Article

Dietary exposure and risk assessment of Aflatoxin M1 for children aged 1 to 9 years old in Serbia

Dragan R. Milićević [1,*] Jelena Milešević [2], Mirjana Gurinović [2], Saša Janković [1], Jasna Đinović – Stojanović [1], Milica Zeković [2], and Maria Glibetić [2]

[1] Institute of Meat Hygiene and Technology, Kaćanskog 13, 11040 Belgrade, Serbia; dragan.milicevic@inmes.rs (D.M.); sasa.jankovic@inmes.rs (S.J.); jasna.djinovic@inmes.rs (J.DJ.S.).

[2] University of Belgrade, Institute for Medical Research, National Institute of Republic of Serbia, Centre of Research Excellence in Nutrition and Metabolism, Serbia; jelena.milesevic@gmail.com (J.M.); mirjana.gurinovic@gmail.com (M.G); zekovicmilica@gmail.com (M.Z.); mglibetic@gmail.com (M.G.);

* Correspondence: dragan.milicevic@inmes.rs (D.M.)

Abstract: The present study was conducted to estimate the exposure and risk characterization of the children population of Serbia to Aflatoxin M1 (AFM1) from milk and milk-based food. A total of 3404 samples comprising of milk and different milk-based food samples were collected from various regions of Serbia from 2017 to 2019. Evaluation of the AFM1 exposure was carried out using the deterministic method, whereas risk characterization was evaluated with the calculation of the Margin of Exposure (MOE) and the risk of Hepatocellular Carcinoma (HCC). Detection rates for AFM1 in milk and milk-based food samples ranged between 2% and 79%, with the highest incidence (79%) and mean level (22.34 ± 0,018 ng kg^{-1}) of AFM1 was detected in pasteurized and UHT milk. According to the three consumption estimates, the values of EDI were higher for toddlers as compared with children. Children aged 1–3 years had the highest risk of exposure to AFM1 in milk, with an estimated daily intake of 0.164 and 0.193 ng kg^{-1} bw day^{-1} using a lower bound (LB) and the upper bound (UB) exposure scenarios, respectively. Such difference could result from the higher consumption versus weight. Based on the EDI found in this study, the risk of AFM1 due to consumption of milk and milk-based food was low since MOE values obtained were >10000. In addition, the risk of HCC cases/year/10^5 individuals of different age groups showed that the value of HCC using potency estimates of 0.0017 (mean) was maximum (0.00034) in the age group 1–3 years which indicates no health risk for the evaluated groups. The present study revealed the importance of controlling and preventing AFM1 contamination in milk through continuous monitoring and regular inspection to reduce the risk of AFM1 exposure, especially in children.

Keywords: aflatoxin M1; milk, dairy products; risk assessment; children

1. Introduction

Mycotoxins are toxic compounds produced as secondary metabolites by certain groups of fungi which constitute a significant hazard to food safety and public health [1]. Under certain environmental conditions (i.e., temperature and humidity) and/or biotic stress, toxigenic fungi and their metabolites may contaminate crops and food commodities in different phases of production and processing [2]. Mycotoxins showed stability against heat processes, which make their occurrence in processed food likely expected even if toxin-producing molds are eliminated during the food preparation process [3]. Consumption of mycotoxin-contaminated food may lead to different health adverse effects, including immune suppression, target organ toxicity, genotoxicity, or carcinogenicity [4]. Moreover, when animals ingest these toxins, their metabolites or unmetabolized compounds may be transferred to products such as milk and further contaminate dairy products. Aflatoxin M1 (AFM1) is a principal hydroxylated metabolite of Aflatoxin B1 (AFB1), that may be found in milk from lactating animals after ingesting

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 November 2021 doi:10.20944/preprints202111.0014.v1

feed contaminated with Aflatoxin B1 (AFB1) [5]. The ubiquitous occurrence of mycotoxins in the food chain has been shown in numerous reports over the last decades [6,7]. Thus, to ensure consumer safety exposure through food, strict regulations and guidelines have been set by different organizations such as the World Health Organization (WHO) and the European Food Safety Authority (EFSA) to control, measure, and diminish occurrence for the major mycotoxins [8].

