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ASSESSMENT OF HUMAN HEALTH RISK OF TWELVE PESTICIDES APPLIED IN DOUBLE DOSEIN AN APPLE ORCHARD

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Abstract. *The aim of this work was to assess the human health risk associated with the consumption of a variety of apples for which five treatments with pesticides in double dose were applied. Six fungicides, five insecticides, and one acaricide were used in the study. Human health risk analyses were based on fruit consumption data released by Freshfel Consumption Monitor of 167.62 g/person/day on average for the European member states (EU-28) in 2014, for 2012. The results of health risk analysis based on consumption data in EU-28 revealed that the pesticide propargite can pose a risk to children health, when applied in double dose, even in two months after harvest. When compared to reference doses (RfDs), the lifetime exposure dose values for each of the analysed pesticides are suggesting that risks to adults and children in their lifetime are negligible.*

Keywords: fruit consumption estimates, lifetime exposure dose, pesticides, risk assessment

1. Introduction

Production of plant protection products based on pesticides continues to be a standard practice in agriculture, since these chemicals play a central role in yields improvement. Despite of numerous disputes among researchers or farmers

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regarding the opportunity of pesticide use for plant protection, the reality is that pesticides protect yields by limiting the losses caused by competitions with weeds and from attacks of insects, or protect plants against diseases. However, the use of pesticides is carefully regulated worldwide by authorities entrusted with this matter, because of the risks that pesticides can generate for human health, as a result of the consumption of agro-food products treated with pesticides [1].

Nowadays, food quality control is being recognized as one of the most important tasks that should be taken into account when human health is under concern. The EU established regulations to ensure the integrated pest management leading to the sustainable use of pesticides so as to diminish or eliminate the impacts and risks that pesticides could generate for environment and human health [2]. All products must be in a strict compliance with legislation in order to guarantee the consumers health. Consumption of pesticide residues along with vegetables, fruits, grains, which have undergone various treatments is a critical aspect of farming practices used to combat diseases and plant pests [3 - 5]. Human exposure to pesticides can result from consumption of fruits and vegetables containing residues of pesticides sprayed on plants or by inhalation and ingestion. Therefore, an important side effect of pesticide use consists in pursuing potential threats to human health and environmental quality.

In recent years the concern for diminishing the risk of pollutants in different environmental compartments grew continuously [6 - 12]. The study of Reiss et al. [13] appraised that almost 20,000 cancer cases per year could be stopped by an increase in fruit and vegetable consumption, while up to 10 cancer cases per year could be due to the excess of pesticide intake along with fruits and vegetables consumption. This contradictory situation imposes acutely the control of the quantity of pesticides in fruits and vegetables by continuous monitoring programs. In many countries, in the last 10 years, authorities have focused on the surveillance of proper pesticides use according to Good Agricultural Practices (GAP) and in compliance with Maximum Residue Levels (MRLs) [4, 5, 14]. On the other side, the use of pesticides in agriculture has several advantages such as controlling the numbers of pests destroying plants or stimulating or inhibiting plant-growth processes. A more detailed list with advantages and disadvantages of pesticide use is compiled in the work of Fenik et al. [15].

Although several studies can be found in literature concerning pesticides in fruit samples from markets [16-21], very few are focusing on the application of pesticides according to fruits phenological growth phases. In this context, Pogacean et al. (2014) [22] addressed the behavior of pesticides used in the treatment of a variety of apples from a Romanian orchard, considering recommended dosages at different stages of fruit development. The estimated lifetime exposure doses for adults and children were below the reference dose

(RfD) for all pesticides, suggesting a negligible risks for consumers. However, there are studies that indicate that double doses of pesticides can endanger organisms' health [23, 24].

Considering the above information, the goals of our study were: i) to investigate the residual concentration of several pesticides used in common practice of apple treatment, applied in double dose and ii) to estimate the human health risk (for adults and children) to each of the analyzed pesticides in apples at harvest and two months after harvest.

2. Materials and methods

2.1. Reagents

Chem Service (West Chester, SUA) and Sigma Aldrich Laborchemikalien GmbH (Seelze, Germany) provided the pesticides analytical standards with a certified purity between 95.1% and 99.7%, while Super Purity Solvents like acetone, petroleum ether, dichloromethane, toluene and isooctane were purchased from Fluka & Riedel-de Haën (Sigma-Aldrich, UK). A Thermo Scientific TKA system (Niederelbert, Germany) guarantees the distilled water used for samples analysis. All standard solutions were dissolved in toluene and later stored in a refrigerator at 4^{o}C. Dafcochim SRL (Tg. Mures, Romania) and Chemark Rom SRL (Tg. Mures, Romania) provided the pesticides which were applied in the field study.

