Evaluation of Pesticides Residues in Fresh Fruits

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The presence of pesticide residues in fruits and vegetables has aroused growing public concerns. In this study the levels and distribution of nine organochlorine pesticides in some fresh fruits cultivated in Dobrogea County (Romania) and in some imported fruits from Greece, Turkey, Chile, Ecuador and Italy were investigated. The method has been validated for different fruit matrices as apple, quince, apricot, peach, nectarine, cherry, sour cherry, orange, lemon, grapefruit, banana, kiwi and mandarin. Recoveries were in the range 96–99%. Repeatability of the method, expressed as the relative standard deviation, was lower than 4.7%. The method quantification limit was in the range 0.2–0.3 μg/kg. The obtained results showed the predominance of aldrin in most of the analyzed samples but levels of studied pesticides didn't exceed the maximum permissible levels established by European Communities regulations.

Keywords: organochlorine pesticides, fruits, matrix-matched standards, GC-ECD

Due to their persistence, organochlorine pesticides can be still found in the global environment, where they cycle between soil, vegetation and air. Many of them are semivolatile, which makes them susceptible to long-range transport and wide geographic distribution. Due to their hydrophobicity and lipophilicity, they accumulate in soils, sediments and in the fatty tissues of living organisms, they are a subject of biomagnifications in food chains and eventually toxic to humans and wildlife. Organochlorines have a wide range of both acute and chronic health effects, including cancer, neurological damage, respiratory illness, Parkinson's disease and birth defects [1].

Pesticides residues are inevitably present in food. The key to their selective toxic effect is that they act against certain organisms without adversely affecting others. The absolute selectivity is difficult to achieve and most pesticides create some risks to human health. Residues left after pesticide treatment may penetrate plant tissues and appear in pulp and juice of fruits and vegetables, although their concentrations are in general lower that those observed in the whole fruits [2].

The convention on persistent organic pollutants (POPs) is a major achievement that is complemented by a number of other chemicals-related global or regional conventions, agreements and action plans. Most recent international agreement for POPs is the Stockholm Convention. 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. The convention is global in scope and multimedia in coverage. It focuses on reducing and eliminating releases of twelve chemicals that can be grouped into three categories: pesticides (aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, mirex, toxaphene), industrial chemicals (HCB, PCB) and secondary products (dioxins and furans). At present, the Stockholm Convention was signed by 151 governments from 23 May 2002, and ratified by 131 (in June 2006). Within these conventions, the establishments of emission inventories for POPs is mandatory and provide the basis for further emission reductions among Parties [3].

In 1948 organochlorine pesticides have been used for the first time in Romania. The first products were based in principal on DDT but had been also used other products based on endrin, dieldrin, aldrin, heptachlor, chlordane and toxafen. Since 1988 these kinds of products are banned or restricted in Romania and in the present only chlorinated insecticides on the base of lindane are used for seeds treatment in Romania, but this substance is not included in the Stockholm Convention list [4].

Determination of pesticides in environmental samples demands the use of complex chromatographic methods but also requires application of sample-extraction procedures (often combined with pre-concentration steps) to isolate the analytes and to remove interfering compounds. Current methods for extraction of pesticides from samples are liquid-liquid extraction (LLE) and solid-phase extraction (SPE) and solid-phase microextraction (SPME) [5].

Analytical procedures to detect pesticides after extraction and purification from environmental or biological matrices are commonly followed by GC, HPLC or GC-MS determination [6-10].

Capillary gas chromatography is the technique most widely used in pesticide analysis. It offers many advantages to the pesticide residue analyzed, high resolution reproducibility of retention time for multi residue studies. Pesticides are identified as trace solely on basis of retention times [11].

The purpose of this study was to determine the residues of nine organochlorine pesticides in some fresh fruits cultivated in Dobrogea County (Romania) and in some imported fruits from Greece, Turkey, Chile, Ecuador and Italy.

Experimental part

Materials and methods

Chemicals and reagents

Standards of pesticides: Lindane, p,p'- DDT, p,p'- DDE, p,p'- DDD, HCB, Aldrin, Dieldrin, Endrin, Hepthaclor were supplied by International Atomic Energy Agency, Monaco laboratory.

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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 (12h) at 130°C before use. Anhydrous sodium sulphate (granulated for residue analysis – Merck) was activated at 200°C for 2h before use. As eluents were assayed two organic solvents: n-hexane, supplied by Merck, Darmstadt, Germany and dichlormethane supplied by J.T. Baker.

Sampling

In the present study, 13 species of fresh fruits at different stages of growing (green, almost ripe and ripe) were collected from 3 different locations in Romania (Dobrogea area): Mereni (rural area) – peach, nectarine, apricot, Cernavoda (urban area) – cherry, sour cherry, Crisan (rural area) – apple, quince.

Mereni is a small village situated at 40 km far away from Constantza city. The town Cernavoda is a Danube fluvial port, and it houses the Cernavodă Nuclear Power Plant, a reactor providing about 10% of Romania's electrical energy output. Crisan is a fishing village situated in Tulcea district on Sulina canal in Danube Delta.