Long-term exposure to mycotoxins is associated with myriad health consequences which belong to non-communicable diseases (NCDs), among which are liver and renal cancer, chronic gastritis, and disorder of the nervous system [9]. Among mycotoxins, AFs represent the major public health concern because they are hepatotoxic, teratogenic, immunosuppressive. These secondary metabolites are produced by some *Aspergillus species*, especially *A. flavus, A. nomius,* and *A. parasiticus* [10]. The International Agency for Research on Cancer (IARC) classified AFs as class 1 carcinogenic compounds to humans [11]. According to the risks associated with mycotoxins, the European Union has established the strictest maximum levels for AFM1 (0,05 μg kg^{-1}) in raw milk, heat-treated milk and milk for the manufacture of milk-based products and 0,025 μg/kg, for Infant formulae and follow-on formulae, including infant milk and follow-on milk [12]. Previous regulations have not eradicated milk AFM1 successfully which resulted in periodic changes to the official regulations. In meanwhile Serbia has set standards for AFs where the maximum regulatory level for AFM1 in raw milk, heat-treated milk, and milk for the manufacture of milk-based products is 0,25 μg kg^{-1} [13]. Dairy products are not included in the Serbian regulation, while for infant formulae and follow-on formulae, including infant milk and follow-on milk, as well as for dietary foods for special medical purposes intended specifically for infants, the permitted level of AFM1 has been set at 0.025 μg kg^{-1}.

An important health effect of AFs is their link with liver cancer. In 2012, about 745 000 deaths were estimated to have been caused mostly by AF-induced hepatocellular carcinoma (HCC) in the world [14]. In the same year, a total of 782 451 new liver cancer cases and 745 533 related deaths were estimated to occur per year [15]. In addition to liver cancer and cirrhosis, aflatoxins have also been linked to growth stunting in children, malnutrition, kwashiorkor, or marasmus diseases, and the suppression of immune responses [16].

Although, the levels of mycotoxins found in the diet are often low, because of their longer life expectancy, children are critically affected by natural contaminants such as mycotoxins and thus prone to develop chronic syndromes in the future (e.g., mycotoxin-related cancers) [17,18]. Moreover, infants and young children are more vulnerable to the deleterious effects of mycotoxins, because of their larger intake/body weight ratio, higher metabolic rate, and lower detoxification capabilities [19,20]. Therefore, it is necessary to evaluate mycotoxin presence in foods and the level of exposure to children [21].

Food security and food safety is an important prerequisite for good health. Milk and dairy products are a source of many nutrients including proteins, fatty acids, calcium, vitamins, and minerals essential for human health, especially in infants and children [22]. However, the risk of contamination by AFM1 is an important food safety concern for milk. Despite the available data on AfM1 occurrence in milk and dairy products, information on exposure and risk assessment of infants and young children in Serbia is lacking. This is due to a combination of limited monitoring systems and a lack of food consumption data. Thus, the extent and health implications associated with mycotoxin exposure of infants and young children need to be evaluated and should be given a priority in Serbia.

Therefore, the objective of the present study was to conduct a preliminary risk assessment and dietary exposure of the children population to AFM1. Probable daily intakes (PDIs) for the AFM1 were determined based on the available data of the occurrence of AFM1 in milk and dairy products, and the consumption data of mentioned food for Serbian children. According to EFSA recommendations, dietary exposure assessment as an integral part of the risk assessment process need to use harmonized and standardized food consumption data from national dietary surveys at an individual level and this will be the first time in Serbia. In present study will be used the data obtained

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 November 2021 doi:10.20944/preprints202111.0014.v1

from the national survey in Serbia: EFSA project-Support to National Dietary Surveys in Compliance with the EU Menu methodology (sixth support) "The children's survey"[23].

The dietary exposure of these population groups will then be compared with existing health-based guidance values. Health-based guidance values (HBGVs), i.e. tolerable daily intake (TDI) or tolerable weekly intake (TWI), define the amount of a specific contaminant that an individual can consume regularly over a lifetime without any appreciable risk to health [24]. The European Food Safety Authority (EFSA) recommended the use of the margin of exposure (MOE) approach to evaluate compounds that are both carcinogenic and genotoxic [24]. The MOE is the ratio between a toxicological threshold obtained from animal studies ($BMDL_{10}$) and the estimated human exposure (EDI). A MOE less than 10,000 for a genotoxic and carcinogenic substance based on the $BMDL_{10}$ is considered to represent a risk to public health [24]. Thus, MOE is a tool used by risk assessors to consider possible safety concerns arising from the presence of food of substances that are both genotoxic and carcinogenic. The results of our study are helpful to risk managers in their prioritization for food monitoring programs as part of risk-based food control, as well as to applying adequate measures to protect the health of children.

2. Materials and Methods

2.1. Sample collection and sample analysis

We conducted a risk assessment for AFM1 by combining the concentration of AFM1 in food commodities from several studies published between 2017 and 2019 [5,25,26]. In brief, a total of 3404 milk and milk-based food samples was randomly collected from various regions of Serbia from 2017 to 2019. The samples consisted of different types of milk, dairy products, and infant formula. The majority of collected samples were from local dairy processing plants that manufacture fluid milk, cheese, cream, or cultured dairy products, while some of the food samples (i.e, infant formula, milk beverages) were from imported sources.

