

# Monitoring Pesticide Residues in Food of Plant Origin

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## *Abstract*

Economic crop production cannot be achieved without the use of pesticides. Pesticide residues express harmful effects on the biological systems and the environment. Especially dangerous is the presence of multiple residues which results in the appearance of different forms of combined toxicity with no predictable effects. There are well organized mechanisms for consumer health protection in developed countries, conducted through the legal standards in food safety, continuous monitoring of pesticide residues in food of plant origin, carrying out actions in accordance with the observed state and international cooperation. Regular monitoring of pesticide residues in food assures compliance of the principles of GAP and consumer risk assessment. By applying novel instrumental techniques with the low limit of detection (LC/MS-MS and GC/MS-MS) significant progress has been made for the detection of some recently registered pesticides whose residues could not be detected by previous standard procedures. Up to now results of monitoring programs have shown that American consumers are under lower risk associated with the toxic effects of pesticides, and the percentage of samples with residues content above MRLs has been below 5%, both in America and the EU, with a trend to diminish. This is the consequence of abandoning the use of persistent compounds with high potential of bioconcentration and bioaccumulation, and switching on the use of less toxic and persistent pesticides which are more effective at lower concentrations, as well as the improved techniques of application. Developing and transition countries are confronting a number of problems in the mentioned field, such as a lack of adequate legal frame, absence of introduction of law in practice or its violation. Higher frequency of samples with pesticide residues above MRLs originating from the third countries is evident from the annual reports of developed part of the world, as well as from the separate reports of these countries, mainly without the coordinated and regular control. Due to the international pressure, higher standards in food safety and control have been implemented in some intensively developing countries. Serbia is in a transition period for applying PPP Regulation in accordance with the EU law, and there are no stable mechanisms of pesticide residues control at the market yet, meanwhile, a permanent border control of imported fruits and vegetables has been performed. Only one, single monitoring program at the national level as a part of the project of the Ministry for Agriculture, Water Management and Forestry, Direction for Plant Protection, under the name "Pesticide residues in food of plant and animal origin, soil and water" was conducted through the period 2004-2006.

**Key words:** pesticide residues, monitoring, MRLs, fruits, vegetables

## *Introduction*

The hunger and poor balanced diet are the main factors involved in the origin and development of many human diseases, such as heart diseases and diabetes. Adequate food supply is no longer the matter of big concern in developed countries, so more attention is paid to the proper nutrition as a powerful tool in the prevention from chronic diseases (Martin and Phillips, 2009). Therefore, one of the primary tasks of the modern society can be accomplished through a well balanced nutrition based on food pyramid, assuming greater intake of fruits and vegetables, which cannot be economically produced without the use of pesticides (Kennedy et al., 2007).

There is an indirect positive impact of pesticides on human health due to increased plant health, higher crop production and availability to human population. Moreover, pesticides protect people and domestic animals from natural toxins produced in crops. In a crop grown without use of pesticides higher level of stress is achieved, as a response to pathogen attacks, predators and competition. Stress is a trigger for the production of plant defence compounds or natural toxins. Produced phenolic compounds, such as flavonoids, stilbens, coumarins and polyflavonoids, are generally considered as promoters of human health and different plant extracts with significant biological activity are commonly used in human therapy (Parr and Bolwell, 2000; Mimica-Dukić et al., 2000, Bošković and Mimica-Dukić, 2012). But in some circumstances they can express toxicity (Mattsson, 2008; Lima and Vianello, 2011).

On the other hand, growing popularity of organically grown food is a result of human effort to avoid exposure to synthetic pesticides, whose residues show toxicity after application. In that sense, selection and development of plant species with a higher level of natural persistence towards insects and fungi has been made. But, although free from pesticides, organically grown food may contain mycotoxins and therefore may represent greater risk for human health than conventionally grown food (Swanton et al., 2011). However, law regulations are mainly related to the toxicological testing of synthetic pesticides, as are tending to protect human health and environment. According to that, determination and detection of pesticide residues in food allow the realization of principles of Good Agricultural Practice (GAP) and setting of Maximum Residue Levels (MRLs; mg/kg, tolerance in USA) in food. Up to now, legal frame demanding systematic analysis of plant food as a source of a potentially toxic substances has not been established (1:10000 law regulations have been related to synthetic pesticides, although man is 10,000 times much more exposed to natural plant toxins) (Mattsson, 2008).

