**Abstract**

Bananas produced for the international trade are among the most pesticide-intensive food crops. The aim of this research was to determine the residual levels of pesticides in bananas, to assess the acute exposure from pesticide residues in bananas and to determine the acute risk to consumers.

In this work, eight pesticides: azinphos-ethyl, carbofuran, chlorfenpinphos, chlorpyriphos, metazachlor, methoxychlor, propham and sulfotep were analyzed in 10 banana samples taken from the market of Bosnia and Herzegovina. Analyses were carried out by the QuEChERS approach, using gas chromatography with mass spectrometry detection (GC-MS). To determine the risk, acute exposure assessment was performed according on International Estimation of Short Term Intake (IESTI) equation. In acute quantitative risk assessment, estimated dietary exposure was compared with the relevant toxicological reference values Acute Reference Dose (ARfD).

In four banana samples the levels of pesticide residues were below detectable limit, in five samples determined levels of pesticide residues were at or below the maximum residue level (MRL), while in one sample level of pesticide residue exceeded MRL. Carbofuran was detected in three samples (concentration range 0.01 - 0.03 mg/kg), chlorpyriphos in five samples (concentration range 0.01 - 0.17 mg/kg), propham in two samples (concentration range 0.01 - 0.02 mg/kg) and metazachlor in one sample at 0.01 mg/kg. For one banana sample where the level of carbofuran residue of 0.03 mg/kg was found above MRL (0.01 mg/kg), acute exposure risk assessment was performed and there was a risk for health, because the calculated dietary intake was above the ARfD.

Risk assessment has established that the intake of certain pesticide residues from bananas in acute exposure conditions is not within the limits of the reference toxicological values. Dietary intake of bananas may pose an acute risk to adults and children, especially if consumed in large quantities.

**Key words:** Bananas, Pesticide residues, Dietary risk assessment.

1. **Introduction**

Pesticides are widely used in agriculture to control and prevent crop pests and diseases. Consequently,
consumers are exposed to pesticide residues through the diet [1]. Exposure of the general population to pesticides occurs primarily through eating food and drinking water contaminated with pesticide residues [2]. According to the World Health Organization, fruit and vegetables are the most frequently consumed food group [3]. Because fruit and vegetables are mainly consumed raw or semi-processed, it is expected that they contain higher pesticide residue levels compared to other food groups of plant origin, such as bread and other foodstuffs based on cereal processing [4]. Along with the increase in use of pesticides in the world, the concerns over their health impacts are rapidly growing. Pesticide use has been associated with several concerns, including the potential risks to human health, association of pesticide’s exposure with the incidence of chronic diseases and genetic damages [5].

According to EU regulations [6], the maximum residue level (MRL) represents the maximum concentration of a pesticide residue (mg/kg) that is legally permitted in food commodities and animal feeds. The proposed MRLs are derived from the field trials conducted under Good Agricultural Practice [7]. To evaluate the safety of consumers regarding pesticide residues, the exposure needs to be assessed and compared to health safety limits. Residue levels above the MRL have to be analysed to determine if the intake could exceed the health-based limits [8]. Dietary exposure assessment combines food consumption data with data on the concentration of pesticide residues in food [9].

The risk assessment of pesticide residues is based on comparison of acute (short-term) and chronic (long-term) dietary intake with the value of two toxicological reference limits: acute reference dose (ARfD) and acceptable daily intake (ADI). Consumer is considered to be adequately protected provided that the estimated dietary intake of a pesticide residue does not exceed the ARfD or the ADI [10]. The International Estimation of Short Term Intake (IESTI) is model that has been used to calculate acute intake. IESTI is based on “the worst-case scenario” and addresses the possibility that consumers sometimes eat large amounts of a food item, and such a large amount might contain residues at highest levels [11]. Bananas produced for the international trade are among the most pesticide-intensive food crops. The aim of this research was to determine the residual levels of pesticides in bananas, to assess the acute exposure from pesticide residues in bananas and to determine the acute risk to consumers.

2. Materials and Methods

In this work, eight pesticides: azinphos-ethyl, carbofuran, chlorfenviphos, chlorpyriphos, metazachlor, methoxychlor, prophpam and sulfofep were analyzed in 10 banana samples taken from the market of Bosnia and Herzegovina. Samples were randomly collected in the period from July to September 2014. Analysis of samples was carried out in the accredited laboratory of the Federal Institute for Agriculture Sarajevo.

Sample preparation method, extraction and clean up were based on the acetonitrile extraction technique, which is known under the acronym name QuEChERS (quick, easy, cheap, effective, rugged and safe) method developed for pesticide residue analysis mainly in fruit and vegetables. Gas chromatography with mass spectrometry detection (GC-MS) was used for the multi-residue analysis of pesticides in bananas, according to standard BAS EN 15662:2011. Samples were analysed using an Agilent 7890A GC system with an Agilent 5975C Series MSD. Synchronous full scan and selected ion monitoring (SIM) modes were used.