The sample analysis was performed with an enzyme-linked immunosorbent assay (ELISA). Preparation of the samples and ELISA test procedure for the determination of AFM1 in milk-based food samples was performed according to the manufacturer's instructions (Tecna S.r.l., Mirandola (MO), Italy). The samples above MRL were confirmed and also quantified by LC-MS/MS analytical techniques. All details related to the analytical methods, validation, and quality assurance have been previously described by Milicevic et al. [5,25,26].

2.2. National Food Consumption Survey on toddlers and children

Serbian National Food Consumption Survey on toddlers and children was conducted between 2017 and 2021 according to the EFSA EU MENU methodology [23]. Valid data was collected from a total of 576 participants with 290 toddlers aged from one to below three years old and 286 children aged from three to nine years old. Data collection was conducted using project-specific national Survey Pack that included: General questionnaire, age appropriate Food Propensity Questionnaire (FPQ) and 24h food diary. The consumed portion sizes were estimated based on natural units, household measures, packaging information and country-specific portion size measurement aid (PMSA) (i.e. previously tested Food Atlas) [27]. Following EFSA guidance on the EU Menu methodology, previously developed and validated innovative nutritional software tool DIET ASSESS & PLAN (DAP) was used [28] for standardised and harmonised food consumption data collection and comprehensive dietary intake assessment. Basic FoodEx2 codes including implicit facets were assigned to all foods and recipes from the Serbian Food Composition Data Base (FCDB) which is integrated into the DAP platform.Weight measurements were performed without shoes and jackets using digital balance and data were recorded to the nearest 0.1 kg. For height measurements, portable stadiometers were applied with 0.1 centimetre accuracy.

2.3. Health Risk Assessment

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 November 2021 doi:10.20944/preprints202111.0014.v1

Deterministic methods (or single point) was employed to derive a worst-case risk estimate. Assessment of cumulative risks posed to the health of children by consumption of milk and milk-based food was done in three stages which comprise exposure assessment, risk characterization, and assessment of liver cancer risk.

2.4. Exposure Assessment

In our study, chronic AFM1 exposure among two age groups was estimated by the deterministic approach involving the Average Probable Daily Intake (APDI) method [29]. Exposure was calculated for all the food categories, and for both consumer groups according to their gender and age to highlight the differences in exposure. The estimated daily intake (EDI) of AFM1 (expressed as ng kg^{-1} bw day^{-1}) was performed based on the concentration of AFM1 detected and the intake rate of analyzed foods, according to the equations as follows:

$$EDI = \Sigma c * C/Bw \quad (1)$$

where Σc is the average concentration of AFM1 (ng kg^{-1}), C is the daily average consumption of the commodity (kg per day); bw – body weight for the male and female children populations (kg).

The mean concentrations of AFM1 in selected milk and dairy products were taken from Table 1. Within the general framework of chemical risk assessment, a difficult step in dietary exposure evaluation is handling concentration data reported to be below the limit of detection (LOD). These data are known as non-detects and the resulting occurrence distribution is left-censored. The left-censored data (data below LOD and LOQ) were processed by applying the substitution method of EFSA [30]. According to this guidance, for dietary exposure assessments, three exposure scenarios were considered. Middle bound (MB), assuming that the not detected results correspond to the half of LOD (ND=2,5 ng kg^{-1}) was used for all AFM1 when a finding with a value <LOD was below in ≤60% of samples. In contrast, when a large percentage of the results are below the LOD (> 60 but ≤80% non-quantified and with at least 25 results quantified) two estimates using a lower bound (LB) scenario, in which zero was assigned to samples showing AFM1 concentration below LOD/LOQ and the upper bound (UB) was obtained assuming the value for the limit of detection of AFM1 (5.0 ng kg^{-1}) for the results of AFM1 reported as not detected (ND = LOD). Furthermore, following the EFSA recommendations exposure calculations at the 95th percentile (P95) of AFM1 concentration (P95), was performed to evaluate the worst-case scenarios [24]. The daily average consumption of these products, and mean body weight, were obtained from the data provided in the food frequency questionnaire by age (Table 2 and 3). The different food commodities were grouped within each food category to better explain their contribution to the total dietary exposure to AFM1.

2.5. Risk characterization

Since AFM1 is considered carcinogenic, there is no Tolerable Daily Intake (TDI) based on a dose of no observable effect (NOEL). Therefore, risk characterization originating from the oral exposure to aflatoxins was calculated using two approaches; the qualitative margin of exposure (MOE) approach established by EFSA [24] for substances that are both genotoxic and carcinogenic and the quantitative approach to liver cancer risk estimation proposed by the FAO/WHO [31].