### ***Toxic effects of pesticides***

The foreign substances entered into a human body by inhalation, adsorption through a skin, and indigestion, alter cell functions and consequently lead to the appearance of various diseases (Coman et al., 2006). Pesticides after application are widely distributed in environmental compartments, contaminating the air, soil and water (Verstraeten et al., 2002; Pucarevic et al., 2002; Pucarević et al., 2003; Pucarevic and Sekulić, 2004; Pucarević et al., 2007; Pucarević et al., 2010a; Bošković et al., 2012). The widespread use of pesticides, their stability and tendency of bioaccumulation, make them particularly dangerous for a man, hence, exposure through consumed fruits and vegetables must be pointed out as a special risk to human health (Fenik et al., 2011).

Great number of diseases and pathological conditions of man are associated with exposure to pesticides, such as demyelization and paralysis of nervous system, asthma, hematuria, proteinuria, leukemia, anemia, multiple myeloma. Furthermore, recent epidemiological studies have brought up possible relationship between human exposure to pesticides and origin and development of different malignant diseases, which is shown in Table 1.

**Table 1.** Possible relationship between human exposure to pesticides and malignant diseases (Dich et al., 1997).

<b>Class of pesticide</b>	<b>Disease</b>
Acid phenolic herbicides	Soft tissue sarcoma
	Malignant lymphoma
Organochlorine insecticides	Soft tissue sarcoma
	Non-Hodgkin lymphoma
	Leukemia
Organophosphorus compounds	Lung and breast cancer (in a smaller number)
	Non- Hodgkin lymphoma,
	Leukemia
Triazine herbicides	Cancer of ovarium

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Children exposure to pesticide is possible very early, in the course of intrauterine life, via trans-placental transfer, and post-natally by the consumption of mother's milk, and at the older age through the contact with the environment, such as pets treated with insecticides, home dust, carpets and gardens. They are especially vulnerable category, which is reflected on the development of congenital malformations, cancer, malabsorption, immunological disorders, endocrine diseases and neurobehavioral defects (Jurewitz et al., 2006). Moreover, exposure to pesticides badly affects parent reproductive health which is especially prominent in undeveloped countries (Aktar et al., 2009).

Many organochlorine pesticides are lipophilic and persistent, with a great ability of bioaccumulation and bioconcentration in the environment. Therefore, use of DDT and many other organochlorine compounds were banned or restricted in the USA and Europe at the second half of the last century (Vassilev and Kamburova, 2006). The main route of human exposure to DDT and its metabolites is intake by food, and they are well absorbed and stored in adipose tissue, with half lives 7-10 years. It was observed relation between these compounds and cancer of liver, diabetes type II, decreased psychomotor and mental development of children. The positive correlation between the level of organochlorine compounds accumulated in human milk and quantity consumed by food was also established (Yu et al., 2009).

Organophosphorus pesticides are considered as less toxic substances and they are rapidly absorbed and transformed in less polar metabolites, excreted by urine with half lives 2-27 hours, and they irreversibly inhibit acetyl-cholinesterase (Borchers et al., 2010). They cause mental and psychomotor disturbances of children, problems with attention, hyperactivity, decreased mental development, mother exposure in the pregnancy leads to lower weight and length of the newborn.

For the difference of organophosphorus compounds, carbamates reversibly inhibit acetyl-cholinesterase, and they are characterized with shorter period of action and less toxicity at the same doses (Geller, 2006). Pesticides from pyrethroid group are rapidly metabolized in the environment and they are less toxic for man. These compounds may cause allergic reactions and asthma exacerbation.

Plant saponins, the natural insecticides, are used due to their low toxicity as an alternative to organophosphorus pesticides, carbamates and pyrethroids. The use of these compounds represents a new approach in crops protection compliant to modern agriculture and horticulture demands (De Geyter et al., 2007). Unfortunately, natural products often cannot adequately respond to the market demands towards biological activity, stability, produced quantity and quality. Therefore, they are mainly used as lead structures for the development of new synthetic compounds with improved characteristics.

