Studii şi Cercetări Ştiinţifice 2011, **12** (3), pp. 229 – 234
Chimie şi Inginerie Chimică, Biotehnologii, Industrie Alimentară

Scientific Study & Research
Chemistry & Chemical Engineering, Biotechnology, Food Industry *ISSN 1582-540X*

ORIGINAL RESEARCH PAPER

PESTICIDE CONCENTRATIONS IN VEGETABLES FROM *Fabaceae* FAMILY♦

Alina Soceanu[1]*, Simona Dobrinas[1], Valentina Coatu[2], Viorica Popescu[1], Gabriela Stanciu[1], Danut Tiberiu Epure[3]

[1]University "Ovidius" of Constanta, Department of Chemistry, 124 Mamaia Blvd, 900527, Constanta, Romania
[2]"Grigore Antipa" National Institute of Marine Research and Development, 300 Mamaia Blvd. Constanta, Romania
[3]University "Ovidius" of Constanța, Department of Economic Sciences, 1 Universitatii Alley, Romania

*Corresponding author: asoceanu@univ-ovidius.ro

Received: July 23, 2010
Accepted: April 07, 2011

Abstract: This study was conducted to assess the concentration levels of nine pesticides in vegetables of the *Fabaceae* family. For this purpose we used chromatography with electron capture detector (GC-ECD) after the extraction and cleaning. The recoveries are between 96 and 99%. The repeatability of the method, expressed by coefficient of variation is less than 4.7%. The limits of quantification of the method are between 0.2 to 0.3 ppb. Residues of these pesticides are distributed differently in vegetables, depending on the organ of the plant and areas where vegetables were grown. For all the vegetables studied, DDD, DDE and DDT were below the limit of quantification. All concentrations of organochlorine pesticides from the samples studied are located within the limits imposed by the regulations of the European Commission.

Keywords: *European Commission regulations, GC-ECD, organochlorine pesticides, vegetables*

♦ Paper presented at the 6th edition of *Colloque Franco-Roumain de Chimie Appliquée*, **COFrRoCA 2010**, 7-10 July 2010, Orléans, France

INTRODUCTION

Several decades ago the production of organochlorine pesticides was at its peak. Because of their persistence, however, all these compounds can still be found in the environment worldwide, where they cyclent between soil, vegetation, and air. Because of their hydrophobicity and lipophilicity, they accumulate in soils, sediments and in the fatty tissue of living organisms; they are subject to biomagnification in food chains and possibly toxic to humans and wildlife. The different classes of pesticides have different types of effects on living organisms [1 – 6].

Many fruit and vegetables sold in supermarkets and greengrocers contain pesticide residues that are above the maximum legal level.

Pesticides are included in a broad range of organic micropollutants that have ecological impacts. Pesticides which are sprayed can become airborne and eventually end up in soil or water. Pesticides that are applied directly to the soil can be washed off into water or can percolate through the soil into the groundwater, a major source of our drinking water. These pesticides are broken down or degraded by the action of sunlight, water, other chemicals or microorganisms. This degradation process often leads to the formation of less harmful residue, but can sometimes produce more toxic products. It is also possible for a pesticide to become resistant to degradation by any means and therefore remain unchanged in the environment for long periods of time. These are called persistent pesticides.

The different classes of pesticides have different types of effects on living organisms. According to the Environmental Protection Agency (EPA), 60 percent of herbicides, 90 percent of fungicides and 30 percent of insecticides are known to be carcinogenic, or cancer causing. Laboratory studies show that pesticides can cause health problems such as: birth defects, nerve damage, cancer, blocking the absorption of important food nutrients necessary for normal healthy growth in children, other long-term effects [1 – 6].

The Stockholm Convention on Persistent Organic Pollutants was adopted in May 2001 with the objective of protecting human health and the environment from persistent organic pollutants. Romania has ratified Stockholm Convention by the Law no 261 of June 2004 and becoming a Party of the Convention on 28 October 2004. As a part of the convention, Romania elaborate National Implementation Plans, document which has been send to the Stockholm Convention Secretariat on April 2006. The overall objective of the National Implementation Plans is to reduce, or eliminate releases from the existing stockpiles and wastes; to eliminate production of POPs; to restrict the use of DDT and to reduce unintentionally releases of Dioxins, HCB and PCBs from the social and economic activities [7].

Various attempts have been made to describe and quantify the negative impacts that pesticides have on the environment and human health [8 – 11].

In the present study, two species of vegetables (green peas and green beans) from *Fabaceae* family were collected from 5 different locations in Romania (Dobrogea County): Mereni and Greci (rural areas) and Constanta, Medgidia, Tulcea (urban areas).

Fabaceae or *Leguminosae* is a large and economically important family of flowering plants, which is commonly called the legume family, pea family, the family of beans or pulse family [12].

