Occurrence and estimation of aflatoxin M1 exposure in milk in Serbia

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ABSTRACT

The occurrence of AFM1 was investigated in 150 cow's, 10 goat's, 5 donkey's, 10 breasts milk and 1 infant formula samples. Analyses were done using Enzyme Linked Immunosorbent Assay (ELISA) method. AFM1 was detected in 98.7% of analyzed cow's milk samples in concentrations ranged from 0.01 to 1.2 μg/kg. Further, even 129 (86.0%) cow's milk samples contained AFM1 in concentration greater than maximum residue levels (MRL) of 0.05 μg/kg defined by European Union (EU) Regulation. Analysis of other types of milk showed that AFM1 was detected in 80.0% goat's, 60.0% donkey's and 60.0% of breasts milk samples.

Although Serbian Regulation for MRL of AFM1 in milk has been changed and harmonized with EU Regulation in 2011, occurrence of AFM1 in milk in Serbia during 2013 resulted in Regulation changes, and MRL were changed from 0.05 to 0.5 μg/kg.

On the basis of the obtained concentrations of the AFM1 in cow's milk, collected information about average milk intake and mean body weight (bw) for different age's categories, mean ingestion of AFM1 in ng/kg per bw per day were estimated. Obtained results showed that all age's categories, especially children, are exposed with high risk related to presence of AFM1 in milk.

There were only a few published data about occurrence of AFM1 in milk in Serbia and none about intake assessment for AFM1.

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1. Introduction

Aflatoxins (AFs) are one of the most known and investigated group of mycotoxins which can be found as contaminants in different types of food and feed. AFs are mainly produced by Aspergillus species in agricultural products from tropical and sub-tropical regions (Ardic, Karakaya, Atasever, & Durmaz, 2008; Decastelli et al., 2007). Among approximately 18 identified AFs, aflatoxin B1 (AFB1) is the most common as well as highly toxic, mutagenic, teratogenic and carcinogenic compound (IARC, 1993).

Aflatoxin M1 (AFM1) is the 4-hydroxy derivative of AFB1, formed in liver and excreted into the milk in the mammary glands of both human and lactating animals that have been fed with AFB1 contaminated diet (Fallah, Jafari, Fallah, & Rahnama, 2009; Curbay et al., 2010). The amount of converted AFB1 from food and feed in AFM1 in milk is influenced by several factors including breed, health, type of diet, milk production, rate of ingestion and digestion, etc (Duarte et al., 2013). Depending on the level of contamination, approximately 0.3–6.2% of AFB1 ingested by livestock is transformed to AFM1 in milk (Ayar, Sert, & Con, 2007). If contaminated feedstuffs are used, AFM1 can be detected in milk 12–24 h after first intake of AFB1, while its concentration decreases to an undetectable level 72 h after the initial intake is stopped (Van Egmond, 1989). Hence, rate of absorption of AFB1 and excretion of AFM1 in milk varies from day to day and from one milking to the next (Duarte et al., 2013). AFM1 shows resistance to heat treatment and mild acidic conditions used in the production of milk and other dairy products (yoghurt, cheese, cream, butter), therefore if milk is contaminated there is a great possibility of AFM1 appearance in dairy products (Colak, 2007; Oruc, Cibik, Yilmaz, & Kalkanli, 2006).

Until 2002, AFM1 was classified in 2B group as a possible carcinogen for human (IARC, 1993). Based on numerous studies regarding carcinogenic, teratogenic, genotoxic and immunosuppressive effect, AFM1 and other AFs (B2, G1 and G2) were reclassified in the first group (IARC, 2002).
Milk has the greatest demonstrated potential for AFs introducing into the human diet since it represents one of the main foodstuffs in human nutrition. Furthermore, infants and young children eat and drink more relative to their size than adults, and due to high intake of milk, children are the most susceptible population to the effects of AFM1 (Erkekoglu, Sahin, & Baydar, 2008).