The MOE value was calculated using Equation:

$$MOE = \frac{BMDL_{10}}{EDI} \quad (2)$$

where BMDL10 is the benchmark dose lower confidence limit (BMDL10) for 10% increased cancer risk. Based on animal data, EFSA concluded, that AFM1 induces liver cancer with a potency one-tenth that of AFB1 (for AFB1 0.4 μg kg^{-1} bw per day^{-1}), hence, a potency factor of 0.1 for the AFM1 risk assessment was used in this study. EDI is the average daily intake used to estimate chronic dietary exposure to AFB1, as calculated in

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 November 2021 doi:10.20944/preprints202111.0014.v1

Equation (1). A calculated MOE value lower than 10,000 implies that exposure to a carcinogenic and genotoxic substance contributes to the risk of HCC and is of concern to public health [24].

2.6. Assessment of Liver Cancer Risk – the carcinogenic potency

Most health concerns for AFs are related to primary liver cancer burden as the ingestion of AFs has been directly linked to hepatocellular carcinoma development particularly in individuals infected with hepatitis virus. To estimate the risk of cancer posed by dietary exposure to AFM1, we used the following equation:

$$\text{Population risk} = \text{EDI} \times \text{Average potency} \quad (3)$$

Regarding the differences in carcinogenic potency, for AFM1, according to JECFA [29], AFM1 induces liver cancer with one-tenth of that potency of AFB1. Therefore, the carcinogenic potency (CP) of AFM1 was calculated to be 0.0562 additional cancer cases per 100,000/year per 1 ng kg^{-1} bw day^{-1} for $HBsAg^+$ populations and 0.0049 additional cancer cases per 100,000/year per 1 ng kg^{-1} bw day^{-1} for $HBsAg^-$ populations. The prevalence of hepatitis-B-infected individuals used was 1% in Serbia based on earlier studies [5]. Thus, the CP of 1 ng AFM1 kg^{-1} bw day^{-1} in a population with a 1% prevalence of HBV infection would be 0.005413 cases per year per 100,000 people according to equation:

$$\text{Average cancer potency} = (0.0049 \times 0.99 + 0.0562 \times 0.01) \quad (4)$$

2.7. Statistical analysis

Data were analyzed using Minitab statistical software version 17 (Minitab Ink., Coventry, UK). AFM1 concentrations for the studied samples were expressed in the form of descriptive statistics and presented in Tables 1, 4-7. A Shapiro–Wilk test of normality was run to check the normality of data and after recording the data as normal, a further test was used for statistical evaluation of the data.

3. Results

3.1. Prevalence of AFM1 in milk and milk-based food

The prevalence of AFM1 in milk and milk products samples collected from various regions of Serbia from 2017 to 2019 is presented in Table 1. Amongst the collected samples, 574/725 pasteurized and UHT milk, 67/201 milk powder, 158/775 fermented milk products, 145/714 milk beverages, 14/92 infant formula, 19/132 sour cream, 13/90 whey, 14/143 butter, and, 7/404 cheese, were contaminated with AFM1, respectively. Overall, the mean levels (ng kg^{-1}) of AFM1 based on the LB mean can be ranged as follows: pasteurized and UHT milk > whey > fermented milk products > milk powder > sour cream > butter > milk beverages > infant formula > cheese. As expected, the highest incidence of contamination (79%) and the mean concentration of AFM1 were observed in pasteurized and UHT milk (22.34 ± 0.02 ng kg^{-1}), while cheese by 1.36±0.01 ng kg^{-1} showed the lowest mean concentration. Among the different milk products, the maximum AFM1 level found in this study was registered in a whey sample, reaching a contamination level of 278 ng kg^{-1}, followed by cheese (276 ng kg^{-1}) and fermented milk products (174 ng kg^{-1}).

The average concentration of AFM1 in the present study is slightly lower compared to the previous studies from Serbia [32-34]. Also, the results of this study are in agreement with the reported AFM1 concentrations in milk and dairy products from global studies, where are the prevalence of aflatoxin M1 in milk worldwide was 79.1% [24]. This could be explained by the fact that preventive and control activities, during harvest, processing, and storage of dairy feeds combined with the improvement of risk management actions in dairy processing industries have been improved in recent years and could be considered as considerable. The variation in the mean of AFM1 contamination in milk previously reported may be attributed to differentiation in carry-over rates of AFB1 in milk which depends on the animal species, but also can vary greatly depending upon nutritional, environmental, and physiological factors such as stage of lactation, systemic

 doi:10.20944/preprints202111.0014.v1

diseases, and local (mammary) infections, level of AFB1 in feed and, rate of feed ingestion, and geographical and seasonal conditions [35]. It is also important to highlight that many of the data have been obtained using different methodologies, with a consequence of different sensitivity and precision.