This study was conducted to assess the concentration levels of nine pesticides in vegetables (green beans and green peas) of the family *Fabaceae* grown in urban and rural areas.

EXPERIMENTAL

Reagents and solutions

Standards of pesticides: Lindane, *p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, HCB, Aldrin, Dieldrin, Endrin, Heptachlor were supplied by International Atomic Energy Agency, Monaco laboratory.
Florisil was assayed for preconcentration step as sorbent material of variable polarities. It (60 – 100 mesh) was obtained from Fluka (packed in Switzerland) and was activated overnight (12 h) at 130 ^{o}C before use. Anhydrous sodium sulfate (granulated for residue analysis – Merck) was activated at 200 ^{o}C for 2 h before use. As eluents were assayed two organic solvents: *n*-hexane, supplied by Merck, Darmstadt, Germany and dichloromethane supplied by J.T. Baker.

Sample preparation

The vegetables analyzed were green peas and green beans grown in rural (Mereni, Greci) and urban areas (Constanta, Medgidia and Tulcea) from Dobrogea County (Figure 1). All samples were handled by Soxhlet extraction and an aliquot of the extract was applied to 5 g of activated florisil column for pesticides, topped with 1 cm of anhydrous sodium sulfate, which was pre-washed with *n*-hexane as described in EPA methods 8270C and 3600C. The columns were eluted with *n*-hexane-dichloromethane (3:1). Each fraction was concentrated to 1 mL using the Kuderna–Danish concentrator. The concentrated aliquot was blown down with nitrogen, the internal standards (2,4,5 trichlorobiphenyl) were added, and the final volume was injected.

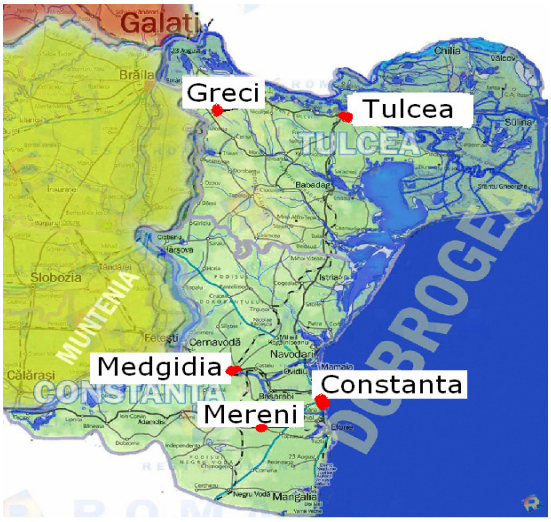

Figure 1. *Sampling sites*

Sample analysis

For OCPs analysis was used a Hewlett-Packard 5890 gas chromatograph (GC) equipped with an electron capture detector (ECD). The GC was equipped with a HP–5 fused–silica capillary column (30 m × 0.32 mm × 0.25 μm), helium was used as the carrier gas with flow rate 1.36 mL.min^{-1} and nitrogen make-up gas at 40 psi. The injector and detector temperatures were 250 °C and 300 °C, respectively. The initial temperature was 60 °C, after 1 min the temperature was increased to 300 °C at

a ramp rate of 20 °C.min^{-1} and then held for 10 min as was described in a previous paper [13].
The LOD and LOQ values were determined using calibration standards. LOD and LOQ were calculated as $(3 \cdot S_a - a)/b$ and $(10 \cdot S_a - a)/b$, respectively, where b is the slope of the calibration curve and S_a is the standard deviation of intercept of regression equation. The accuracy of the method was further assessed by recovery studies. Satisfactory results were found, with recoveries between 96 and 99% indicating the high accuracy of the proposed method. These percentage recoveries were ranged between the limits imposed by Horwitz equation (85 – 110%) for the established concentration range.
Precision has been assessed on the basis of the relative standard deviation calculated from results generated under repeatability (RSD) conditions. The RSD values were less than 4.7 for all the studied pesticides, indicating that RSD are below those given by the Horwitz equation.
Table 1 shows the quality parameters of the method and the maximum residue levels of pesticides in vegetables adopted by European Commission [14].

Table 1. *Quality parameters of the method*

Pesticides	**Retention time [min]**	**LOQ [ppb]**	**Maximum Residue Levels [mg/kg] European Commission**
Lindane	11.15	0.3	0.01
p,p'-DDT	16.98	0.2	ΣDDT – 0.05
p,p'-DDE	15.44	0.2	
p,p'-DDD	16.22	0.2	
HCB	10.53	0.3	0.01
Aldrin	13.42	0.2	0.01
Dieldrin	15.78	0.2	0.01
Endrin	15.97	0.3	0.01
Heptachlor	11.35	0.2	0.01

RESULTS AND DISCUSSION

The levels of organochlorine pesticide residues found in vegetables grown in rural and urban areas from Dobrogea County are given in Tables 2 and 3.
Examination of the data show that, in some analyzed samples, residues of some of the tested pesticides (DDD, DDE, DDT and dieldrin) were below the quantification limits. The other samples contain variously different levels of the residues depending of the growing sites.
In accordance with European Communities regulations [14], the maximum residues levels of pesticides in vegetables are showed in Table 1. Our results show that all pesticides concentrations of analyzed fruits are below maximum levels imposed by European Communities regulations.
The highest value was obtained for lindane (0.851 ppb) found in pods of green peas grown in rural area Greci.