To determine the risk, short-term exposure assessment was performed according on the IESTI equation, described by FAO/WHO [12], and EFSA [13], as follows:

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

Where: \(U\) = unit weight (kg); \(HR\) = highest residue (mg/kg); \(v\) = variability factor; \(LP\) = large portion (kg); \(bw\) = body weight (kg).

Since Bosnia and Herzegovina has not done national dietary study of food consumption, largest food portion for children and adult population and body weight are used from European Food Safety Authority (EFSA) database. For the purpose of risk estimation, acute exposure calculation was conducted using computer model “EFSA PRIMo-Pesticide Residue Intake Model revision 2”, created as Excel application. Acute exposure assessment, which relates over a period of 24 hours, was performed for samples with MRLs exceeding. Risk evaluation was carried out for two consumer groups, adults and children. In acute quantitative risk assessment, estimated dietary exposure was compared with the relevant toxicological reference values ARfD. If the result is less than the ARfD (up 100%), the risk is considered acceptable. If the result is greater than the ARfD (> 100%) the risk is considered unacceptable.

3. Results and Discussion

3.1 Results

From the total of 10 analysed samples, in four banana samples the levels of pesticide residues were below detectable limit, in five samples determined levels of pesticide residues were at or below the maximum residue level, while in one sample level of pesticide residue exceeded MRL. Three samples of bananas contained multiple pesticide residues.
A total of four pesticides were detected: carbofuran (three samples), chlorpyriphos (five samples), propham (two samples) and metazachlor (one sample), while azinphos-ethyl, chlorfenvinphos, sulfotep and methoxychlor were not detected. Carbofuran was detected in three samples in the concentration range 0.01 - 0.03 mg/kg, chlorpyriphos in five samples in the concentration range 0.01 - 0.17 mg/kg, propham in two samples in the concentration range 0.01 - 0.02 mg/kg and metazachlor in one sample at 0.01 mg/kg (Table 1). One banana sample contained level of carbofuran residue at 0.03 mg/kg which exceeded MRL (0.01 mg/kg) prescribed by national regulation of B&H [14], which is harmonised with Regulation (EC) 396/2005 [6].

Table 1. Concentration of pesticide residues in bananas

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Mean ± standard deviation (mg/kg)</th>
<th>Range (mg/kg)</th>
<th>MRL* (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbofuran</td>
<td>0.016 ± 0.011</td>
<td>0.01 - 0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>Chlorpyriphos</td>
<td>0.054 ± 0.068</td>
<td>0.01 - 0.17</td>
<td>3.0</td>
</tr>
<tr>
<td>Propham</td>
<td>0.015 ± 0.007</td>
<td>0.01 - 0.02</td>
<td>0.05</td>
</tr>
<tr>
<td>Metazachlor</td>
<td>0.01</td>
<td>-</td>
<td>0.1</td>
</tr>
</tbody>
</table>

*Regulation on maximum residue levels of pesticides in or on food and feed of plant and animal origin ("Official Gazette of B&H", no. 89/12 [14]).

For one banana sample where the level of carbofuran residue of 0.03 mg/kg was found above the MRL (0.01 mg/kg), acute exposure risk assessment was performed and there was a risk for health of adults and children, because the calculated dietary intake was above the ARfD, which is 0.00015 mg/kg body weight according to EFSA 2009 [15]. Acute dietary intake for adults and children was estimated at 273.01% and 1671.95% of ARfD (Table 2). These results highlight the importance of residual data on carbofuran to enable further dietary risk assessment for consumers.

Table 2. Acute risk assessment of pesticide residues in bananas

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Fruit</th>
<th>Category</th>
<th>U (g)</th>
<th>HR (mg/kg)</th>
<th>v</th>
<th>LP (g)</th>
<th>bw (kg)</th>
<th>I (mg/kg bw)</th>
<th>% ARfD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbofuran</td>
<td>Banana</td>
<td>Adults</td>
<td>100</td>
<td>0.03</td>
<td>7</td>
<td>260.00</td>
<td>63</td>
<td>0.00041</td>
<td>273.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Children</td>
<td>100</td>
<td>0.03</td>
<td>7</td>
<td>127.30</td>
<td>8.7</td>
<td>0.00251</td>
<td>1671.95</td>
</tr>
</tbody>
</table>