Considering that AFM1 was included in first group by carcinogenicity and milk and its derivatives are consumed daily, most countries have set up maximum residue levels (MRL) of AFM1 in milk. MRL of AFM1 in milk varies from 0.05 μg/kg in European Union (European Commission, 2006b) to 0.5 μg/kg established in United States (FDA, 2011). Regulation for MRL of AFM1 in milk in Serbia (Serbian Regulation, 2011) was recently adopted and harmonized with European Union (EU) Regulation. However, presence of AFM1 in milk during January and February 2013 resulted in Regulation changes. During March 2013, relevant authorities changed previously MRL of AFM1 from 0.05 μg/kg to 0.5 μg/kg (Serbian Regulation, 2013).

Currently, there are more than 200 registered dairies in Serbia, but only 123 of them are active. Other dairies operate in the field of gray economy (nonregistered analytical zone). Big dairy farms own 90% of total capacity, medium-sized 6.0%, whereas small dairy companies only have 4.0% of total capacity. Major milk producers in Serbia are family farms as well as commercial farms both private and state owned (Berkum, 2010). Cow milk is the main milk type produced and its share accounts about 90% of the total milk production in Serbia. Average milk production for the previous years in Serbia has been amounted about 1.6 million tons per year. According to data provided by the Ministry of Agriculture, Forestry and Water Management about 52% were delivered to dairy plants for further processing, while the rest of fourth group. The third group included the following samples: 10 goat milk samples from small dairy producers, and 12 pasteurized, 31 UHT and 4 organic milk samples from big dairy producers were a part of fourth group.

The prevalence of AFM1 in milk has been reported in many countries (WHO, 2010). However, there is a lack of data regarding AFM1 presence in milk from Serbia. Hence, the aim of this report was to investigate the occurrence of AFM1 in different types of milk and to evaluate human exposure to AFM1 in Serbia.

2. Materials and method

2.1. Samples

Present study examined 176 samples of different types of milk. All samples were collected during first part of 2013 and classified into four groups. First, second, third and fourth group of milk samples were collected during February, March, April and May, respectively.

The first group of milk samples included 55 samples of heat-treated milks. Eleven pasteurized, 39 ultra-high temperature (UHT) and 2 organic milk samples were collected from six largest dairy producers in Serbia, while 5 pasteurized milk samples were collected from 5 small dairy producers in Vojvodina (Northern Province of Serbia). From 40 different small milking farms in Vojvodina, 40 raw milk samples were collected and included in second group. The third group included the following samples: 10 goat's and 5 donkey's raw milk samples from small milking farms, 10 breast milk samples and 1 sample of infant formula. Breast milk samples were taken from women who had a mixed diet and only one sample of infant formula was taken from producer, who is the only producer of this type of product in Serbia. Eight pasteurized milk samples from small dairy producers, and 12 pasteurized, 31 UHT and 4 organic milk samples from big dairy producers were a part of fourth group.

The heat-treated milk samples from first and fourth group were collected from supermarkets in original packaging and included different types of milk: skimmed, semi-skimmed, full, milk without lactose, organic, milk enriched with vitamins and full milk for coffee. Samples from second and third group were collected in sterile glass bottles. Immediately after collection samples from all groups were transported to the laboratory and analyzed. Sampling was performed according to EU requirements (European Commission, 2006a).

2.2. Aflatoxin analysis by ELISA

Determination of AFM1 was done by Enzyme Linked Immunosorbent Assay (ELISA) method using 1 screen AFLAM1 test kit (Tecna S. r. l., Trieste, Italy). Analyses were performed according to the test kits instructions. Procedure is based on binding of free AFM1 from samples and standard solutions to the anti-AFM1 antibodies during first incubation. Any unbound substance is removed in a washing step. A second incubation is performed with an aflatoxin-HRP conjugate, which covers all the remaining free binding sites of the antibody. The bound enzyme activity is determined by adding a fixed amount of a chromogenic substrate. The enzyme converts the colorless chromogen into a blue product during the third incubation. The addition of the stop reagent leads to a color change from blue to yellow. The absorbance is measured by a microplate reader (Thermolabsystem, Thermo, Finland) at 450 nm. The color development is inversely proportional to the AFM1 concentration in the sample. Concentration of AFM1 was calculated from calibration curve which was obtained using 7 standards with the following concentrations: 0, 5, 10, 25, 50, 100 and 250 ng/l. Samples with AFM1 concentration greater than 250 ng/l were diluted with sample diluent solution from the test kit and analyzed again.

