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DIETARY EXPOSURE RESIDUES OF PESTICIDES FROM CITRUS FRUITS AND RISK ASSESSMENT

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Abstract

The aim of this research was to determine the residual levels of pesticides in citrus fruits, to assess the exposure and to determine the acute risk to consumers. In this work, eight pesticides: *azinphos-ethyl*, *carbofuran*, *chlorfenvinphos*, *chlorpyrifos*, *metazachlor*, *methoxychlor*, *propham* and *sulfotep* were analysed in 23 samples of citrus fruits, taken from the market of Bosnia and Herzegovina. Analyses were carried out by the QuEChERS approach developed for pesticide residue analysis in food, using gas chromatography with mass spectrometry detection. To determine the risk, acute exposure assessment was performed according on *International Estimation of Short Term Intake (IESTI) equations.* In quantitative acute risk assessment, estimated dietary exposure was compared with the toxicological reference values Acute Reference Dose (ARfD). In four samples of oranges the levels of pesticide residues were below detectable limit, in six samples determined levels of pesticide residues were at or below the MRL, while in one sample level of pesticide residues exceeded MRL. In six samples of grapefruits the levels of pesticide residues were below detectable limit, in five samples determined levels of pesticide residues were at or below the MRL, while in one sample level of pesticide residues exceeded MRL. In one sample of grapefruit where the level of *chlorpyrifos* residue of 0.61 mg/kg was found above MRL, an acute exposure risk assessment was performed and there was not a risk for health of adults and children, because the value of the calculated dietary intake was below the ARfD.

Keywords: "citrus fruits"; "pesticide residues"; "risk assessment".

1. Introduction

Pesticides are a very diverse group of chemical substances with different toxicological characteristics and effects used in agriculture for the prevention, destruction and control of pests, weeds or plant diseases. Although the use of pesticides is regulated through very strict regulatory processes, they can pose a serious health risk as a result of exposure to pesticide residues in food and drink water. The correlation between exposure to pesticides and the incidence of various chronic diseases has been demonstrated. EU regulations, Regulation (EC) 396/2005 (EC, 2005), regulate the maximum permitted levels (MRLs) of residues of certain pesticides in foodstuffs. Determination of MRLs is based on tests of a particular pesticide under the prescribed conditions of use (recommended quantity and time of use) with compulsory compliance with the principles of good agricultural practice. Excess MRLs for certain active substance indications are needed to determine the acute and chronic exposure of consumers. In assessing the acute and chronic risk of pesticide residuals, the estimated dietary exposure is compared with the relevant toxicological values: acute reference dose (ARfD) and acceptable daily intake (ADI). The aim of this study was to determine the level of residual amounts of pesticides in citrus fruit samples, to calculate the pesticide residue in acute exposure and to estimate the likelihood of adverse health effects due to acute exposure to citrus fruit pesticide residues.

2. Material and Methods

Sampling and sample analysis

The random method selection from the BH market in July-August-September 2014 took 23 samples of fresh citrus fruit: 11 orange samples and 12 grapefruit samples. All samples were analyzed for the presence of eight types of pesticides: *azinphos-ethyl*, *carbofuran*, *chlorfenvinphos*, *chlorpyrifos*, *metazachlor*, *methoxychlor*, *propham* and *sulfotep*. Sample analysis was done in an accredited test laboratory of the Federal Institute of Agriculture, Sarajevo.

Determination of pesticide residues in fruit was performed by mass spectrometric gas chromatography (GC-MS) according to BASEN 15662:2011. Before analysis, homogenisation and preparation of samples was carried out - acetonitrile extraction and purification by QuEChERS method. The purified and acidified extract was transferred to an auto-sampler vial for multiresidual analysis on GC-MS technique. Chromatography was performed on the GC-MS Agilent 7890A/5975C instrument under the conditions of the "PESTICIDI Gcabc_A"method. M". In this study, the quantification limit (LOQ) was 0.01 mg/kg.

Method of risk assessment

For the calculation of the acute intake of pesticide residues, the International Estimation of Short Term Intake (IESTI) was proposed at the Joint Meeting of the FAO/WHO (FAO/WHO, 2006), accepted by EFSA (EFSA, 2007). IESTI was calculated using the following formula:

$$\text{Intake (mg/kg bw)} = U \times HR \times v + (LP - U) \times HR / bw$$

where is:
U = mass of the sample unit (kg)
HR = maximum specific percentage of pesticides (mg/kg)
v = variation factor
LP = large portion (kg)
bw = body weight (kg)

Bosnia and Herzegovina does not have its national consumption database, so the values for LP are taken from the EFSA consumption database. The estimated value of the calculated intake was compared with the acute reference dose (ARfD), the amount of substance for which it was scientifically determined to be consumed in a short period, usually during one meal or one day without any perceived risk to consumer health. If the estimated risk is up to 100% of the ARfD value, it is considered that there is no real risk for the observed population. A risk assessment was performed for pesticides where the MRL values were exceeded in the analyzed samples for which ARfD values were defined.