Synthetic analogues of natural fungicides, strobilurins have shown a higher level of biological activity and photochemical stability in the field conditions than analogue natural compounds (Hutter, 2011). Synthesis and application of powerful, selective agonists of nicotin acetylcholine receptors of insects, neonicotinoids have opened a new perspective in development of insecticides (Millar and Denhlom, 2007). Despite their general toxicology and ecotoxicology, they are considered as safer and their use is in the expansion. Negative effects on bees and bumbles in the course of use of neonicotinoids have been observed, their presence has been detected in honey, and recently one case of a fatal human poisoning has been reported (CCD Steering Committee, 2007; Gross, 2008; Mommaerts et al., 2010; Fidente et al., 2005; Iyyadurai et al., 2010).

### ***Multiple residues of pesticides***

There is a serious negative impact of pesticides on human health associated with exposure to multiple residues resulting in the appearance of different forms of combined toxicity (Table 2). According to data in Table 2, mode of action of CMG compounds can serve as an indicator of the harmful effects on biological systems. End effects of interactions can be stronger, relaying on the synergism, amplifying effects or supra-additivity, or weaker if antagonism and inhibition are running on.

**Table 2.** The influence of different forms of combined toxicity on biological systems (EFSA, 2008; Boobis et al., 2008).

Combined toxicity	Mode of action	Effect on the biological systems
Dose addition	Compounds act in the same way but with different intensity (CMG -common mechanism group)	Simple sum of actions of each compound
Effect addition	Compounds act independently each of the other, the nature and site of action are completely independent	Results of independent action of each compound
Interaction	All forms not included in previously mentioned groups	Stronger or weaker effect than that of each compound separately

A mixture of CMG compounds expresses dose addition at lower concentrations, but all forms of combined toxicity may be achieved at higher concentrations. A mixture of compounds out of CMG group does not show dose addition below NOEL (no observable adverse effect level) concentrations for each compound, but at higher concentrations all forms of the combined toxicity can be present (Moretto, 2008).

Only the first form of combined toxicity, the dose addition has been investigated until now. Empirical data for the other two forms are still missing. A majority of present investigations on the xenobiotics, and plant natural products have been limited on the exploration of toxicity of only one, single substance (Sattar, 2007). This simple approach does not represent real exposure to a mixture of different chemicals in the environment. For example, it has been considered that rotenone, paraquat, dieldrin and maneb are involved in some way in the origin and development of Parkinson`s disease. Their mode of action in the body could not be connected with their chemical structure, because they are basically completely different compounds (Hatcher et al., 2008). Thus, in some further investigations adequate strategy for the estimation of risk of human exposure to a mixture of different compounds in the environment should be applied. For the same purposes in the approval procedure of active substances for plant protection, cumulative and synergistic effects of pesticide residues must be considered, after their application in compliance with good practice of plant protection, and in the realistic conditions, if the data are available (EC, 2009).

### *Monitoring in European Union (EU)*

Every country member of EU conducts the national monitoring program as a regular part of control and participates in the EU coordinated monitoring program to estimate the level of pesticide residues in food of plant origin. Pesticide MRLs have been harmonized in 2008 at EU level and the schedule of coordinated monitoring program was made for the period of next three years which can be seen in table 3. 15-50% of total intake of plant food by EU consumers was covered by the plant commodities analyzed during 2008.

There was an evident decrease of percentage of samples with residues above MRLs, as well as higher percentage of samples of crops imported from the third countries with residue above MRLs, and increased percentage of samples with multiple residues during presented monitoring period (2006-2008) in EU (Table 4).

Four-year-long investigation carried out in Germany brought up linkage between the time of harvesting, agricultural practice in a specific climate and pesticide residues content (Table 4). An average residues concentration (0.41 mg/kg) determined in this investigation was the usual one (Looser et al., 2006). In cooperative monitoring project during 2007 carried out in five countries (Table 4) residues of thiabendazole (29% of samples), imazalil and chlorpyrifos were the most often detected (Hjorth et al., 2011). Findings from this investigation have shown how important continuous monitoring is, especially in the case of imported fruits and vegetables.

**Table 3.** Coordinated monitoring plan for the three years (EFSA, 2010).

\*completely represents intake of food of plant origin by EU consumers and can be used for the risk assessment on human exposure to pesticide residues.