The analytical quality of the ELISA method was assured by the use of certified reference material (CRM) as well as by participation in interlaboratory study. Partially defatted raw lyophilized milk with certified AFM1 content of 0.053 μg/kg was used as CRM (MI1142-1/C1, Progetto Trieste, Test Veritas, Padova, Italy) for validation of I screen AFLAM1 test kit. The validation parameters (Table 1) were calculated and expressed using European Official Decision procedure for screening methods (European Commission, 2002b) and their values were in accordance with recommendations given in Commission Decision (European Commission, 2006a). Further, obtained Z value of 0.67 in interlaboratory study confirmed analytical quality of the results obtained by ELISA method (Ring Test 2013, Mycotoxins, Aflatoxin M1, Test Veritas, Padova, Italy).

<table>
<thead>
<tr>
<th>Table 1 Validation parameters.</th>
<th>I screen AFLAM1 test kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOD</td>
<td>1.50</td>
</tr>
<tr>
<td>LOQ</td>
<td>5.00</td>
</tr>
<tr>
<td>RSD</td>
<td>3.54</td>
</tr>
<tr>
<td>RSDr</td>
<td>4.97</td>
</tr>
<tr>
<td>Recovery</td>
<td>107.6</td>
</tr>
</tbody>
</table>

LOD: limit of detection (ng/kg).  
LOQ: limit of quantification (ng/kg).  
RSD: relative standard deviation calculated under repeatability conditions.  
RSDr: relative standard deviation calculated under reproducibility conditions.
2.3. Sample preparation

Milk samples were prepared according to the manufacturer's instructions. Samples were centrifuged at 3000 g for 10 min. The upper creamy layer was removed by Pasteur pipette and 100 μl from the lower phase was used for the analysis.

2.4. Calculation of estimated daily intake

Recent data about average milk intake in Serbia are not available, therefore we approached calculating average milk intake for people in Serbia. The calculation was based on questionnaire examination that included 1500 people of different age's categories and economical status. In order to estimate mean ingestion of AFM1 in ng/kg per body weight (bw) per day following parameters were used: concentrations of the AFM1 in cow's milk obtained in the present study, mean intake of milk and mean bw for different age's categories obtained from questionnaire.

3. Results

3.1. Occurrence of AFM1 in different type of milk

In this study a total of 176 milk samples were analyzed to determine concentration of AFM1. Obtained results were summarized and shown in Table 2.

As can be seen, all examined samples from first group were contaminated with AFM1. Further, only 1 UHT and 2 organic milk samples had AFM1 concentration lower than 0.05 μg/kg. Even 52 (94.6%) of analyzed samples from first group had AFM1 concentration greater than MRL defined by EU Regulation (European Commission, 2006b) and Serbian Regulation that was valid at the time of examination (Serbian Regulation, 2011). Analysis of 40 raw milk samples collected from small milking farms showed that among 13 pasteurized and 40 raw milk samples, 100% and 75.0% were not suitable for human consumption according to EU Regulation, and 38.5% and 12.5% according to Serbian Regulation, respectively. Comparing AFM1 levels in organic and others cow's milk, it was found that organic milk samples were contaminated in a lower frequency. One (16.7%) out of six organic milk samples exceeded European MRL. On the other hand, the highest concentrations of AFM1 were found in raw (0.90 μg/kg) and pasteurized (1.20 μg/kg) milk samples from milking farms.