Aflatoxins contamination of foods of animal origin (milk, dairy products, eggs, and edible animal products) represent a global public health and economic concern. The presence of AFM1 in milk and milk products samples is most probably the consequence of feeding dairy cows a diet contaminated with AFB1. Since their presence has been responsible for significant adverse health and economic issues affecting consumers and farmers worldwide, has been triggered the formulation of regulations to control their presence in animal feed [8]. Various investigations conducted in Serbia in the last decade have revealed a significant presence of AFs in maize [3,36]. In general, the reported concentration of AFB1 in maize and consequently the presence of AFM1 in milk showed year-to-year variations of AFM1 prevalence [26]. Therefore, to estimate the risk of illness in the Serbian population exposed to aflatoxins, a series of survey studies have been conducted to monitor the incidence of AFM1 contamination particularly in raw milk in Serbia.

Albeit mean concentrations of AFM1 in our study were lower in whey and cheese than in the milk samples the highest concentration of AFM1 in whey and cheese was also observed in previous studies which concluded that during cheese production, 60% of the initial content of AFM1 accumulates in the whey, while 40% of the AFM1 remains in the curd or fresh cheese [25,37,38]. It might be due to the water-soluble nature of AFM1 and its affinity to form a hydrophobic bond with the hydrophobic part of casein that is subsequently concentrated in cheese [39]. Furthermore, AFM1 concentration was in soft cheeses generally 2.5–3.3 times higher and in hard cheeses, 3.9–5.8 times higher than in the milk from which the cheeses were made [34,37,40]. Most studies have reported that AFM1 concentrations in milk products are strongly dependent on the AFM1 concentrations in milk, hence could be a good predictor of the AFM1 concentration in cheese and whey. Noticeable, in the present study, AFM1 was found in 20% (158/775) of fermented milk products samples (ranging from 25 to 174 ng kg^{-1}, mean 9.58±0.02 ng kg^{-1}). The presence of AFM1 in fermented milk products may be due to manufacturers usually using imported dry milk for producing dairy products that were contaminated with AFM1. However, this low level of AFM1 in fermented milk products samples could be attributed to the function of lactic acid bacteria, during fermentation [41]. Results indicated that the incidence and mean AFM1 values obtained in the present study are low to moderate. Hence the risk of AFM1 exposure could not be a public health concern for the general population. However, as children use milk and dairy products in their diets frequently and are more sensitive to the adverse effects of AFs compared to adults, ingestion of low doses of AFM1 in milk over long periods must be considered a risk, and should not be underestimated or neglected.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 November 2021 doi:10.20944/preprints202111.0014.v1

Table 1. Aflatoxin M1 incidence and concentration in milk and dairy products samples included in the study.

Type of samples	n/N (%)	Mean (ng kg^{-1}±SD) of all samples				Mean positives (ng kg^{-1}±SD)	Median positives (ng kg^{-1})	Q1 positives (ng kg^{-1})	Q3 positives (ng kg^{-1})	Range (ng kg^{-1})
		LB	MB	UB	P95					
Infant formula	14/92 (15.2)	1.6±0.004	3.76±0.003	5.88±0.002	12.5	10.0±0.002	11.0	8.00	13.00	8.0-14.0
Fermented milk products	158/775 (20.3)	9.58±0.02	11.5±0.02	13.56±0.02	57.0	47.0±0.022	38.0	34.0	56.25	25.0-174.0
Clotted cream	0/48	-	-	-		-	-	-	-	<5.0
Butter	14/143 (10)	5.20±0.01	7.44±0.016	9.70±0.01	47.0	53.0±0.016	47.0	41.75	58.0	39.0-92.0
Milk beverages	145/714 (20)	4.22±0.01	6.21±0.011	8.20±0.01	23.0	20.77±0.02	14.0	10.50	22.50	5.0-117.0
Sour cream	19/132 (14)	6.90±0.02	9.04±0.018	11.18±0.02	48.0	47.95±0.002	39.0	31.0	60.0	25.0-103.0
Cheese	7/404 (2)	1.36±0.01	3.82±0.014	6.28±0.01	5.0	7.89±0.08	49.0	40.0	58.0	39.0-276.0
Pasteurized and UHT milk	574/725 (79)	22.34±0.02	22.87±0.018	23.40±0.01	53.0	28.22±0.016	25.00	19.00	35.0	5.0-132.0
Milk powder	67/201 (33)	9.12±0.02	10.79±0.020	12.46±0.02	47.0	27.37±0.03	16.00	9.00	36.00	5.0-155.0
Whey liquid	13/90 (14)	14.82±0.05	16.96±0.05	19.10±0.05	70.0	102.6±0.10	70.0	20.50	211.0	5.0-278.0
Total	**1012/3404 (29.7)**	**9.47±0.02**	**11.23±0.02**	**12.99±0.02**	**44.0**	**31.86±0.026**	**27.00**	**16.00**	**38.00**	**5.0-278.0**