3.2 Discussion

In Spain, 11 pesticides were analysed in 57 banana samples taken from the local markets of the Canary Islands. Chlorpyrifos was detected in 50 samples (88%) in the concentration range 0.03 - 0.65 mg/kg, malathion in five samples (8.8%) in the concentration range 0.16 - 0.17 mg/kg, fenitrothion in four samples (7.0%) in the concentration range 0.02 - 0.10 mg/kg and buprofezin in one sample (1.8%) at 0.15 mg/kg. All these values were below the MRLs established for these compounds except for two samples containing fenitrothion. Among the studied pesticides only chlorpyrifos had a high occurrence in the samples, but authors concluded that the levels of these residues cannot be considered a serious public health problem [16]. According to EFSA, in 2015 the reporting countries analysed 84,341 samples for 774 different pesticides. Overall, 97.2% of the samples analysed fell within the legal limits, 53.3% of the samples tested were free of quantifiable residues (residue levels below the limit of quantification), and in 2.8% of the samples the residue levels exceeded the MRLs (2,366 samples). The foods with the highest percentage of samples with multiple residues were bananas 58.4% (701 samples), up to 9 different pesticides were reported in an individual bananas sample. MRL exceedance rate was identified for bananas at 0.3%. In total, 1,201 samples of bananas were analysed; in 323 samples (26.9%) no quantifiable pesticide residues were found, while 878 samples (73.1%) contained one or several pesticides in quantified concentrations. EFSA performed a short-term (acute) dietary risk assessment for the 11 food products. For 244 samples of 16,197 samples screened for potential short-term consumer health risks, the estimated dietary intake exceeded the toxicological reference value (ARfD). The highest number of cases with exceedances of the ARfD was identified for bananas (120 determinations). The most frequent cases of exceedance of the ARfD in the risk assessment screening for bananas were related to residues of chlorpyrifos (57 determinations), imazalil (33 cases) and acrinathrin (21 determinations) [17]. The monitoring programme of pesticide residues in foods was conducted on the Danish market in the period 2004 - 2011. The analytical programme included in total 17,309 samples. Overall residues above the MRLs were found in 2.6% of the samples. In total, 425 banana
samples were collected and analysed. It was found that 85% of the banana samples contained 14 different pesticide residues; no residues were above the MRLs. Most of the frequencies were 80 - 70 and 50% of the samples contained more than one residue [18]. Monitoring of pesticide residues in fruits and vegetables was conducted in Ghana. A total of 350 locally produced fruits and vegetables were analysed for organochlorine and pyrethroid residues. The results obtained showed that 37.5% of the fruit and vegetable samples analysed contained no detectable level of the monitored pesticides, 19.0% of the samples gave results with levels of insecticides residues above the MRL, while 43.5% of the samples showed results below the MRL. A total of 25 samples of bananas were analysed. It was found that 67% of the samples contained one or more residues. Concentration range of chlorinated pesticide residues in bananas were: methoxychlor 0.004 - 0.012 mg/kg, dieldrin 0.013 - 0.203 mg/kg, endrin 0.004 - 0.012 mg/kg and p,p’-DDT 0.005 - 0.062 mg/kg. Concentration range of synthetic pyrethroid pesticide residues in bananas were: cyfluthrin 0.006 - 0.022 mg/kg, cypermethrin 0.007 - 0.012 mg/kg and deltamethrin 0.008-0.040 mg/kg. The residual concentration level of dieldrin in bananas exceeded MRL values [19].

Carbofuran, an anticholinesterase carbamate, is commonly used as an insecticide, nematicide, and acaricide in agricultural practice throughout the world. Due to its widespread use in agriculture, contamination of food, water, and air has become imminent, and consequently adverse health effects are inevitable in humans, animals, and wildlife [20]. Adverse health effects of acute pesticide poisoning caused by carbamates are: malaise, weakness, dizziness, sweating, headache, salivation, nausea, vomiting, diarrhoea, abdominal pain, confusion, dyspnea, dermatitis, pulmonary oedema [21]. Carbofuran, a systemic N-methyl carbamate pesticide, is a potent acetylcholinesterase (AChE) inhibitor and is highly toxic through the oral and inhalation routes of exposure, with predominantly contact and stomach action [22]. Carbofuran is rapidly and completely absorbed and distributed. Onset of clinical signs, significant for an AChE inhibition, is rapid, within 1 hour, and result in: neurotoxicity, tremors, oral discharge, fasciculation, swollen cheeks, ataxia and convulsions. Classification for acute toxicity is needed and the proposed risk phrases are: T+, R26/R27 "Very toxic if swallowed and via inhalation", N2, R21 "Harmful in contact with skin" and T, 39/41 "Danger of very serious irreversible effects" and "Risk for serious damage to eyes" [15]. According to the results of this research, the acute consumer risk assessment of carbofuran residues indicates the ARfD is significantly exceeded for bananas consumed by children and by adults. Since the results showed a great exceedance of the ARfD further consumer risk assessment will be necessary.

4. Conclusions

- Risk assessment has established that the intake of certain pesticide residues from bananas in acute exposure conditions is not within the limits of the reference toxicological values. Dietary intake of bananas may pose an acute risk to adults and children, especially if consumed in large quantities.
- An adverse health effects of carbofuran include an acute risk of neurotoxicity based on a significant inhibition of the acetylcholinesterase. Continuous monitoring for pesticide residues in food, especially in fruit and vegetables, is very important in determining the food safety and the risk associated with consumption of fruit and vegetables as they constitute major part of human diet contributing nutrients and vitamins.
- Total dietary study in Bosnia and Herzegovina will provide data about dietary habits that is essential for the exposure and risk assessment. Constant survey and monitoring programs for pesticide residues in all food commodities are required in order to protect consumers.

5. References