3. Results

From the total number of orange samples (11 samples), in the four samples (36.4%) no quantifiable pesticide residues were found, while seven samples (63.6%) contained one or several pesticides in quantified concentrations. Multiple residues were detected in one sample (9.1%). In the analyzed samples the presence of residues of five types of pesticides was found: *azinphos-ethyl*, *carbofuran*, *chlorpyrifos*, *metazachlor* and *methoxychlor* (Figure 1).

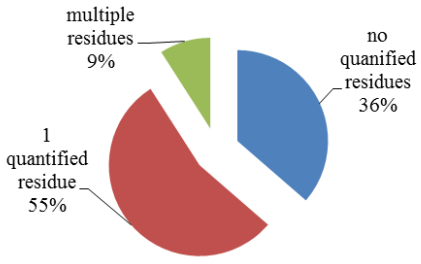

Figure 1: Quantified residues in orange samples

From the total number of grapefruit samples (12 samples), in the six samples (50%) no quantifiable pesticide residues were found, while six samples (50%) contained one or several pesticides in quantified concentrations. Multiple residues were detected in three sample (8%). In the analyzed samples the presence of residues of four types of pesticides were found: *sulfotep*, *chlorpyrifos*, *metazachlor* and *methoxychlor* (Figure 2).

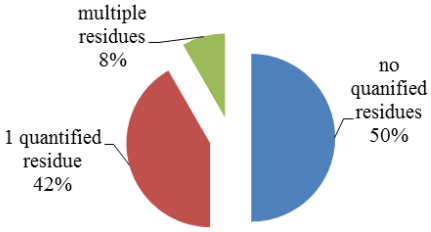

Figure 2: Quantified residues in grapefruit samples

The established concentrations of pesticide residues in the analysed samples are shown in Table 1. In one orange sample, the residual value of *azinphos-ethyl* of 0.14 mg/kg was determined, which is above the MRL. In one orange sample, the residual value of *carbofuran* residue was 0.01 mg/kg. In two orange samples, the level of residual *chlorpyrifos* was 0.01 mg/kg. In two samples of orange, the residue level of *metazachlor* ranged from 0.01 to 0.02 mg/kg. In two samples of orange, the level of *methoxychlor* was 0.01 mg/kg. In one sample of grapefruit the residual level of sulfotep was 0.01 mg/kg. In five grapefruit samples, the level of residual *chlorpyrifos* ranged from 0.01 to 0.61 mg/kg, where in one sample the level of 0.61 mg / kg exceeded the MRL value. One grapefruit sample had a residual *methazachlor* residue value of 0.01 mg/kg. In two samples, the level of *methoxychlor* residue ranged from 0.01 to 0.02 mg/kg, where the established residue value of 0.02 mg/kg *methoxychlor* exceeded the MRL.

Table 1: Concentrations of pesticide residues in analyzed samples

Pesticide type	Average concentration in the orange mg / kg (range)	
	Orange	Grapefruit
Azinphos-ethyl	0.14	-
Carbofuran	0.01	-
Sulfotep	-	0.01
Chlorpyrifos	0.01	0.13±0.26 (0.01-0.61)
Metazachlor	0.015±0.007 (0.01-0.02)	0.01
Methoxychlor	0.01	0.015±0.007 (0.01-0.02)

Table 2. shows levels of MRL values for pesticides where determined level of residues in oranges and grapefruit was above the MRL, in accordance with the regulations (Official Gazette, 2012).

Table 2: The concentrations of pesticide residues are above the MRL

Pesticide	Sample type	MRL (mg/kg)	Concentration (mg/kg)
Azinphos-ethyl	Orange	0.02	0.14
Chlorpyrifos	Grapefruit	0.3	0.61
Metoxychlor		0.01	0.02

The sample was analyzed *chlorpyrifos* residue level of 0.61 mg/kg in grapefruit was determined; the value which is above the MRL (0.3 mg/kg). The assessment of risk due to acute exposure was performed and it is found that there is no a health risk for adults and children. Due to the fact that the value of the calculated input was below 100% ARfD, which is 0.1 mg/kg body weight/day the risk was not found. The estimated level of exposure for adults and children accounted for 12.2% and 54.4% compared to the ARfD (Table 3).

