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Professional paper

DETERMINATION OF PESTICIDE RESIDUES (INSECTICIDES) IN APPLES AND DIETARY RISK ASSESSMENT

Vezirka JANKULOSKA[1*], Ilija KAROV[2], Gorica PAVLOVSKA[1]

[1]Faculty of Technology and Technical Sciences - Veles, "St. Kliment Ohridski" University-Bitola, Republic of North Macedonia*
[2]Faculty of Agriculture, University Goce Delchev – Shtip, Republic of North Macedonia
**Corresponding Author: e-mail: vezirka.jankuloska@uklo.edu.mk*

Abstract

Fruit is an inevitable part of a balanced diet due to the presence of bioactive components, dietary fibres, vitamins, and minerals. Apples, as a crop primarly grown in the Resen region, are frequently consumed by both local residents and a wider global audience, as they are exported to other countries from this region. Throughout their cultivation, apples are treated with pesticides to ensure disease protection and maximize income. Consequently, the quantity of pesticide residues, as well as the quality and safety of apples, consistently garner significant attention within the realm of science, particularly in the context of risk assessment and their potential impact on health. To address these concerns, an analysis of thiamethoxam, acetamiprid and imidacloprid in apples was conducted using the UPLC-TQ/MS method, following prior extraction through the QuEChERS method. Risk assessments were performed using established benchmarks such as the Acceptable Daily Intake (ADI) and the Acute Reference Dose (ArfD). After processing the data and performing the assessment, it can be noted that the value of HQa (acute risk), HQc (chronic risk) as well as the cumulative risk of most pesticides (HI) detected in apples is less than 1 and there is no risk to the health of adults and the children. We suggest a reasonable use of pesticides and respecting the pre-harvest interval (PHI) to better protect the health of consumers.

Keywords: pesticides, risk assessment, apples, nutrition, consumer health

1. Introduction

Pesticides encompass a diverse group of active substances used for plant protection. As of May 2023, the European Commission has reviewed 1483 active substances, of which 449 have been approved,while the remainder are pending approval or have been rejected/disallowed (European Commission, 2023).

Thiamethoxam, acetamiprid and imidacloprid belong to the group of non-nicotinoid insecticides. Their chemical structures act as potent agonists of nicotinic acetylcholine receptors in insects (Wallace, 2014). Thiamethoxam is regulated by the European Commission with Reg. 07/6/EC, 2010/21/EU, Reg. (EU) 2022/801, Reg. (EU) No. 2018/524, Reg. (EU) No. 2018/785, Reg. (EU) No. 485/2013, Reg. (EU) No. 487/2014, Reg. (EU) No. 540/2011. However, concerns about its impact on bees persist, leading to its disapproval for use since 30/04/2019.

Acetamiprid is used to control insects such as aphids that infest plant leaves. According to the EU, acetamiprid has been authorized for use from 2018 to 2033 and is governed by 04/99/EC, Reg. (EU) 2016/2016, Reg. (EU) 2018/113, Reg. (EU) No. 1197/2012, Reg. (EU) No. 540/2011 (European Commission EU Pesticides Database (v.2.1)).

Imidacloprid has found widespread application in various crops. According to Sheets (2014), it has been established that imidacloprid does not possess reproductive or developmental toxicity, teratogenicity, mutagenicity, immunotoxicity, or carcinogenicity. However, as indicated by El-Gendy et al. (2010), imidacloprid can induce oxidative stress and damage essential biological molecules such as proteins, lipids, and DNA (deoxyribonucleic acid). Initially approved by the European Commission in 2009, the use of imidacloprid was discontinued on December 1, 2020, as indicated in regulations (2008/116/EC, 2010/21/EU,

Reg. (EU) 2017/195, Reg. (EU) 2020/1643, Reg. (EU) 2022/801, Reg. (EU) No. 485/2013, Reg. (EU) No. 540/2011, Reg. (EU) No. 2018/783)."