Year	Fruits and vegetables	Total contribution in the nutrition
2008	beans without pods, carrots, cucumbers, mandarins, oranges, pears, potatoes, rice, spinach	39-95%*
2009	aubergines, bananas, cauliflower, grapes, orange juice, peas without pods, sweet peppers, wheat	
2010	apples, head cabbage, leek, lettuce, peaches, oats, swine meet, strawberries, tomatoes	

**Table 4.** Review of the results of different monitoring studies conducted in the EU.

Year	Country	Number/kind of samples	% of samples with residue above MRLs % with multiple residues	Reference
2006	25 Member States and the three EFTA States	65810/fruits, vegetables, grains and plant products including baby food* 10906/ eight commodities**	- 4.4% - 6.4% from the third countries, and 2.2% from EU - The highest percentage in aubergines (4.3%), peppers (3.5%) and grapes (3.2%)**	Commission of the European Communities, 2008
2007	27 EU Member States and the two EFTA States	74305 samples/ 350 kinds of plant food	- 3.99% - 6.8% from the third countries, and 2.31% from EU - 0.6% in baby food, and 1.24% in organic grown fruits, vegetables and cereals. - The highest percentage in oat (3.8%), peaches (3.4%), strawberries and lettuce (2.9% each)** - The most often found in strawberries, apples, peaches, and lettuce (66%, 60.9%, 47.1% and 44.9%)** - Multiple residues of pesticides in 26.2% of samples	EFSA, 2009
2008	27 EU Member States and two EFTA States	- 70143/ nearly 200 different kinds of food* - 11610/ nine commodities**	- 3.5 % of surveillance samples* - 7.6 % from the third countries* - 10.3% of enforced samples* (chosen from the suspicion in product safety and previous violations of law) - 0.2 % in baby food and 0.9 % in organic grown fruits and vegetables* - 2.2 %** - The most often in spinach (6.2%), oranges (3%), rice (2.4%), cucumbers (2.1%), mandarins (2%), carrots (1.8%), pears (1.6%), beans (0.8%) and potatoes (0.5%)** - 27% of samples with multiple residues*/** -The highest number of pesticide residues found in the samples of table grapes - 26	EFSA, 2010
2002-2005	Germany	539/ conventionally grown strawberries from Germany, Italy and Morocco	- 17% (not from the Germany) in accordance with German legislative and legislative harmonized with EU - 93% with multiple residues	Looser et al., 2006
2007	Danish, Estonia, Finland, Norway and Sweden	724/ 46 kinds of fruits and vegetables imported from eight South American countries	- 8.4% - Pesticide residues content was at the higher rate above MRLs in the South American apples than in the apples from the Nordic countries	Hjorth et al., 2011

\*national programs; \*\*coordinated monitoring

## *Monitoring in the USA*

Monitoring of pesticides in food of plant origin has been performed in the USA since 1987 (US FDA, 2010a). The published reports have shown pesticide residues level in food to be far below the maximum allowed by the standards for safety of food.

Total of 334 samples of fruit and 713 samples of vegetables of the US origin, 771 samples of fruit and 1,839 samples of vegetables of foreign origin were analyzed during 2008. In 1.7% of samples of fruits of the US origin illegal residue were found (use of the unauthorized substances), and in 4.8% of fruits of foreign origin (in 2 samples the level of residues had exceeded MRLs, and in 34 samples it was from the use of unauthorized substances). In 4.4% of samples of foreign vegetables illegal residue were observed, thereof in 12 samples residue were above MRLs, and in 68 samples the use of unauthorized substances for the plant protection was reported (US FDA, 2010b).

Total of 3,429 samples of 180 commodities of plant food were analyzed in monitoring program in California during 2009 (CDPR, 2010a). In 2.4% of samples illegal residues were detected (among them in 12.3% of samples residues above MRLs, and in 87.7% of samples the presence of unauthorized pesticides for the specific crop with no established MRLs). Traditionally, the higher ratio of illegal residues, but at low level was present in the samples from the specific locations (Mexico, China, and Guatemala). By applying the novel instrumental techniques with low limit of detection (liquid LC/MS-MS and gas chromatography GC/MS-MS) an advance has been made for the detection of pesticide residues in agricultural crops, fruit, vegetables, and food (Fintschenko et al., 2010). A number of samples with detected residues of pesticides were increased by application of LC/MS analysis in the monitoring program in California during 2010 (CDPR, 2010b). Residues of some recently registered pesticides which could not be detected with prior methods of analysis were found. Illegal residues were present in 2.4% samples.