N = Number of analyzed samples. n- number of positive samples (AFM1 > LOD). % = Percentage of positive samples. The limit of detection (LOD) for AFM1 is 5.0 ng kg^{-1}. Lower bound (LB) = assuming that the not detected results are equal to 0 (ND = 0). Middle bound (MB) = assuming that the not detected results correspond to the half of LOD (ND=2.5 ng kg^{-1}). Upper bound (UB) = assuming that the not detected results correspond to the LOD (ND= 5.0 ng kg^{-1}). P95-95th percentile. 1st quartile (Q1) 25% of the data are less than or equal to this value. 2nd quartile (Q2)he median. 50% of the data are less than or equal to this value. 3rd quartile (Q3) 75% of the data are less than or equal to this value.

Table 2. Characteristics of the study sample [23]

Age group	Body weight (kg)	N

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 November 2021 doi:10.20944/preprints202111.0014.v1

	Male	Female	Male	Female
Toddlers. 1-3 y	14	13	98	91
Children. 3-9 y	24	24	159	150

N-Number of participants

Table 3. The average intake of food groups by children population (g/day) [23]

Age group		Infant formula	Fermented milk products	Clotted cream	Butter	Milk beverages	Sour cream	Cheese	Pasteurized and UHT milk	Whey. liquid
Toddlers. 1-3 y	M	31.51	133.4	9.94	4.88	230.0	15.0	22.5	102.87	-
	F	29.93	115.76	8.72	5.3	213.3	17.47	24.4	112.05	-
Children. 3-9 y	M	-	153.24	13.31	7.95	220.83	16.58	27.19	99.55	250.0
	F	-	150.5	13.6	8.02	199.75	15.05	26.94	93.58	-
Average		30.72	138.23	11.41	6.54	215.97	16.02	25.25	102.01	250.0

BW-Body weight (kg). M-male. F-female. Food groups were categorized according to a national survey

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 November 2021 doi:10.20944/preprints202111.0014.v1

3.2. Dietary exposure assessment

Risk assessment through dietary exposure is the process of estimating the magnitude and the probability of a harmful effect on individuals or populations from specified agents or activities. Per definition, exposure assessment, as one component of risk assessment methodology combines mycotoxin levels in food with consumption patterns, and therefore, provides valuable information for risk management in which mycotoxins compromise food safety and health hazards, at either an individual or a population level [9]. Following the recommendations of EFSA [30] the current study is the most comprehensive (chronic) exposure scenario to assess the EDI of AFM1 by Serbian children, were taken into account a range with LB, and UB values.

Based on the data described before (*Section 2.4.*) estimated daily intake of AFM1 (ng kg^{-1} bw day^{-1}) through milk and dairy product consumption in different age categories is calculated and presented in Table 4. It is widely considered that the LB scenario generally underestimates contamination and exposure levels and that the UB scenario overestimates them [30]. As can be seen from Table 4 the exposure of AFM1 differs from product to product, also a significant difference ($p<0.05$) between the exposure values assessed, considering the UB, and LB scenarios were found within products. In consequence for these purposes, the middle-bound approach should be applied. In this study, the highest estimated dietary intake of AFM1 0.164 -0.172 ng kg^{-1} bw day^{-1} (LB-UB) and 0.193 - 0.202 ng kg^{-1} bw day^{-1} was found in the consumption of pasteurized and UHT milk in male and female toddlers, respectively.

The trend of AFM1 exposures for all the food types was pasteurized and UHT milk > whey > fermented milk products > milk beverages > sour cream > infant formula > butter > cheese. The food categories pasteurized and UHT milk (46 to 48%) and fermented milk products (27 to 31%) were the main contributors to the overall AFM1 mean exposure throughout both age groups (figure 1). Due to the limited number of consumption and concentration data for milk powder and clotted cream these food categories have not been taken into account for risk assessment.

The findings obtained in this study showed a remarkably lower exposure of the Serbian children population in comparison with the estimate of AFM1 intake reported by Kos et al., [32] and Milićević et al., [5]. These contradictory results regarding the EDI could be attributed to some uncertainties, including occurrence data (sampling strategy, low number of samples, seasonal effects, lack of sensitivity of some analytical methods) and exposure modeling. Also, data from the Health survey of the population of Serbia indicate a negative trend in the consumption of milk and dairy products. At least 41.8% of the population consumed milk and dairy products on a daily basis, which is significantly less than in 2013, when accounted for 51.7%.

