residues above MPC [1, 12]. Similar results are also found in the 2014 report, when 378 samples were analyzed [3]. In the reports it can be seen that the average in the EU country from 2013 to 2014 increased by 1%. In 2013, the EU average was 1.6%, while the EU average in the EU in 2014 was 2.6% [11]. It was emphasized the use of resources that were allowed for use but not for the products they were found to be. Such an example is stated in the 2014 report, where the strawberry was found to be an active substance chlorpyrifos which has no license for its use [3]. They also mention the finding of active pyrazophos (on tomatoes) that is banned in Croatia and the EU. Pesticide residues, as stated in both reports, are most commonly present in orange and strawberries, while pesticide residues in wheat flour, baby food and bread are not found or are at the limit of quantification [3, 12].

Pesticide residues are most commonly present in orange and strawberries, while pesticide residues in wheat flour, baby food and bread are not found or are at the limit of quantification

On the basis of this data it can be estimated that consumer exposure in the Republic of Croatia is extremely low because none of the samples listed in the reports have passed the MDK or the samples analyzed for the purpose of this paper. It should also be taken into account that MDK values are set several times lower than concentrations of pesticides that are harmful to the health of consumers themselves [3, 12].

CONCLUSIONS

The use of pesticides is a worldwide problem and today's hygiene could not be imagine without them. Pesticides are a necessary today, but their use must be strictly controlled. The presence of DDT and vinclozoline is elevated but within the allowed limits. We can conclude that pesticide concentrations do not exceed the permissible concentration in a single sample and can not cause food poisoning. After the food test was carried out on randomly selected samples in the Public Health Institute's labs of Primorsko-goranska County, it was found that the food was safe and had no adverse effect on humans and their health.

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