2.2. Gas Chromatography - Mass Spectrometry (GC-MS) Analysis

The residual pesticides analysis was assessed by Agilent 7890 gas chromatograph type with 2 ovens coupled with a mass spectrometer with flight time, CG*GC-TOF-MS Pegasus 4.21 (LECO, SUA). The conditions for gas chromatography analysis were: capillary column Rxi-Ms (30m*0.25mm*0.25µm) as main oven and BPX-50 (1.6m*0.1mm*0.1µm) as secondary oven. Helium was used as carrier gas and make-up gas, at a flow rate of 1.0 mL/min. The injector temperature was set at 250^{o}C. The oven temperature was programmed as follows: main oven, 70^{o}C hold for 1 min, ramp at 20◦C/min to 140^{o}C, hold for 1 min, ramp at 5^{o}C/min to 310^{o}C, hold for 4 min; secondary oven, 95^{o}C hold for 1 min, ramp at 20^{o}C/min to 165^{o}C, hold for 1 min, ramp at 5^{o}C/min to 330^{o}C, hold for 4 min. The injection volume of the GC was 1.0 µL. The conditions used for the mass spectrometer analysis were: ion source temperature, 220^{o}C, ionization mode EI, 70 eV, detector Voltage 1800, Start mass 40, End start 450, Acquisition Rate *spectre/second, 5, temperature of transfer, 280^{o}C and time of analysis, 43 min. The high-performance auto sampler software enables the syringe washing with several solvents (at least four different solvents in the same washing phase) to end the contamination. The major ions (m/z) and retention time (t_R) were considered for pesticide identification and are described in detail by Pogacean et al. [22].

2.3. Field survey

A field survey was conducted from May 2012 to August 2012 at the Phytosanitary Office Mureş (Romania). We have applied six fungicides (captan, folpet, myclobutanil, tebuconazole, chlorolothalonil and triadimenol), five insecticides (bifenthrin, deltamethrin, alpha-cypermethrin, lambda-cypermethrin, chlorpyrifos-methyl) and one acaricide (propargite), in double dose in 5 treatments in an orchard of Jonathan apples according to the phenological growth phases considering the BBCH scale (Biologische Bundesanstalt, Bundessortenamt and Chemical industry) (Table 1). The BBCH scale represents a system for a uniform coding of phenologically-similar growth stages of all mono- and dicotyledonous plant species [25, 26].

We have ensured a buffer zone for the apples subjected to the experiment. Samples were collected in 15 days after pesticides application and were prepared according to the procedure presented in Pogacean et al. [22]. All samples were analyzed at the GC-MS.

Table 1) Phenological growth stage of apples and application dates

No	*BBCH scale*	*Fruit size*	*Pesticides application date*
1	72-73	20-25 mm	28.05.2012
2	74	30-40 mm	20.06.2012
3	75	1/2 normal size	13.07.2012
4	76-79	2/3 normal size	5.08.2012
5	81-85	at ripening	27.08.2012
6	91-99	at harvest	-
7	-	2 months after harvest	-

2.4. Human Health Risk Assessment

We have estimated the human health risk considering the pesticides concentration in apples at harvest and two months after harvest. According to Freshfel Consumption Monitor which analyses trends in the production, trade and supply of fresh fruits and vegetables across the EU-28, per capita fruit consumption in 2012 was estimated as 167.62 g/capita/day [27]. Food consumption analysis is essential for risk estimation. Moreover, the estimated lifetime exposure dose (mg/kg/day), food consumption (kg/person/day) and body weight (kg) were used to determine if there are any health risks to consumers posed by pesticide residues in apples, when applied in double dose.

Based on food consumption rate for fruits in Europe, the estimated lifetime exposure dose (mg/kg/day) was obtained by multiplying the residual pesticide concentration (mg/kg) in the apple samples with the food consumption rate (kg/person/day), and dividing the product by the body weight (kg) [5, 22, 28].

A comparison of exposure estimates with toxicological endpoints such as Reference Doses (RfDs) was also addressed. The RfD is defined as an estimate of daily or continuous exposure of human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime [29-31]. When RfDs values were not available, the analogous to RfD, Acceptable Daily Intake (ADI) values, were used as substitutes.