## *Monitoring in developing and transition countries*

**India.** Presence of three different groups of pesticides, namely organochlorine insecticides, synthetic pyrethroids and organophosphorus insecticides were investigated in three different kinds of fruit, produced in Hysar, in India (Kumari et al., 2006). Detected level of insecticides was within the frame allowed by FAO/WHO. In all samples, HCH (0.017-0.034 mg/kg), DDT (0.03-0.075 mg/kg) and endosulfan (0.021-0.082 mg/kg) were detected. The main contaminant from the class of organophosphorus insecticides, hinalpos was detected at concentration of 0.045-0.046 mg/kg because of insufficient period between its application and the time of harvesting.

The presence of 48 pesticides (13 organochlorine, 17 organophosphorus, 10 syntetic pyrethroids and 8 herbicides (triazine, triazole, organochlorine, dinitroaniline, and phenylpyrazole) in 20 types of fruits collected from local markets in Lucknow, India, were determined by multiresidual QuEChERs method, during 2009 (Srivastava et al., 2011). 23 pesticides at the level of 0.005-12.35 mg/kg (HCH, dicofol, endosulfan, fenpropathrin, permethrin II, beta-cyfluthrin-II, fenvalerat, dichlorvos, dimethoat, diazinon, malathion, chlorfenvinfos, aniliphos, dimethachlor) were detected. Residues content above MRLs were detected in radish, cucumbers, cauliflower, cabbage and okra. Low level of pesticides detected was the result of replacement of persistent organochlorine pesticides with easy degradable organophosphorus and synthetic pyretroids, over the last decade in India.

**China.** The presence of eight organophosphorus pesticides (fonofos, methyl-parathion, malathion, chlorpyrifos, fenthion, chlorfenvinfos, phorate, and dimethoate) were determinated in four types of vegetables from Nanjing, China (Wang et al., 2008). Residues of chlorfenvinfos found at the concentrations of 0.131 and 0.094 mg/kg in carrot samples, were more than allowed ones according to Chinese regulative (MRL = 0.05 mg/kg). That implicates incorrect use of the pesticide at a higher rate

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than recommended. Besides that, residues of malathion (0.0342-0.0526 mg/kg) in Shanghai green and phorate in Shanghai green (0.0257 mg/kg) and Chinese cabbage (0.0398 mg/kg) were detected despite the fact that these two insecticides are prohibited in China and many other countries due to their high toxicity. Detected level of organophosphorus pesticides (dimethoate, chlorpyrifos and methyl-parathion), the most frequently used in the vegetable production in China, showed much more professional use of these compounds.

The presence of residues of 22 actually used pesticides (organophosphorus insecticides, pyrethroids, triazoles and chloronitriles (fungicides) were analyzed in the fruits and vegetables collected through the period from October 2006 to March 2009 in Xiamen, in China. In 37.7% of samples pesticide residues were found. Pesticide residues were the most often detected in pakchoi cabbage, legumes and leaf mustard (in 17.2%, 18.9%, and 17.2% of samples they exceeded MRLs, respectively), and cypermethrin was the most often found (in 18.7% of samples) (Chen et al., 2011).

**Saudi Arabia.** Residues of 23 pesticides (13 insecticides, 3 fungicides, 2 acaricides, 1 rodenticide and 1 nematicide) were determined in 160 species of vegetables grown in the greenhouses, collected at four supermarkets in western part of Saudi Arabia. 44.4% of samples were pesticide-free, and in 55.6% of samples residues were detected, thereof in 59.6% of samples above MRLs. Residues of carbaryl were most often above the MRL (17 samples). Pesticide residues were the most often found in cabbage (11 samples) and at the highest number (16). Seven of 23 pesticides were with no established MRLs (Osman et al., 2011). According to these findings, there was no risk associated with consumer health but it was clear direction for further regular control of vegetables grown in greenhouses.