While it is inevitable for the population to be exposed to pesticides through food, excessive levels of pesticide residues can have detrimental effects on human health. Assessing the risk to human health resulting from pesticide exposure requires careful consideration of various factors, including the method of pesticide entry into the body, duration of exposure, pesticide toxicity, and underlying mechanisms. Extensive literature indicates that pesticides can lead to adverse health outcomes, such as cancer, congenital disorders, immunological and respiratory issues, neurobehavioral problems, and reproductive deficits. Furthermore, pesticides can induce genetic damage, epigenetic changes, mitochondrial dysfunction, oxidative stress, endoplasmic reticulum stress, and endocrine disruption in various tissues and organs (Eddleston et al. 2008; Hernandez et al. 2013).

In recent years, there has been an increasing consumer awareness of healthy food choices and nutrition, leading individuals to consciously avoid processed foods and choose fresh and unprocessed alternatives, such as fruits and vegetables. Fruits, including apples, are particularly valued for their high content of vitamins, minerals, dietary fibers, and antioxidants, which contribute to reducing the risk of various diseases such as heart disease, diabetes, cancer, and inflammatory conditions (Madureira et al. 2023). Nutritionists recommend and encourage consumers to incorporate at least five portions of fruits and vegetables into their daily diet (Jankuloska et al. 2020).

According to O'Neil et al. (2015), the average consumer's consumption of apple products is 222.2±3.9 g, and one apple is equivalent to 143±3.8 g (approximately 1 cup). The dietary intake of pesticide residues in apples is calculated by multiplying the residue level in the apple by the quantity of apple consumed (Jankuloska et al. 2020).

The aim of this study was to determine pesticide residues in apples and estimate the dietary risk assessment for both adults and children.

2. Material and methods

This study focused on the detection of insecticides (thiamethoxam, acetamiprid, and imidacloprid) in Idared apples cultivated in Resen, Republic of North Macedonia. The samples underwent analysis using ultra-performance liquid chromatography-triple quadruple mass spectrometry (UPLC-TQ/MS) (Agilent UPLC 1290; temperature: 35°C; flow rate: 0.4 ml/min; injection volume: 0.7µl; with a DAD and TQ) following the QuEChERS method for residue extraction, as outlined in the MKC EN 15662:2011 standard (Anastassiades et al. 2013; Jankuloska et al. 2020). The obtained data were processed using XLSTAT software, version 2018.1.49561, developed by Addinsoft (Paris, France).

The values of Acceptable Daily Intake (ADI) and Acute Reference Dose (ARfD) were sourced from the European Pesticide Database of the European Commission. To evaluate associated risks, various equations were utilized, including Estimation of Daily Intake (EDI), Estimation of Short-term Intake (ESTI), Chronic Hazard Index (HQc), Acute Hazard Index (HQa), and cumulative risk assessment - Hazard Index (HI) (as shown in Table 1)

Table 1. Equations for risk assessment calculation

No. Equation	Equation	References
(1)	$\mathrm{EDI}=\sum \frac{\mathrm{FxRL}}{\mathrm{body}_{\mathrm{weight}}(\mathrm{bw})}$	WHO, 1997 Łozowicka *et al.* 2013 Liu *et al*. 2023
(2)	$\mathrm{ESTI}=\sum \frac{\mathrm{FxHR}.P}{\mathrm{body}_{\mathrm{weight}}(\mathrm{bw})}$	Tsoutsi *et al.* 2008 Hawari *et al.* 2019 El-Sheikh *et al.*2023

(3)	$HQc = \frac{EDI}{ADI}$	Łozowicka *et al.* 2013 Hawari *et al.* 2019 Liu *et al.* 2023
(4)	$HQa = \frac{ESTI}{ARfD}$	Tsoutsi *et al.* 2008 Hawari *et al.* 2019 Liu *et al.* 2023
(5)	$HI = \sum HQc$	Hawari *et al.* 2019 Tripathy *et al.* 2022

*RL is the average residual of pesticide in mg/kg; F is the food consumption data in kg/day; bw is the consumer body weight (60 kg) for adults and (15 kg) for children; HR.P is highest residue level

3. Results and Discussion

3.1. Detected pesticide residues in apples: The concentration of pesticide residues (insecticides) in the analyzed apples are given in table 2. Additionally, the ADI and ArfD for thiamethoxam, imidacloprid and acetamiprid are also given.