For human health risk assessment, we have considered the U.S Environmental Protection Agency's guidelines: 1) maximum absorption rate is 100% and 2) bioavailability rate is 100% [30, 32]. The average body weight of adults in Europe was estimated as 70.8 kg [33] (Walpole et al., 2012) and as 23.1 kg for children (age group, 3 to < 10 years [34]).

For a more accurately human health risk estimation of pesticide residues from Jonathan apples we have assessed the hazard indices (HI) for adults and children based on the ratio between estimated pesticide exposure doses and the corresponding RfDs [5, 22]. For a HI higher than 1, the apples subjected to analysis can be considered a risk to consumers, while a HI lower than 1 is considered as an acceptable limit with no risk to human health [5, 10, 22, 32].

3. Results and discussion

The pesticides concentration in apples determined in 15 days after application in double dose correlated with the corresponding BBCH stage of apple growth is presented in Table 2. As seen from the field survey data presented in this Table, the highest concentration at harvest (BBCH 91-99) is found for chlorothalonil and propargite. The Maximum Residue Level (MRL) established by the European Union legislation for these pesticides is lower than 1 mg/kg for chlorothalonil and < 3 mg/kg for propargite (https://secure.pesticides.gov.uk/MRLs). In addition to chlorothalonil and propargite, the pesticides that exceed the MRL at harvest and could pose a threat to human health when applied in double dose are tebuconazole, bifenthrin and lambda-cyhalothrin. In two months after harvest, chlorothalonil, tebuconazole and bifenthrin still exceeded the MRL. Table 3 includes lifetime exposure doses for each of the analyzed pesticide applied in our study, at harvest and two months after harvesting, considering both adults and children.

Table 2. Pesticides concentration (mg/kg) in Jonathan apples according to BBCH scale

Pesticides	*MRL* (mg/kg)*	*BBCH scale*						
		72-73	*74*	*75*	*76-79*	*81-85*	*91-99*	*2 months after harvest*
Captan	<3	5.06	4.34	3.99	4.19	2.4	1.72	0.63
Folpet	<3	5.25	4.91	4.62	5.47	4.43	2.11	0.96
Triadimenol	0.2	0.41	0.5	0.38	0.45	0.22	0.08	0.03
Myclobutanil	<0.5	1.01	1.15	0.6	1.05	0.92	0.27	0.09
Chlorothalonil	<1	15.75	14.52	7.25	8.01	6.27	3.52	1.09
Tebuconazole	<1	2.12	2.65	1.78	2.63	2.16	1.94	1.14
Chlorpyrifos-methyl	<0.5	0.65	0.55	0.54	0.66	0.22	0.09	0.05
Bifenthrin	<0.3	0.57	0.80	0.55	0.07	0.43	0.36	0.31
Alfa-cypermethrin	<0.2	0.78	0.18	0.21	0.36	0.19	0.11	0.07
Lambda-cyhalothrin	<0.1	0.26	0.27	0.33	0.44	0.22	0.11	0.07
Deltamethrin	<0.2	0.47	0.44	0.32	0.42	0.11	0.08	0.01
Propargite	<3	2.12	5.62	4.8	5.39	4.89	3.47	2.85

**MRL - Maximum Residue Level set by European Union legislation (https://secure.pesticides.gov.uk/MRLs)*

Table 3. Lifetime exposure dose calculated for pesticides applied in apples at harvest and 2 months after harvest

Pesticides	*Reference dose (mg/kg/day)*	*Lifetime exposure dose (mg/kg/day)*			
		At harvest		*2 months after harvest*	
		Adults	*Children*	*Adults*	*Children*
Captan	0.13	0.0040	0.0124	0.0014	0.0045
Folpet	0.1	0.0049	0.0153	0.0022	0.0069
Triadimenol	0.05*	0.0002	0.0006	7.1E-05	0.0002
Myclobutanil	0.003*	0.0006	0.0019	0.0002	0.0006
Chlorothalonil	0.015	0.0083	0.0255	0.0025	0.0079
Tebuconazole	0.03*	0.0045	0.0140	0.0026	0.0082
Chlorpyrifos-methyl	0.01*	0.0002	0.0006	0.0001	0.0003
Bifenthrin	0.015	0.0008	0.0026	0.0007	0.0022
Alfa-cypermethrin	0.01	0.0002	0.0007	0.0001	0.0005
Lambda-cyhalothrin	0.005	0.0002	0.0007	0.0001	0.0005
Deltamethrin	0.01*	0.0001	0.0005	2.3E-05	7.2E-05
Propargite	0.02	0.0082	0.0251	0.0067	0.0206

**ADI*

These data demonstrate that the lifetime exposure dose values for each of the analyzed pesticides do not exceed the RfD, suggesting that risks to adults and children in their lifetime could be considered as negligible.