Although AFs contamination in food occurs in many countries around the world, the nations that have been identified to be substantially exposed to AFM1 (sometimes dramatically) are primarily in sub- Saharan Africa and South Asia (Iran and Pakistan), of particular concern, are populations of children [42]. Generally, the mean dietary AFM1 exposure from milk and dairy products consumption in European populations is comparatively low, which may be the result of strict regulations on these mycotoxins in feed and milk products and from the adoption of integrated food safety management system. In comparison to international studies, our results were lower than the results of several studies. In addition, the current EDI values have not exceeded the previously established international TDI limit (0.2 ng kg^{-1} bw day^{-1}) [43]. Although the estimated AFM1 exposure levels for milk and dairy products consumers in the present study is relatively low, owing to the genotoxic and carcinogen nature of aflatoxins, the approach of "As Low as Reasonably Achievable" (ALARA) could be adopted in forthcoming regulations to protect Serbian consumers against the health effects caused by AFM1.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 November 2021 doi:10.20944/preprints202111.0014.v1

Table 4. Estimated dietary intake (ng kg^{-1} bw day^{-1}) of AFM1 in selected food products for two age categories

Food group	Exposure (ng kg^{-1} bw day^{-1})											
	Toddlers, 1-3 y						Children, 3-9 y					
	Male			Female			Male			Female		
	LB	UB	P95	LB	UB	P95	LB	UB	P95	LB	UB	P95
Infant formula	0.004	0.014	0.029	0.004	0.014	0.029						
Fermented milk products	0.091	0.129	0.543	0.085	0.121	0.508	0.061	0.087	0.364	0.060	0.085	0.358
Butter	0.002	0.003	0.016	0.002	0.004	0.019	0.002	0.003	0.016	0.002	0.003	0.016
Milk beverages	0.069	0.135	0.378	0.069	0.134	0.377	0.039	0.075	0.212	0.035	0.068	0.191
Sour cream	0.007	0.012	0.051	0.009	0.015	0.065	0.005	0.008	0.033	0.004	0.007	0.030
Cheese	0.002	0.010	0.008	0.003	0.012	0.009	0.002	0.007	0.006	0.001	0.007	0.005
Pasteurized and UHT milk	0.164	0.172	0.389	0.193	0.202	0.457	0.093	0.097	0.220	0.087	0.091	0.207
Whey liquid							0.154	0.177	0.199			
Total	**0.340**	**0.475**	**1.415**	**0.365**	**0.501**	**1.463**	**0.201**	0.277	0.850	0.190	0.262	0.807

Lower bound (LB) = assuming that the not detected results are equal to 0 (ND = 0). Middle bound (MB) = assuming that the not detected results correspond to the half of LOD (ND=2.5 ng kg^{-1}). Upper bound (UB) = assuming that the not detected results correspond to the LOD (ND= 5.0 ng kg^{-1}). P95-95th percentile. M-male. F-female.

 doi:10.20944/preprints202111.0014.v1

3.3. Risk characterization/ Cancer risk attributable to AFM1

The risk of exposure to AFM1 through milk and dairy product consumption was characterized using MoE (Table 5), and the liver cancer risk approach (Table 6 and 7). According to the EFSA scientific committee guidance [24] when the MoE value is ≥10,000, it is considered that there is a low risk of a negative impact on public health. Our results showed that MoE values for LB and UB exposure scenarios to AFM1 were far higher than 10000 in toddlers, and other children which indicates no health concern due to exposure to AFM1 through consumption of milk and dairy products. However, as children consume more milk relative to their body weight, children's exposure risk to AFM1 in milk and dairy products should be a continuous focus of attention.