Also, were analyzed fresh fruits from Constantza market – lemon, orange, grapefruit, kiwi, banana, mandarin. The lemon has been imported from Greece, the orange from Romania green house, grapefruit from Turkey, kiwi from Chile, banana from Ecuador and mandarin from Italy. The collected samples were kept in aluminum foil and frozen until analysis.

Sample preparation

To determine the OCPs from fruits an analytical procedure was used, based on extraction step, a clean up step, followed by the injection of concentrated extracts in gas chromatograph. Samples were handled by Soxhlet extraction and an aliquot of the extract was applied 5g of activated florisil column for pesticides, topped with 1cm of anhydrous sodium sulfate, which was prewashed with n-hexane as described in EPA method 8270C [12]. The columns were eluted with n-hexane-dichlormethane (3:1). Each fraction was concentrate to 1ml using the Kuderna Danish concentrator. The concentrated aliquot was blown down with nitrogen, the internal standard (2,4,5 trichlorobiphenyl) was added and the final volume was injected.

Sample analysis

For OCPs analysis all samples were analyzed on a Hewlett-Packard 5890 gas chromatograph (GC) equipped with an electron capture detector (ECD). The GC was installed with an HP–5 fused–silica capillary column (30m x 0.32mm x 0.25μm), helium was used as the carrier gas with flow rate 1.86 mL/min and nitrogen makes-up gas at 40 psi.

Method analytical performance

Since plant matrices with certified concentrations of pesticides are not available, fortified ecological plants (representative matrices) were analyzed during the validation study to verify the recovery, linearity, precision, limit of detection and limit of quantification. Validation was made in agreement with quality criteria described in document SANCO No 3131/2007 [13] concerning the pesticide residues in food and feed. Representative matrices were spiked, prior the extraction step, using solutions containing the pesticides at five levels of concentrations (peach- representative for fruits and vegetables with high water content and orange-representative for citrus fruits with high acid content).

Specificity and selectivity are measures that assess the reliability of measurements in the presence of interferences [14].

For the repeatability conditions, the quantification of the same representatives matrices spiked with a solution containing the 9 pesticides at 1 mg/L level of concentration was performed ten times on the same day.

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 the 9 pesticides, indicating that RSD are below those given by the Horwitz equation.

Linearity was assessed on the base of the coefficient of determination (R^2), calculated by linear regression after having plotted the targeted spiking levels against the mean introduced concentrations. The calculated R^2 values were above 0.998 for all the 9 pesticides, indicating the linear relationship between targeted spiking levels and mean introduced concentrations, within the working range of concentrations.

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 (standard addition method). The recovery were determined on blank (free of pesticides) samples of peach and orange spiked with pesticides at three concentration levels, each test being performed six times.

Results and discussions

The working concentration range (0.01 - 0.4 μg/kg) was established by analyzing (n=10) each of two concentrations (the lowest and the highest concentration values of proposed concentration range). The homogeneity variance test was applied for these values.

Limit of quantification (LOQ), precision, recovery and linearity (R2) are indicated in the table 1.

Table 1
VALIDATION PARAMETERS

Pesticides	LOQ (μg/Kg)	RSD% (repeatability data)	Linearity (R^2)	Recovery (%)
Lindane	0.3	4.67	0.9998	98
HCB	0.3	3.67	0.9999	99
Heptachlor	0.2	1.11	0.9999	97
Aldrin	0.2	2.17	0.9999	97
Dieldrin	0.2	1.29	0.9999	98
Endrin	0.3	1.48	0.9999	99
p,p'-DDD	0.2	3.27	0.9999	98
p,p'-DDE	0.2	2.704	0.9999	96
p,p'-DDT	0.2	3.105	0.9999	97

Table 2
OCPs CONCENTRATIONS IN FRUITS FROM RURAL AREAS (MERENI AND CRISAN)

Pesticide (μg/Kg)	*Prunus armeniaca* **Apricot 2005**			*Prunus persica* **Peach 2006**			*Prunus persica var, nucipersica* **Nectarine 2006**			*Cydonia oblonga* **Quince 2005**			*Malus domestica* **Apple 2006**		
	Mereni			Mereni			Mereni			Crisan			Crisan		
	Green	Almost ripe	Ripe	Green	Almost ripe	Ripe	Green	Almost ripe	Ripe	Green	Almost ripe	Ripe	Green	Almost ripe	Ripe
Lindane	<LOQ	1.283	<LOQ	0.432	0.753	1.44	0.607	1.08	1.33	0.803	1.054	1.541	0.786	1.258	<LOQ
HCB	<LOQ	1.025	<LOQ	1.21	1.295	0.474	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.42	1.272	<LOQ	<LOQ
Heptachlor	<LOQ	1.941	<LOQ	0.601	0.571	0.464	1.011	1.0127	1.036	<LOQ	<LOQ	1.201	0.603	1.63	1.057
Aldrin	0.32	1.253	0.487	0.359	0.670	0.698	0.564	0.910	0.785	0.254	0.374	0.606	1.779	1.33	1.18
Dieldrin	0.279	<LOQ	0.307	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Endrin	0.472	1.065	<LOQ	0.507	0.641	0.758	1.134	1.197	1.089	1.194	0.479	1.227	1.141	1.029	1.06
p,p'-DDD	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
p,p'-DDE	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
p,p'-DDT	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