As seen from Fig. 1, a health risk to children is posed due to HI > 1 for chlorothalonil and propargite residues in apples at harvest. In two months after harvest (Fig. 2), only propargite residues can pose a threat to children health.

These results indicate that there is a risk associated with apples consumption if pesticides are applied in double dose, particularly for children. With respect to the other pesticides, although their concentration exceeds in some cases the MRLs, the hazard indices were less than 1, indicating that these pesticides do not represent a hazard to human health.

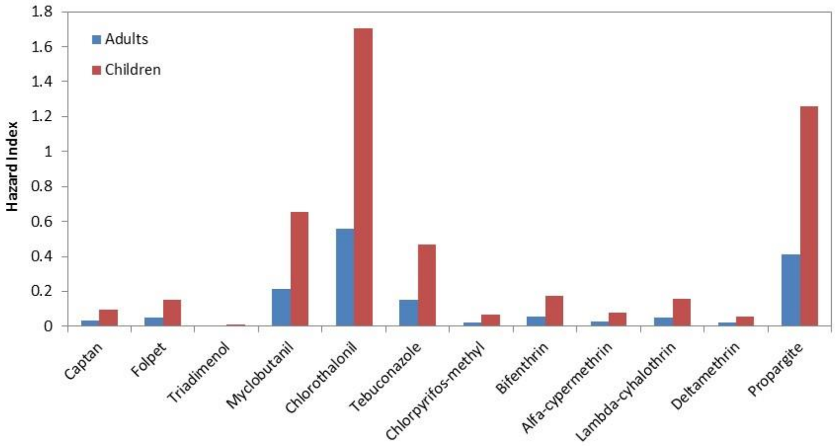

Fig. 1. Hazard Index calculated for pesticides concentration in apples at harvest

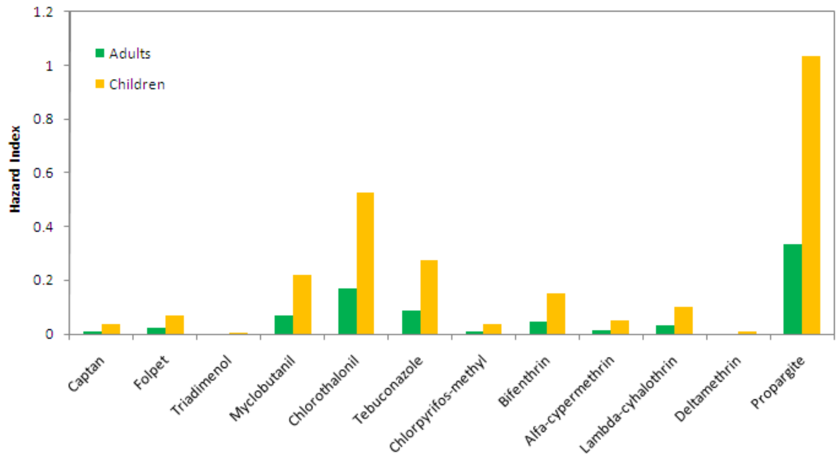

Fig. 2. Hazard Index calculated for pesticides concentration in apples at 2 months after harvest

Conclusions

Data analysis of health risks assessment indicate that, although some of the pesticides exceed the MRLs, only chlorothalonil and propargite residues in apples at harvest could pose a threat to children health due to a value of HI > 1. Considering data analyzed in two months after harvest, our results indicate that only the propargite residues in apples can pose a threat to children health.

Therefore, the application of chlorothalonil and propargite in double dose on apples could pose a threat to children health (age group 3 to < 10 years), although when compared to RfDs, the lifetime exposure doses don't exceed these values, suggesting that risks to adults and children in their lifetime are negligible.

However, control strategies are opportune in this context to ensure as few possible side effects on beneficiaries. In this respect, the exploitation of existing knowledge regarding the side effects of pesticides on agro-food products together with new results, obtained following the development of new research could be an advantageous way to promote strategies associated to Integrated Pest Management.

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