**Egypt.** Monitoring of food contaminants has been performed by different institutions in Egypt in the past few years. Up to now, content of pesticide residues in fruits and vegetables was not analyzed in a sense that it can be dependent on the type of production. For that reason pesticide residues were determined in the conventional, organic and greenhouse-produced cucumbers from different locations in Giza, in Egypt (Mansour et al., 2009). In more samples, residues of organochlorine pesticides (HCH, heptachlor, aldrin, endrin, dieldrin, o- i p-DDT) and organophosphorus pesticides (chlorpyrifos-methyl, thiometon, phorate) were detected above MRLs. Lindane was detected in 33.3%, 50% and 25% of cucumber samples (conventionally grown, grown in greenhouses, and organically grown, respectively) and insecticide methamidophos in 66.7%, 41.7% and 50.0%, of cucumber samples (the same order of production of cucumbers as above) below the MRL. The highest residue content was found in cucumbers from greenhouses (1.016 mg/kg), followed by organically grown (0.442 mg/kg) and conventionally produced ones (0.415 mg/kg).

**Croatia.** In the frame of the monitoring program harmonized with the EU law, 866 samples of 28 kinds of Croatian domestic and imported fruit and vegetables were analyzed during three year period (2007-2009) (Knežević et al., 2012). In 66.2% of samples (including all samples of cabbage, onion, leek and spinach) residues were not found, in 28.5% of samples they were detected within MRLs (according to Croatian end the EU legislative). In 5.3% of samples residues exceeded MRLs (most often in apples). The highest content of pesticide residues was detected in oranges (27.9 mg/kg; imazalil). Risk assessment on consumer exposure to pesticide residues from fresh fruits and vegetables was completed. In 12 different types of the samples possible exceeding of Acute Reference Dose (ArfD) was observed if crop should be consumed for a shorter period of time.

**Serbia.** Besides regular border control of imported and exported fruits, vegetables and crops, in Serbia there was only one, single monitoring of pesticide residues at the national level as a part of the project of the Ministry for Agriculture, Water Management and Forestry, Direction for Plant Protection, under the name "Pesticide residues in food of plant and animal origin, soil and water" (Lazić et al., 2005; Pucarević, 2008). The project was conducted during the period 2004-2006. Next large amount of data about the presence of organochlorine and organophosphorus pesticides, triazines,

azoles, dithiocarbamates, and other specific compounds in 778 samples of food, crops, fruit and vegetables were obtained at the Institute for Soil and Crop in Novi Sad, in the course of four year study (2003-2007). Samples were collected by the official inspection, private persons, producers and exporters. In 7.9% of samples residues content was above the allowed one. In 50% of tomato samples residues had exceeded MRLs (vinclozolin and endosulfan were the most often detected) and in 13% of raspberries samples in which chlorpyrifos, vinclozolin and endosulfan were the most frequently found (Pucarević, 2008). Samples of tomato and raspberries were analyzed as the part of above mentioned national monitoring project.

In 53.7% of apple samples from Serbian market analyzed during the period 2004-2007 pesticide residues were found, which showed a need for the regular control in scope to prevent pesticide adverse effects on human and animal health. Residues above EU MRLs were found in 7.41% of samples (Lazić et al., 2009).

Pesticide residues several times above MRLs (metribuzin, trifluralin, pendimethalin, bifenthrin, chlorpyrifos and cypermethrin) were observed in tomato, peppers, potato, and onion samples in the analysis of fruits and vegetables collected from agricultural fields of Belgrade area (Marković et al., 2010). The level of chlorpyrifos in onion samples was more than 20 times higher (1.18 mg/kg) than allowed by national and international regulations. The authors pointed out inappropriate use of pesticides as a possible reason of crop contamination, which presented a potential risk for consumer health. The samples of analyzed crops were taken from private farms, and distributed on the city market immediately after harvesting, with no previous control, which showed on urge for pesticide residues determination before the distribution of food of plant origin in the city markets. The observed high level of chlorpyrifos in onion samples can be explained by interference of onion sulphur compounds with the organophosphorus pesticides. Such possible false positive results can be restrained by microwave inactivation of enzyme alliinase in onion (Zhang et al., 2008).