Table 2. Detected insecticides in the analysed sample

Pesticide	Concentration (mg/kg)	ADI (mg/kg bw)	ArfD (mg/kg bw)
Thiamethoxam	0.02	0.026	0.5
Acetamiprid	0.0125	0.025	0.025
Imidacloprid	0.0038	0.06	0.08

3.2. Health Risk assessment:

3.2.1. Short -term (acute) intake risks assessment: Consumers are consistently exposed to pesticides through their daily consumption of fruits and vegetables, which can result in acute effects with short-term exposure and chronic effects with prolonged exposure (Hawari et al. 2019; Jankuloska et al. 2020). To ensure consumer safety remains a priority, our focus will be on conducting a risk assessment specifically addressing short-term (acute) intake.

By considering the obtained values (see Table 3), we can assess whether consumers face any acute risks when consuming apples containing the mentioned pesticide concentrations. To calculate the acute exposure risk (HQa), it is crucial to perform the Estimate of Short-Term Intake (ESTI), as detailed in equation 2.

Table 3. Estimate of Short-Term Intake (ESTI) (mg / kg bw) from exposure to pesticide residues in adults and children

Pesticide	$ESTI_{a1}$	$ESTI_{ch1}$	$ESTI_{a2}$	$ESTI_{ch2}$	$ESTI_{a3}$	$ESTI_{ch3}$
Thiamethoxam	$1x10^{-4}$	$4x10^{-4}$	$1{,}5x10^{-4}$	$6x10^{-4}$	$2x10^{-4}$	$8x10^{-4}$
Acetamiprid	$3.3x10^{-5}$	$1.3x10^{-4}$	$5x10^{-5}$	$2x10^{-4}$	$6.6x10^{-5}$	$2.6x10^{-4}$
Imidacloprid	$1.6x10^{-4}$	$6.6x10^{-4}$	$2.5x10^{-4}$	$1.0x10^{-4}$	$3.3x10^{-4}$	$1.3x10^{-3}$

*$ESTI_{a1}$ - (100 g apples); $ESTI_{a2}$ - (150 g apples); $ESTI_{a3}$ - (200 g apples) for adults;
*$ESTI_{ch1}$ -(100 g apples); $ESTI_{ch2}$ - (150 g apples); $ESTI_{ch3}$ - (200 g apples); $ESTI_{ch}$ for children;

Considering the values outlined in Table 4, it can be deduced that children face the highest acute risk when consuming 200 g of apples. This is evident from the HQa of 1.6x10-2 for imidacloprid and 1.04x10-2 for acetamiprid. Notably, the HQa values for imidacloprid and thiamethoxam in adults are nearly equivalent, but

for children, they are lower, ranging from approximately 4x10-4 to 8x10-4. These values are the lowest among all the pesticides, indicating that children exhibit the lowest acute risk in relation to thiamethoxam.

Table 4. Acute exposure risk (HQa) from pesticide residues in adults and children

Pesticide	HQa_{a1}	HQa_{ch1}	HQa_{a2}	HQa_{ch2}	HQa_{a3}	HQa_{ch3}
Thiamethoxam	$2x10^{-3}$	$8x10^{-4}$	$3x10^{-3}$	$1.2x10^{-3}$	$4x10^{-4}$	$1.6x10^{-3}$
Acetamiprid	$1.32x10^{-3}$	$5.2x10^{-3}$	$2x10^{-3}$	$8x10^{-3}$	$2.64x10^{-3}$	$1.04x10^{-2}$
Imidacloprid	$2x10^{-3}$	$8.25x10^{-3}$	$3.12x10^{-3}$	$1.25x10^{-3}$	$4.16x10^{-3}$	$1.6x10^{-2}$