LOQ –quantification limit

Pesticides (μg/Kg)	*Prunus avium* **Cherry 2005**			*Prunus cerasus* **Sour cherry 2006**		
	Cernavoda			Cernavoda		
	Green	Almost ripe	Ripe	Green	Almost ripe	Ripe
Lindane	0.875	0.744	0.304	<LOQ	<LOQ	<LOQ
HCB	<LOQ	<LOQ	0.412	<LOQ	<LOQ	<LOQ
Heptachlor	1.061	1.123	1.043	3.751	4.489	4.211
Aldrin	1.124	0.406	1.108	1.014	1.033	1.078
Dieldrin	<LOQ	<LOQ	<LOQ	<LOQ	0.543	0.433
Endrin	0.384	0.758	0.449	<LOQ	<LOQ	1.030
p,p'-DDD	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
p,p'-DDE	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
p,p'-DDT	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Table 3
OCPs CONCENTRATIONS IN FRUITS FROM URBAN AREA (CERNAVODA)

LOQ –quantification limit

Tabel 4
OCPs CONCENTRATIONS IN IMPORTED FRUITS

Pesticides (μg/Kg)	*Citrus lemon* **Lemon Greece 2003**		*Citrus aurantium* **Orange Romania 2003**		*Citrus paradisi* **Grapefruit Turkey 2003**		*Actinidia deliciosa* **Kiwi Chile 2003**		*Musa paradisiaca* **Banana Ecuador 2003**		*Citrus nobilis* **Mandarin Italy 2003**	
	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel
Lindane	1.80	1.091	1.257	1.080	1.181	1.495	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.256
HCB	1.035	2.026	1.002	1.005	2.012	1.017	<LOQ	1.039	<LOQ	<LOQ	<LOQ	1.066
Heptachlor	3.010	3.034	1.031	1.015	1.020	1.052	1.088	1.339	1.017	2.016	1.027	1.165
Aldrin	1.043	0.872	0.430	0.629	1.116	1.169	0.692	0.450	1.123	1.155	2.152	1.319
Dieldrin	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Endrin	0.820	0.907	0.710	0.809	1.223	1.148	2.034	2.117	1.019	1.054	1.023	1.013
p,p'-DDD	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
p,p'-DDE	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
p,p'-DDT	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

LOQ – quantification limit

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.

The levels of organochlorine pesticide residues found in different stage growing of fruit samples are given in tables 2- 4.

In concordance with European Communities regulations [15], the maximum residues levels of pesticides in fruits are: heptachlor 0.01 mg/kg, lindane 1 mg/kg, endrin 0.01 mg/kg and ΣDDT 0.1 mg/kg. Our results show that all pesticides concentrations of fruits analyzed are below maximum levels imposed by European Communities regulations.

It can be noticed that while p,p'-DDT, p,p'-DDE and p,p'-DDD were below quantification limits, a high concentration of heptachlor was found in almost ripe sour cherry (4.489 μg/kg) grown in Cernavoda city. Regarding imported fruits, the highest concentration of pesticides was found also for heptachlor (3.034 μg/kg) in lemon's peel imported from Greece. The obtained results showed the predominance of aldrin in most of the analyzed samples.

There were large differences in the concentrations detected in different stage growing of fruits. In all green fruits studied the concentrations of OCPs are smaller than

those in ripe and almost ripe fruits. This can suggest that OCPs concentrations are not the result of soil pollution and the possibility of contamination is air pollution.

Despite the fact that cherry and sour cherry were growing in urban area the OCPs concentrations are not all significantly very high comparable with those from peach, nectarine, apricot, apple and quince grown in rural area, but there are some differences for some samples maybe because of town pollution.

Many researchers studied organochlorine pesticides in fruits produced in different countries. Examination of the data shows that, while in some analyzed samples, residues of some of the tested pesticides were below the quantification limits, the rest of samples contained variously different levels of the residues.

In our study, the highest concentration for HCB (2.026 μg/kg) was found in lemon peel while DDT, DDE were below quantification limits. Dieldrin was found only in green and ripe apricot, in almost ripe and ripe sour cherry and lindane concentration is between below quantification limit and 1.80 μg/kg. These results show lower concentrations of lindane than those found by [16] (4.5 ng/g in apple). Also, the concentration values of the 73 analyzed pesticides (0.01-0.1 mg/kg) by the researchers [17] are higher that our results. On the other hand, Sanghi and Tewari [18] have not detected DDT, DDE, HCB and dieldrin in India, for peach, apple, banana and lemon.

Conclusions

This study was conducted to reveal and draw attention to the great problem of environmental pollution, in particular by pesticide residues in 13 types of fruits from Romania, to ensure safety and quality. The obtained results showed the predominance of aldrin in most of the analyzed samples but levels of studied pesticides did not exceed the maximum permissible levels established by European Communities regulations.

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