3.2. Estimated daily intake of AFM1

After questionnaire examination of 1500 people of different age's categories we obtained results in terms of average body weight (bw) and average intake of milk (Table 4). Further, we also investigated what kind of milk is most commonly used in Serbia. Only 10 (0.67%) out of 1500 examinees do not drink cow's milk, while 6 of them drink other milk types (soy and goat) and 2 have allergic reaction on cow's milk.

Since cow's milk is mainly used (99.3%) and 98.7% of analyzed cow's milk samples were contaminated with AFM1, exposure of human regarding AFM1 was investigated. Two samples (1.3%) of raw cow's milk with AFM1 concentration lower than 0.005 μg/kg were not included in this estimation. Table 4 represents data regarding calculated ingestion of AFM1 in terms of minimum (0.01 μg/kg), average (0.21 μg/kg) and maximum (1.2 μg/kg) AFM1 concentration obtained in this study.

4. Discussion

Hot and dry weather conditions described in our previous study (Kos, Mastilović, Hajnal, & Sarić, 2013) could be the possible reason

### Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Producers</th>
<th>Sample category</th>
<th>No</th>
<th>Range of concentration (μg/kg)</th>
<th>Average ± SD</th>
<th>Min–Max</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.005</td>
<td>0.005–0.05</td>
<td>0.05–0.50</td>
</tr>
<tr>
<td>1</td>
<td>Big</td>
<td>Pasteurized</td>
<td>10</td>
<td>–</td>
<td>–</td>
<td>10 (100)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>UHT</td>
<td>38</td>
<td>–</td>
<td>1 (2.60)</td>
<td>37 (97.4)</td>
</tr>
<tr>
<td></td>
<td>Organic</td>
<td>2</td>
<td>–</td>
<td>2 (100)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Small</td>
<td>Pasteurized</td>
<td>5</td>
<td>–</td>
<td>–</td>
<td>1 (20.0)</td>
</tr>
<tr>
<td>2</td>
<td>Farms</td>
<td>Raw</td>
<td>40</td>
<td>2 (5.00)</td>
<td>8 (20.0)</td>
<td>25 (62.5)</td>
</tr>
<tr>
<td></td>
<td>Goat</td>
<td>10</td>
<td>2 (20.0)</td>
<td>4 (40.0)</td>
<td>4 (40.0)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Donkey</td>
<td>5</td>
<td>2 (40.0)</td>
<td>3 (60.0)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Breast milk</td>
<td>10</td>
<td>4 (40.0)</td>
<td>6 (60.0)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>Big</td>
<td>Infant formula</td>
<td>1</td>
<td>1 (100)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Big</td>
<td>Pasteurized</td>
<td>12</td>
<td>–</td>
<td>–</td>
<td>11 (91.7)</td>
</tr>
<tr>
<td></td>
<td>UHT</td>
<td>31</td>
<td>–</td>
<td>5 (16.1)</td>
<td>26 (83.9)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Organic</td>
<td>4</td>
<td>–</td>
<td>3 (75.0)</td>
<td>1 (25.0)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Small</td>
<td>Pasteurized</td>
<td>8</td>
<td>–</td>
<td>–</td>
<td>7 (87.5)</td>
</tr>
</tbody>
</table>

No: number of samples.
Range of concentration: number (percentage).
Average ± SD: average concentration (μg/kg) ± standard deviation (μg/kg).
Min–Max: minimum and maximum concentrations (μg/kg).
for high contamination frequency of AFM1 in milk from Serbia. Prolonged drought noted during maize growing season 2012 was favorable for Aspergillus molds growth and AFs productions. Presence of AFs was detected in 68.5% of examined maize samples in the concentration range from 1.01 to 86.1 μg/kg (Kos et al., 2013). Occurrence of AFs in maize and feed were also confirmed in our second report (Lević, Đuragić, Kos, Varga, & Bagić, 2013). AFs were analyzed in 302 maize, 49 maize silage and 27 complete feed samples and detected in 69.9%, 38.7% and 37.0% of analyzed samples, respectively. In Serbia, maize is mainly (80.0%) used as a component of animal feed (Maslaci, 2011), therefore if maize is contaminated there is a great possibility of AFM1 appearance in milk and milk products.