*aHI_{a1} - (100 g apples); aHI_{a2} - (150 g apples); aHI_{a3} - (200 g apples); aHI_a for adults;
*aHI_{ch1} - (100 g apples); aHI_{ch2} - (150 g apples); aHI_{ch3} - (200 g apples); aHI_{ch} for children;

Values of HQa < 1 are generally regarded as acceptable, indicating no immediate threat to short-term health. However, if the HQa values exceed 1, it signifies an unacceptable level of risk. Moreover, higher HQa values correspond to increased exposure to acute risks. The obtained values presented in table 4 for HQa indicate that they are less than 1 and we can conclude that the risk to the health of adults and children is insignificant. Similar results were obtained by Liu *et al.* (2023), where HQa is in the range of $1.17x10^{-4}$-$2.66x10^{-1}$, while our values for children are in the range of $1.04x10^{-2}$-$8x10^{-4}$, and for adults is in rank of $1.32x10^{-3}$- $4x10^{-4}$ but still less than 1.

3.2.2. Long - term (chronic) intake and cumulative risk assessment: We will utilize the outcomes of the pesticide residue analysis in apples (Table 2) to conduct a comprehensive risk assessment concerning human health within a diet containing pesticides. Tables 5 and 6 present the findings for the Estimated Daily Intake (EDI) and the chronic exposure index (HQc) for adults and children under the influence of insecticides when consuming varying quantities of apples each day: 100 g, 150 g, and 200 g.
Given the absence of published and available information on the National Estimated Daily Intake (NEDI) (mg/kg bw) within our country, this study has taken into consideration assumed amounts—specifically, the highest quantities of apples that could be consumed daily (Jankuloska et al. 2020). In a similar vein, Chen et al. (2021) calculated a recommended fruit intake of 0.15 kg for children and 0.35 kg for adults to establish the maximum risk.

Table 5. Estimated daily intake (EDI) (mg x kg bw) from exposure to pesticide residues in adults and children

Pesticide	EDI_{a1}	EDI_{ch1}	EDI_{a2}	EDI_{ch2}	EDI_{a3}	EDI_{ch3}
Thiamethoxam	$3.3x10^{-5}$	$1.3x10^{-4}$	$5x10^{-5}$	$2x10^{-4}$	$6.6x10^{-5}$	$2.6x10^{-4}$
Acetamiprid	$2.08x10^{-5}$	$8.3x10^{-5}$	$3.13x10^{-5}$	$1.25x10^{-4}$	$4.16x10^{-5}$	$1.66x10^{-4}$
Imidacloprid	$6.3x10^{-6}$	$2.53x10^{-5}$	$9.5x10^{-6}$	$3.8x10^{-5}$	$1.26x10^{-5}$	$5.06x10^{-5}$

*EDI_{a1} for 100 g apples; EDI_{a2} for 150 g apples; EDI_{a3} for 200 g apples; EDIa for adults
*EDI_{ch1} for 100 g apples; EDI_{ch2} for 150 g apples; EDI_{ch3} for 200 g apples; EDI_{ch} for children

As it is shown in table 5, the EDI values for both adults and children who consume varying quantities of apples remain below the published Acceptable Daily Intake (ADI). Nevertheless, it is discernible that EDI values escalate with higher apple/fruit consumption, with a pronounced increase among children. According to WHO guidelines from 1997, the projected dietary intake of pesticide residues, stemming from pesticide application and other sources, should ideally remain lower than the established ADI.

This aligns with findings from Liu et al. (2023), who encountered similar outcomes while analyzing pesticides in four distinct types of fruit.