Presence of 93 pesticides in 71 samples of fruit and 210 samples of vegetables of domestic production, collected from Serbian market were analyzed in 2009 (Pucarević et al., 2010b). In 50% of peach samples, 29% of apple samples and 25% of raspberries samples, residues content was below the EU MRLs, and in vegetables, in 63 % of cabbage samples, 30% of onion samples, 12% of peppers samples, and 11% of cucumber samples. Pesticide residues content above MRLs were found in peaches (oksamil 0.561 mg/kg), cauliflower (bifenthrin 1mg/kg), cucumbers (tiametoksam 0.5 mg/kg), cabbage (oksamil 0.113 mg/kg) and peppers (oksamil 0.107 mg/kg). In 8% of samples of fruits multiple residues were found, thereof 50% in apples, and 9% in vegetables, mostly in the tomato (32%) and cabbage (21%). By comparing these results with the ones from previous years, percentage of fruits and vegetables with residues content was decreased.

By investigation of level of dithiocarbamate in 139 samples of fruits and vegetables taken from the Serbian market, in 72% of samples residues were detected. The highest content  $\leq 0.8$  mg/kg was found in raspberries, and there was no evident presence of the pesticide in blueberries (Pucarevic et al., 2010c).

### *Legal frame of monitoring*

- ✓ In the EU, coordinated monitoring program for pesticide residues in food has been running in accordance with the law regulative of EU since 1996, as an aim to protect consumer health and assure the respect principles of GAP (EC, 2004; EC, 2005). EFSA (European Food Safety Authority) as an independent body publishes annual reports with recommendations for the future investigations and decisions about the critical points in the producer-consumer chain. A risk assessment on consumer health is based on:
  - ✓ toxicological characteristics of pesticides,
  - ✓ expected maximum level of residues in food, and types of diets common in the EU (Directorate General for Health and Consumers, 2008; RAPID, 2008).

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In the USA, EPA (Environmental Protection Agency) sets up allowed level of pesticide residues in food, meanwhile monitoring program is controlled by USDA (United States Department of Agriculture) and FDA (Food and Drug Administration) (IFT, 2009; US EPA, 2011).

By comparing the annual data on pesticide residues in food from the USA and EU, it can be concluded that American consumers are under lower risk associated with the undesirable effects of pesticide residues in food of plant origin. This seems as a logical fact if we consider the longest practice of America in that field. The results of recent monitoring studies from the EU and the USA have shown pesticide residues above MRLs in less than 5% of samples.

Monitoring of pesticide residues in food at the national level represents also a response on the international obligations taken by assignment of Stockholm Convention (2008). Hence, it must be considered as a tool for indirect determination of level and trends of movement of persistent organic pollutants (POPs) in human population and environment and their effects on human health and environment.

Through harmonization of methodologies and inventory of sources of POPs and analytical techniques of measurement, straightening the national scientific and technical capacities for investigations, especially in developing countries and transition ones, with adequate exchange of information, and right on the time, in accordance with the law public informing, the Convention demands can successfully be fulfilled.

According to monitoring programs in the USA and other countries, decreased percentage of pesticide residues exceeding the legally set up limits must be viewed as a result of the use of less toxic and persistent pesticides and improved techniques of their application (Seiber, N.J., Article ASAP). On the contrary, in developing countries with insufficient consciousness about harmful effects of the use and exposure to pesticides, banned or restricted pesticides are actually used (EJF, 2003). Pesticide labelling is unclear, on foreign languages, without warnings and instructions for handling and together with low education level of farmers, often incorrectly interpreted. Pesticides are used to frequently and in concentrations greater than is allowed (Akinloy et al., 2011; Bempah and Donkor, 2011; Bempah et al., 2011, Latif et al., in press). Poisoning with food contaminated with pesticides and presence of pesticides in human milk and blood serum of the consumers and agricultural workers from Arabic countries were recorded particularly from that reasons at the end of last and beginning of this century (El-Nahhal, 2004).

- ✓ The developing countries and countries in transition, including Serbia, possess a small number of reports, mostly from separate works of scientific institutions, with no scheduled and coordinated monitoring at the national or international level. According to the results from well organized monitoring studies as well as from the separate investigations from less developed part of the world, next problems should be emphasized:
- ✓ a high percentage of samples with pesticide residues above MRLs in some countries, sometimes many times above EU-MRLs caused by the lack of adequate legal frame and/or funds for regular control of pesticide residues in food,
- ✓ frequent use of prohibited or unregistered PPPs,
- ✓ improper use of PPPs and insufficient period of time between applying PPPs and crop harvesting.