Furthermore, Serbian Regulation (Serbian Regulation, 2010a) for MRL of AFs in maize intended for animal consumption had to be tested on AFB1, while Serbian Regulation for high contamination frequency of AFM1 in milk from Serbia (Serbian Regulation, 2010a) AFs 0.05 0.01

Table 3 Occurrence of AFM1 in milk samples.

<table>
<thead>
<tr>
<th>Producers</th>
<th>Sample category</th>
<th>No</th>
<th>Range of concentration (μg/kg)</th>
<th>Min–Max</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt;0.005</td>
<td>0.005–0.05</td>
</tr>
<tr>
<td>Big</td>
<td>Pasteurized</td>
<td>22</td>
<td>–</td>
<td>21 (95.5)</td>
</tr>
<tr>
<td></td>
<td>UHT</td>
<td>69</td>
<td>–</td>
<td>63 (91.3)</td>
</tr>
<tr>
<td></td>
<td>Organic</td>
<td>6</td>
<td>–</td>
<td>1 (16.7)</td>
</tr>
<tr>
<td>Small</td>
<td>Pasteurized</td>
<td>13</td>
<td>–</td>
<td>8 (61.5)</td>
</tr>
<tr>
<td>Farms</td>
<td>Raw</td>
<td>40</td>
<td>2 (5.00)</td>
<td>25 (62.5)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>150</td>
<td>2 (1.30)</td>
<td>118 (78.7)</td>
</tr>
</tbody>
</table>

No: number of samples. Range of concentration: number (percentage). Average ± SD: average concentration (μg/kg) ± standard deviation (μg/kg). Min–Max: minimum and maximum concentrations (μg/kg).

Table 4 Estimate of AFM1 human exposure.

<table>
<thead>
<tr>
<th>Age categories</th>
<th>Body weight (kg)</th>
<th>Intake of milk per day (l)</th>
<th>Average ingestion of AFM1 ng/kg bw/day</th>
<th>Maximum ingestion of AFM1 ng/kg bw/day</th>
<th>Minimum ingestion of AFM1 ng/kg bw/day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>1–5</td>
<td>14</td>
<td>14</td>
<td>0.44</td>
<td>0.43</td>
<td>6.45</td>
</tr>
<tr>
<td>5–15</td>
<td>38</td>
<td>37</td>
<td>0.42</td>
<td>0.32</td>
<td>2.34</td>
</tr>
<tr>
<td>15–25</td>
<td>74</td>
<td>55</td>
<td>0.44</td>
<td>0.11</td>
<td>1.26</td>
</tr>
<tr>
<td>25–35</td>
<td>90</td>
<td>60</td>
<td>0.21</td>
<td>0.18</td>
<td>0.49</td>
</tr>
<tr>
<td>&gt;55</td>
<td>89</td>
<td>71</td>
<td>0.22</td>
<td>0.23</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Average ingestion of AFM1 calculated for obtained mean concentration of AFM1 (0.21 μg/kg). Minimum ingestion of AFM1 calculated for obtained minimum concentration of AFM1 (0.01 μg/kg). Maximum ingestion of AFM1 calculated for obtained maximum concentration of AFM1 (1.20 μg/kg).

In the same year Janković et al., (2009) and Glamočić (2010) have shown that none of 70 analyzed cow's milk samples were contaminated with AFM1 in concentration greater than 0.05 μg/kg. In the same year Janković, Vukojević, Lakicević, Mitrović, and Vuković (2009) analyzed 23 milk samples and in 3 (13.0%) samples concentration of AFM1 was higher than 0.05 μg/kg. Lower contamination frequency of AFM1 in previous reports from Serbia in comparison to the obtained results in this study can be explained with the absence of AFs in maize and other feed material in Serbia in previous years (Kokić et al., 2009; Kos et al., 2013; Matić et al., 2010).