Table 2. *Pesticides concentrations [ppb] in green peas and green beans grown in rural areas from Dobrogea county (Mereni and Greci)*

Pesticides	Green peas		Green beans		Green peas		Green beans	
	Rural area Mereni				Rural area Greci			
	pods	pea	pods	bean	pods	pea	pods	bean
Lindane	0.615	0.583	0.552	0.548	0.851	0.734	0.603	0.510
HCB	<LOQ	<LOQ	0.372	<LOQ	0.411	<LOQ	0.532	0.382
Heptachlor	0.815	0.753	0.431	0.356	0.531	0.497	0.617	0.523
Aldrin	0.631	0.475	0.388	<LOQ	0.432	0.411	0.443	0.415
Dieldrin	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Endrin	0.413	0.409	0.349	<LOQ	<LOQ	<LOQ	0.357	<LOQ
DDD	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
DDE	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
DDT	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Table 3. *Pesticides concentrations [ppb] in green peas and green beans grown in urban areas from Dobrogea county (Constanta, Medgidia and Tulcea)*

Pesticides			Lindane	HCB	Heptachlor	Aldrin	Dieldrin	Endrin	DDD, DDE, DDT
Green peas	Urban area Constanta	pods	0.439	<LOQ	0.706	0.652	<LOQ	<LOQ	<LOQ
		pea	0.385	<LOQ	0.431	0.422	<LOQ	<LOQ	<LOQ
Green beans		pods	0.516	<LOQ	0.511	0.772	<LOQ	0.396	<LOQ
		bean	0.509	<LOQ	0.503	0.713	<LOQ	0.311	<LOQ
Green peas	Urban area Medgidia	pods	0.439	<LOQ	0.802	0.412	<LOQ	<LOQ	<LOQ
		pea	0.611	<LOQ	0.784	<LOQ	<LOQ	<LOQ	<LOQ
Green beans		pods	<LOQ	0.375	0.562	0.354	<LOQ	<LOQ	<LOQ
		bean	<LOQ	0.356	0.498	0.332	<LOQ	<LOQ	<LOQ
Green peas	Urban area Tulcea	pods	0.441	0.413	0.451	0.603	<LOQ	0.444	<LOQ
		pea	0.440	<LOQ	0.338	0.417	<LOQ	0.398	<LOQ
Green beans		pods	0.319	<LOQ	<LOQ	0.519	<LOQ	0.551	<LOQ
		bean	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.372	<LOQ

Protecting most tree fruits or vegetables from attack by insects or diseases usually requires some pesticide application. The maximum result with the least amount of pesticide applied can result if the spraying is done correctly. The highest values obtained for samples grown in rural area can be due the fact that most gardeners don't have the proper equipment to do the job right. When pesticide solutions are sprayed by ground spray equipment or aircraft, droplets are produced by the nozzles of the equipment. Many of these droplets can be so small that they stay suspended in air and are carried by air currents until they contact a surface or drop to the ground. A number of factors influence drift including weather conditions, topography, the crop or area being sprayed, application equipment and methods, and decisions by the applicator [15]. Recent studies in Germany show a link between heavy pesticide use in rural areas and incidence of childhood leukemia. Pesticides, along with PCBs, dioxin and other environmental contaminants may act as endocrine disrupters interfering with hormonal action and body functions. This makes them possible risk factors for hormone-related

cancers such as prostate and breast cancer. Recent studies by the National Cancer Institute in Hawaii suggest that repeated exposure to the endocrine-disrupting chemicals, chlordane/heptachlor and 1,2-dibromo-3-chloropropane, may play a role in the development of breast cancer [16].
The concentration values of the 73 analyzed pesticides (0.01-0.1 mg/kg) by Hetherton *et al.* [17] are higher that our results. On the other hand, Sanghi and Tewari [18] haven't detected DDT, DDE, HCB and dieldrin in India, for some vegetables studied that is in concordance with our results.

CONCLUSIONS

This study was conducted to reveal and draw attention to the great problem of environmental pollution, in particular by pesticide residues in vegetables grown in Dobrogea County from Romania, to ensure safety and quality. The highest value was obtain for lindane (0.851 ppb) found in pods of green peas grown in rural area Greci, but levels of studied pesticides didn't exceed the maximum permissible levels.

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