Table 3: Assessment of risk due to acute exposure to pesticide residues in grapefruit

Pesticide	Category	U (g)	HR (mg/kg)	v	LP (g)	TM (kg)	I (mg/kg bw)	% ARfD
Chlorpyrifos	adult	160	0.61	7	301	63	0.0122	12.2
	children	270.5	0.61	5	358.6	16.15	0.0544	54.4

In one sample of orange, wherein a concentration of the *azinphos-ethyl* was above the MRL, risk due to acute exposure was not performed because ARfD value for this pesticide was not defined. Also, in the samples where determined grapefruit value *methoxychlor* residues was above the MRL risk assessment acute exposure to that pesticide was not performed because ARfD was not defined.

4. Discussion

Many studies in the world have reported pesticide residue levels in fruit above the MRL, and the MRL exceedance rate was identified for fruit at 0.3% in Denmark (Poulsen & Andersen, 2003), 5% for fruit in Spain (Berrada *et al.*, 2006), 4% for fruit in Italy (Zicari *et al.*, 2011), 5.9% for fruit in Poland (Łozowicka *et al.*, 2013). In Switzerland, a total of 240 samples of citrus fruits were analysed and pesticide residues were detected in 207 (86%) samples. In totality, 27 samples were non-compliant to the Swiss legislation, six cases exceeded the MRLs, three organic products were contaminated with pesticides and 18 samples did not respect the written indication "without post-harvest treatment" (Ortelli *et al.*, 2005). In the study in Turkey, at least one type of pesticide residue was found in 50% of the total citrus fruit samples (105 of 210 samples). None of the 105 citrus fruit samples (50% of the total samples) examined containing pesticide residue. One or more pesticide residue was detected in 31 orange samples (44.3%) at or under the MRLs. In 5 lemon samples (2.4%), the residue levels were found above the MRLs (Taga & Bilgin, 2010). Based on analysis of the 2015 pesticide monitoring results published by EFSA, overall 97.2% of the 84.341 samples analysed were free of residues or contained residues within the legally permitted levels (53.3% of the samples tested were free of quantifiable residues, while 43.9% of the samples analysed contained measurable residues not exceeding the permitted residue concentrations). A total of 2.8% of the samples exceeded the MRLs permitted in the EU legislation. EFSA calculated the short-term (acute) and long-term (chronic) exposure, and based on the results EFSA concluded that the residues of pesticides are not likely to pose a health risk of European citizens, and the probability of negative health outcomes was low. The results are comparable with the year 2014: 97.1% of samples within the legal limits, and 53.6% free of quantifiable residues (EFSA, 2017). In Croatia during a period 2007-2009, a total of 866 samples of 28 different kinds of fresh fruits and vegetables from import and domestic production were analyzed. In 67.1% of the samples no residues were found, 27.3% of samples contained pesticide residues at or below MRL, and 5.6% of samples contained pesticide residues above MRL. The short-term exposure assessment revealed that for 12 food samples analyzed the ARfD might have been exceeded if the food sample was consumed in high amounts. The short-term risk assessment could not be performed for seven pesticides because there are no available data on acute reference doses (Knezevic *et al.*, 2012). In the other study in Croatia, a total of 650 samples of fruits, vegetables and cereals were analysed, and it was found that 625 samples did not contain measurable values of the active substances and 25 samples showed the presence of some active ingredients of pesticides above the legal tolerance limits. An acute risk exposure was calculated and results showed that out of 650 analysed samples MRL exceed 4% and acute risk was found for 1% of the samples. It was concluded that acute risk for Croatian consumers can be considered rare, except for oranges and lettuce with high concentrations of pesticides for all consumers groups, especially for children (Milos *et al.*, 2014). A total of 147 samples of fruits were collected from Egyptian markets throughout 2007, and samples were examined for residues of 86 pesticides. Of the analysed samples, 53% contained detectable residues whereas 47% had no detectable amounts. In two samples pesticide residue levels exceeded MRL (*cypermethrin* in one sample of grapefruit and *deltamethrin* in one sample of plum). An acute dietary exposure assessment was performed, and the values of estimated short-term intake as a percentage of ARfD ranged from 0 up to 14% indicating a minimum acute risk from the detected pesticides. No health hazard was found associated with the consumption of the studied fruits for adults (Gad *et al.*, 2013). According to Brazilian pesticide residue monitoring programs between 2001 and 2010, a preliminary acute exposure assessment for organophosphates and carbamates has shown that the intake by individuals ≥10 years old accounts for 100% of the ARfD, indicating a need to further investigate the exposure through the consumptions of other crops and group of pesticides, mainly for children (Jardim *et al.*, 2012). A total of 815 samples were analysed during the monitoring period 2008-2009 in Greece, and only 3.06% of samples from all food

commodities exceeded the MRLs. The risk assessment was performed for the four most frequently detected chemical categories. In samples from biological cultivations (grapes and olive oil) the values of estimated short-term intake as a percentage of ARfD ranged from 0.01 up to 0.82 indicating a minimum acute risk from the detected pesticides (Tsakiris *et al.*, 2011). In Poland short-term (acute) exposure was estimated, and the results showed that total % of ARfD for all pesticides was low, for adults did not exceed 3%, while for young children was slightly above 7.5% (Łozowicka *et al.*, 2012).