Published studies regarding presence of AFM1 in organic milk give contradictory information in terms of lower or higher levels of AFM1 in comparison to conventional milk (Ghidini et al., 2005; Vallone, Boscariol, & Dragoni, 2006). Organic milk samples collected during 2009–2010 in Greece (Tsakiris et al., 2013) and 40 samples in Portugal during 2011 (Duarte et al., 2013), only 1.0% and 5.0% of samples exceeded European MRL, respectively. Results from Italy (Meucci, Razzuoli, Soldani, & Massart, 2010) and Spain (Cano-Sancho, Marin, Ramos, Peris-Vicente, & Sanchís, 2010) showed that all analyzed samples were in accordance with EU Regulation. It should be noted that there is still no published data from Europe regarding occurrence of AFM1 in milk from 2012 to 2013 period, which was characterized with favorable weather conditions for Aspergillus species growth and AFs production, especially in Southeast Europe.

Presence of AFM1 in milk from Serbia was published in only few reports. Polovinski-Horvatović, Jurić, and Glamočić (2010) have shown that none of 70 analyzed cow's milk samples were contaminated with AFM1 in concentration greater than 0.05 μg/kg. Published studies regarding presence of AFM1 in organic milk give contradictory information in terms of lower or higher levels of AFM1 in comparison to conventional milk (Ghidini et al., 2005; Vallone, Boscariol, & Dragoni, 2006).
analyzed in present study were taken from the only producer of that type of milk in Serbia. More rigorous control of feed material and raw milk before processing could be the possible explanation for better obtained results in comparison to conventional cow’s milk samples.

There are a several data regarding occurrence of AFM1 in other types of milk (except cow’s). Previous studies confirmed our results that milk from others animals are very often less contaminated with AFM1 than cow’s milk, because of the different digestive apparatuses and mechanism of AFB1 assimilation of animals and for different feeding used in cow’s breeding (Barbierioli et al., 2007; Fallahi, Rahnama, Jafari, & Saei-Dehkordi, 2011; Hussain, Anwar, Asi, Munawar, & Kashif, 2010). Also, it should be noted that data about occurrence of AFM1 in donkey’s milk are very limited.

Since AFs and AFM1 from contaminated food can be transported to breast milk and cause development of several diseases in newborn child (Gurbay et al., 2010), breast milk samples were analyzed in present study. Analysis of 10 breast milk samples showed that determined AFM1 concentration in 6 samples ranged from 0.006 to 0.022 μg/kg. Although, founded AFM1 concentrations in breast milk were lower than 0.025 μg/kg which is allowed MRL for milk and infant formula intended for infant and young children (European Commission, 2006b; Serbian Regulation, 2010b), it should be pointed that any level of AFM1 represent risk to infant since milk is their main food. Breast milk remains the best source of nutrition for infants and World and Health Organization (WHO) recommends exclusive breast-feeding for the first two years of life (Dowling et al., 2012). However, when breast-feeding is not possible or enough, many different infant formulas are available for infants and young children. In Serbia there is only one milk industry that produces infant formulas. Analysis of infant formulas sample showed that concentration of AFM1 was in accordance with Serbian and EU Regulations (European Commission, 2006b; Serbian Regulation, 2010b). Unfortunately, great numbers of infant and young children in Serbia immediately after breast-feeding start to drink cow’s milk. Possible reasons for early introduction of cow’s milk are economic situation in Serbia and/or traditional diet. Early intake could provoke a variety of allergic reactions (Ivakhnenko et al., 2013) and could represent great risks related to AFM1.