These problems have been resolved by international pressure in some countries seriously interested in food export. For example, by assigning agreement with the USA in December 2007, China implemented stronger standards and inspections for 10 products exported on the American market which were contaminated at the past (Al-Taher, 2009). Recently, a network of monitoring at the national level has been established in China, covering 16 provinces (65% of the population) but product quality is still far away from the desired one by international standards. Standards have been implemented only for 7% of active substances used in vegetable production, and MRLs for 35.6% of pesticides (Wang et al., 2009). Meanwhile, India has been controlling residues of 97 actually used, banned or restricted pesticides in three types of fruits (grapes, pomegranate, and mango) intended for export (Savant and Banrjee, 2010).

Despite implementation of principles of Good Management Practice (GMP) and system of quality control (HACCP) in the Arab GCC (Gulf Cooperation Council) countries, they have not been completely integrated in the system of inspection. The standard procedure for monitoring of pesticide residues in food completely allowing the risk assessment on consumer health is successfully implemented only in the UEA (Al-Kandari and Jukes, 2009). Recently, in Sub-Saharan countries, food control at the national level has been established, mostly inappropriate to international standards; therefore placement of their products on the international market has not yet been possible (Bagumire et al., 2009).

### *Relation EU-Serbia*

Meanwhile, regular and strong control of food quality based on Codex and FAO/WHO standards regarding the contamination with pesticides has been systemically applied in the EU, in Serbia, coordinated monitoring of pesticide residues in food of plant origin is still missing (Mojašević, 2010). International MRLs are not often used; the absence of independent body verifying and accepting results can be outlined as additional problem. Furthermore, there is inappropriate understanding of MRLs, false positive results in control quality, analysis of more than 90% substances out of the field of laboratory accreditation, and a great influence of interested groups. According to Gorton et al. (2011) during 2007, 29 phytosanitary inspectors out of the total of 34 existing ones were engaged at the border posts. So around 600,000 family farms in Serbia which produce fruit and vegetables for the local market stayed almost completely uncovered with the control.

The Regulation of Plant Protection Products (PPPs) harmonized with the EU law (Official Gazette RS 41/09), entered into the force in may 2009, demands monitoring of residues of PPPs in food, animal food and environment, professional development of persons responsible for storing, putting on market and applying PPPs. The user of PPPs is obliged to deliver the data for harvesting in a period shorter than necessary from applying of PPPs, and there is no possibility for the placement of such products without previous investigation of safety in accordance with the Regulation of Food Safety (Official Gazette RS, No. 41/09). Transitioning and closing remarks of the Regulation put in obligation corporate bodies to conform their actions to the Regulation in the two-year-period from the date when the Regulation entered into the force, while for the civilian this period is the three years. In that sense, the control and punishment for irregular and unscrupulous use of pesticides are problematic in Serbia in the following period. Maximum level of pesticides remaining in food and feed is defined by the Rulebook on "Maximum allowed of remains of pesticides in food and feed" (Official Gazette RS No.25/10 and 28/11).

For the closer approach to the EU, there is a need for a coordinated systematic analysis of pesticide residues in food of plant origin which quality directly affects on the public health. Besides that, in that way Serbia can fulfil international obligations taken by recognizing Stockholm Convention (Official Gazette RS- International agreement No. 42/09) which is applied through Regulation of Chemicals (Official Gazette RS No. 36/09,) and Regulation of Biocide Products (Official Gazette RS No. 36/09).

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The content of pesticide residues in the analysed samples was assayed with the use of the QuEChERS (Quick Easy Cheap Effective Rugged Safe) method combined with HPLC-MS/MS (high performance liquid chromatography with tandem mass spectrometry) analysis. The estimation of contaminants and chemical residues in food of plant origin assumes a significant importance, which is related with the progress of science and results from the critical attitude of consumers towards the applied methods of agricultural production and to the environmental pollution [1]. Accumulation of pesticide residues in food may cause toxic and allergic effects for human health and life as. ESI-MS/MS was operated in scheduled multiple reaction monitoring mode (MRM), in both