5. Conclusion

In the investigation, a total of 23 citrus fruit samples were analyzed and the level of pesticide residues exceeded the MRL value in two samples. Based on the results of the research, it can be concluded that the presence of most of the pesticides in citrus fruits from the BiH market is not a threat to human health. Quantitative risk assessment found that the entry of certain pesticide residues under acute exposure conditions in the examined population is within the limits of the reference toxicological values. Research findings indicate the importance of continuous monitoring of pesticide residues with the aim of assessing the dietary intake and assessing the likelihood and seriousness of negative health effects.

References

[1] Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC.

[2] JMPR, 2006, Short-term dietary intake assessment: uncertainties in the International Estimated Short-Term Intake (IESTI) calculation and its interpretation. In: Pesticide residues in food 2006. Report of the Joint Meeting of the FAO panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues, Rome, Italy, 3-12 October 2006. FAO Plant Production and Protection Paper 187: 8-12

[3] EFSA, 2007, Opinion of the Scientific Panel on Plant protection products and their Residues on a request from the Commission on acute dietary intake assessment of pesticide residues in fruit and vegetables. *EFSA Journal*, 538, pp 1-88.

[4] Pravilnik o maksimalnim nivoima ostataka pesticida u i na hrani i hrani za životinje biljnog i životinjskog porijekla („Službeni glasnik BiH“, broj 89/12).

[5] Poulsen, M. E., J. H. Andersen, 2003, Results from the monitoring of pesticide residues in fruit and vegetables on the Danish market, 2000-01. *Food Addit Contam*, 20(8), pp 742-757.

[6] Berrada H., Fernández M, Ruiz M.J., Moltó J.C., Mañes J., 2006, Exposure assessment of fruits contaminated with pesticide residues from Valencia, 2001-03. *Food Addit Contam*, 23(7), pp 674-682.

[7] Zicari G., Soardo V., Cerrato E., D. Rivetti D., 2011, Results from the monitoring of pesticide residues in fruits and vegetables marketed in Piedmont (Italy), 2000-2008. *Ig Sanita Pubbl*, 67(2), pp 149-168.

[8] Łozowicka B, Kaczyński P., Rutkowska E., Jankowska M., Hrynko I., 2013, Evaluation of pesticide residues in fruit from Poland and health risk assessment. *Agricultural Sciences*, 4(5B): pp 106-111.

[9] Ortelli D, Edder P., Corvi C., 2005, Pesticide residues survey in citrus fruits. *Food Addit Contam*, 22(5): pp 423-428.

[10] Taga O., Bilgin B., 2010, Determination of 107 Pesticide Residues in Citrus Fruits by Gas Chromatography/Mass Spectrometry. *Asian J Chem*, 22(3): pp 2367-2374.

[11] EFSA, 2017, The 2015 European Union report on pesticide residues in food. *EFSA Journal*, 15(4), pp 4791.

[12] Knežević Z, Serdar M., Ahel M., 2012, Risk assessment of the intake of pesticides in Croatian diet. *Food Control*, 23(1), pp 59-65.

[13] Miloš S., Jurković Z., Raspudić E., Lončarić Z., 2014, Risk assessment of pesticide residues in food on Croatian market: Should we worry? *Journal of Hygienic Engineering and Design*, 8, pp 74-81

[14] Gad Alla Sohair A, Ayoub M.M., M. Amer A.,Thabet W.M.,. 2013, Dietary Intake of Pesticide Residues in some Egyptian Fruits. *J Appl Sci Res*, 9(1), pp 965-973.

[15] Jardim A.N.O., Caldas E.D., 2012, Brazilian monitoring programs for pesticide residues in food e Results from 2001 to 2010. *Food Control*, 25(2), pp 607-616.

[16] Tsakiris I.N., Toutoudaki M., Kokkinakis M., Paraskevi M., Tsatsakis A.M, 2011, A Risk Assessment Study of Greek Population Dietary Chronic Exposure to Pesticide Residues in Fruits, Vegetables and Olive Oil. In: Stoytcheva M., editor. Pesticides - Formulations, Effects, Fate, InTech, pp 253-268.

[17] Łozowicka B., Rutkowska E., Jankowska M., Kaczyński P., Hrynko I., 2012, Health risk analysis of pesticide residues in berry fruit from North-Eastern Poland. *J Fruit Ornam Plant Res*, 20(1), pp 83-89.

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