Kuiper-Goodman (1990) expressed 0.2 ng/kg bw/day as tolerable daily intake (TDI) of AFM1. Based on the results shown in Table 4 it could be noted that calculated ingestion for obtained average and maximum concentrations for all age’s categories are significantly higher than TDI. For obtained minimum concentration of AFM1, calculated ingestion is higher than 0.2 ng/kg bw/day only for children from 1 to 5 years. Different intake of milk and bw for different age’s categories influenced significant differences in daily ingestion of AFM1.

Although International expert committees (JECFA, 2001) did not specify TDI for AFs, they concluded that daily exposure even with concentration lower than 1 ng/kg bw contributed to the risk of liver cancer. Further, JECFA (2001) established intake of AFM1 for five regions based on the regional consumption of milk, assuming bw (60 kg) and weighted mean concentrations of AFM1 in milk obtained in various monitoring programs (Table 6). It should be pointed, that results from JECFA did not include data from Serbia. From the showed data it can be stated that currently calculated daily intake of AFM1 in Serbia are higher than daily intake of AFM1 in other five world regions.

The most of the previously estimated intakes of AFM1 through milk consumption in some countries were calculated for adults, and some of these are following: 0.08 ng/kg bw/day in Brazil (Shundo, Navas, Lamarro, Ruvieri, & Sabino, 2009), 0.01 ng/kg bw/day in France (Leblanc, Tard, Volatier, & Verger, 2005), 0.305 ng/kg bw/day in Spain (Cano-Sancho et al., 2010), and 3.26 ng/kg bw/day in Morocco (Zinedine et al., 2007). In the present study, estimated intakes of AFM1 for adults were expressed for minimum, average and maximum obtained concentration of AFM1 and those values ranged from 0.02 to 0.06, 0.42 to 1.26, and from 2.39 to 7.18 ng/kg bw/day, respectively.

Obtained ingestion of AFM1 in this study greater than TDI value of 0.2 ng/kg bw/day calculated by Kuiper-Goodman (1990) indicate high risk related to AFM1 since this toxin has carcinogenic, teratogenic, genotoxic and immunosuppressive effect and could cause DNA damage, gene mutation, chromosomal anomalies and cell transformation (IARC, 2002).

5. Conclusion

Obtained results indicate high contamination frequency of AFM1 in examined cow’s milk samples. Since milk represents one of the main foodstuffs in human diet in Serbia there was a concern regarding human exposure to AFM1 from February to May 2013. Furthermore, infants and children represent the most susceptible population to the effects of AFM1 since they drink more milk relative to their size. In order to avoid risk related to presence of AFM1 in milk there is a need for permanent control and harmonization of Serbian Regulations with EU Regulations in terms of AFM1 in milk and AFs in feed.

Acknowledgments

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References


<table>
<thead>
<tr>
<th>Region</th>
<th>Milk intake (kg/day)</th>
<th>Weighted mean AFM1 in milk (μg/kg)</th>
<th>AFM1 intake (ng/person per day)</th>
<th>AFM1 intake (ng/kg bw/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>European Union</td>
<td>0.290</td>
<td>0.023</td>
<td>6.8</td>
<td>0.11</td>
</tr>
<tr>
<td>Latin America</td>
<td>0.150</td>
<td>0.022</td>
<td>3.5</td>
<td>0.058</td>
</tr>
<tr>
<td>Far East</td>
<td>0.032</td>
<td>0.360</td>
<td>12</td>
<td>0.20</td>
</tr>
<tr>
<td>Middle East</td>
<td>0.120</td>
<td>0.005</td>
<td>0.6</td>
<td>0.10</td>
</tr>
<tr>
<td>Africa</td>
<td>0.042</td>
<td>0.002</td>
<td>0.1</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Results are taken from Joint Expert Committee on Food Additives (JECFA, 2001).

Table 6

Daily intake of AFM1 in milk in the five regional diets.


IARC. (2000). Monograph on the evaluation of carcinogenic risk to humans (Vol. 82 (pp. 171). Lyon, France: World Health Organization, IARC.