Table 6. Chronic exposure index (HQc) and hazard index (HI) from exposure to pesticide residues in adults and children

Pesticide	HQc_{a1}	HQc_{ch1}	HQc_{a2}	HQc_{ch2}	HQc_{a3}	HQc_{ch3}
Thiamethoxam	$1.2x10^{-3}$	$5x10^{-3}$	$1.9x10^{-3}$	$7.6x10^{-3}$	$2.5x10^{-3}$	$1x10^{-2}$
Acetamiprid	$8.32x10^{-4}$	$3.32x10^{-3}$	$1.25x10^{-3}$	$5x10^{-3}$	$1.66x10^{-3}$	$6.64x10^{-3}$
Imidacloprid	$1.05x10^{-4}$	$4.2x10^{-4}$	$1.58x10^{-4}$	$6.3x10^{-4}$	$2.1x10^{-4}$	$8.4x10^{-4}$
HI	$2.137x10^{-3}$	$8.74x10^{-3}$	$3.308x10^{-3}$	$1.323x10^{-2}$	$4.37x10^{-3}$	$1.748x10^{-2}$

*HQc_{a1} (100 g apples); HQc_{a2}- (150 g apples); HQc_{a3} - (200 g apples) HQc_a for adults;
*HQc_{ch1} - (100 g apples); HQc_{ch3} - (150 g apples); HQc_{ch3} - (200 g apples) HQc_{ch} for children;

HQc serves as an indicator of risk acceptability, with values surpassing 1 signaling an unacceptable risk, and higher values correlating to increased risk levels. When HQc falls below 1, the risk is classified as acceptable, posing no sustained health threat over the long term (Liu et al. 2023). As evident from our findings, HQc values for both children and adults are below 1, indicating an acceptable risk level. However, it is notable that for the pesticide thiamethoxam, the HQc values are higher when consuming 200 g of apples, reaching 1 x 10-2 for children. Meanwhile, for imidacloprid, the HQc value is 6.3x10-4 when consuming 150 g of apples for children and 8.4x10-4 when consuming 200 g of apples for children.

The chronic impact of pesticides is more pronounced in children compared to adults, primarily due to their lower body mass relative to adults.

Calculating the cumulative impact of pesticides is crucial when assessing the effects of long-term exposure. The Hazard Index (HI) is used to determine whether the cumulative effect of multiple pesticides warrants concern. As highlighted by Liu et al. (2023), when the Hazard Index (HI) exceeds 1, the fruit should be regarded as posing a risk to consumers. Conversely, if the HI is below 1, the fruit is considered safe for consumption. El-Sheikh et al. (2023) reported elevated HI values above 1, indicating that consuming contaminated tomatoes could result in adverse non-carcinogenic effects.

Table 6 presents the Hazard Index (HI) values for the analyzed apples. As observed in this study, all pesticide-related HI values are below 1. Similar findings were reported by Tripathy et al. (2022) and Liy et al. (2023). This implies that the consumption of these apples does not pose a risk to consumer health. However, caution should still be exercised due to the potential consumption of various types of fruits and vegetables throughout the day. These products, if conventionally produced, might contain pesticide residues at varying concentrations.

4. Conclusions

Based on the conducted research, we can deduce that three insecticides were identified in apples from the Resen region. Their concentrations fall within permissible limits and are below the maximum allowable levels. Nonetheless, due to the heightened vulnerability of children as a sensitive demographic, it is imperative to exercise caution regarding the selection of fruits and vegetables for consumption, ensuring their safety.

Our findings indicate that children exhibit greater susceptibility to pesticide-related risks compared to adults. However, both acute and chronic pesticide risks remain at a low level, thus signifying that these apples can be consumed without apprehension. Going forward, we recommend implementing more frequent monitoring of pesticide levels in fruits and vegetables. Additionally, educating manufacturers about approved and disallowed active substances and advocating adherence to specified preharvest intervals (PHI) is crucial.

These results hold the potential to inform the development of future control programs and serve as a foundation for implementing preemptive measures to curtail human health risks.

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