The results of the characterization of HCC risk (cases per 100,000 individuals per year) for different age groups due to AFM1 exposure based on the calculation of the risk by Pcancer and EDI, are presented in Tables 6 and 7. The additional cancer risk due to mean exposure to AFM1 associated with milk and dairy product consumption in toddlers using potency estimates of 0.0017 (mean) for LB scenario ranged from 0.00032 to 0.00001 and from 0.00038 to 0.00001 cases per 100,000 individuals per year for male and female, respectively. For other children, the mean estimated number of liver cancer cases for the LB scenario ranged from 0.00030 to 0.00012 cases per 100,000 individuals per year for males and from 0.00017 to 0.00001 cases per 100,000 individuals per year for females. The main contribution of HCC risk due to AFM1 exposure was caused by the consumption of pasteurized and UHT milk 0.00038 and 0.00039 cases per 100,000 individuals per year for the lower and upper bound scenario, respectively. Our results are considerably lower than those reported in an assessment by EFSA [24] where the estimated cancer risk (mean and upper bound) ranged between 0.002–0.035, 0.008–0.032, 0.003–0.018, 0.001–0.006, 0.001–0.004, and 0.001–0.003 aflatoxin-induced cancers per 100,000 person-years for infants, toddlers, other children, adolescents, adults, and the elderly, respectively. Globally the standardized annual incidence rate for liver cancer is 15.3 per 100,000 among men and 5.4 per 100,000 among women [44]. Several studies conducted in African and South Asian countries have investigated the health impacts of early dietary exposure to aflatoxins. Prolonged exposure to aflatoxin might be the underlying cause of congenital disabilities and child growth impairment. Most of the studies have reported that exposure to AFs might be the underlying cause of child growth impairment [45,46]. Nonetheless, the possible association between chronic exposure to AFs in early life and the early onset of hepatic cancer has been explored by several studies. AFB1 is the most potent human hepatocarcinogen, accounting for around 4.6–28.2% of the total hepatocellular carcinoma (HCC) cases worldwide [47]. Further, there is a strong synergistic association between AFB1 and hepatitis B virus (HBV) infection in the etiology of HCC. Recent results from a national study [48] revealed that the rate of acute cases of HBV infection continued to decline (incidence of 1.25/100,000 inhabitants) over the last few years (2010–2019), which is following global trends and most likely reflects the impact of national vaccination programs. On the other hand, there is an increasing trend of registered cases of chronic viral hepatitis B and C. Improving the health and well-being of children are priorities health policies of each country. It is necessary to provide children with stability an environment for growth and development that includes good health and proper nutrition. Numerous epidemiological studies link childhood health with health outcomes in adults, and investing in children's health becomes one one of the most important measures that society can take to improve the health of the entire population. In summary, future work in this area would focus on the survey of occurrence and exposure to AFM1 to identify geographic regions where AFB1 levels in staple food are high enough to cause concern for human populations.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 November 2021 doi:10.20944/preprints202111.0014.v1

Table 5. The margin of exposure (MOE) values are based on dietary exposure to AFM1 for two age categories.

Food group	MOE											
	Toddlers, 1-3 y						Children, 3-9 y					
	Male			Female			Male			Female		
	LB	**UB**	**P95**	**LB**	UB	**P95**	**LB**	**UB**	**P95**	**LB**	**UB**	**P95**
Infant formula	1076492	292923	137791	1085686	295425	138968						
Fermented milk products	43819	30958	7365	46890	33127	7881	65393	46200	10991	66566	47028	11188
Butter	2206810	1183032	244158	1886792	1011476	208752	2322206	1244894	256925	2301937	1234028	254682
Milk beverages	57696	29692	10586	57851	29772	10614	103015	53015	18901	113886	58610	20896
Sour cream	541063	333930	77778	431381	266237	62011	839146	517899	120627	924455	570549	132890
Cheese	1830065	396320	497778	1567020	339355	426230	2596110	562215	706142	2679887	580358	728929
Pasteurized and UHT milk	24368	23264	10271	20773	19832	8756	43166	41211	18195	45920	43840	19356
Whey liquid							25911	22642	20105			
Average	**825759**	**327160**	**140818**	**728056**	**285032**	**123316**	**856421**	**355439**	**164555**	**1022109**	**422402**	**194657**

MoE calculations were based on benchmark dose ($BMDL_{10}$) for AFB1 of 0.4 μg/kg bw per day and potency factor for AFM1 of 0.1 [24]. Lower bound (LB) = assuming that the not detected results are equal to 0 (ND = 0). Middle bound (MB) = assuming that the not detected results correspond to the half of LOD (ND=2.5 ng kg^{-1}). Upper bound (UB) = assuming that the not detected results correspond to the LOD (ND= 5.0 ng kg^{-1}). P95-95th percentile

Table 6. Cancer risk estimates were calculated from the chronic dietary exposure to AFM1. Scenario 1 (mean)

Food group	Liver cancer risk (case/100.000 persons)	
	Toddlers, 1-3 y	Children, 3-9 y

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 November 2021 doi:10.20944/preprints202111.0014.v1

	Male			Female			Male			Female		
	LB	**UB**	**P95**	**LB**	**UB**	**P95**	**LB**	**UB**	**P95**	**LB**	**UB**	**P95**
Infant formula	0.00001	0.00003	0.00006	0.00001	0.00003	0.00006						
Fermented milk products	0.00018	0.00025	0.00106	0.00017	0.00024	0.00099	0.00012	0.00017	0.00071	0.00012	0.00017	0.00070
Butter	0.00000	0.00001	0.00003	0.00000	0.00001	0.00004	0.00000	0.00001	0.00003	0.00000	0.00001	0.00003
Milk beverages	0.00014	0.00026	0.00074	0.00013	0.00026	0.00074	0.00008	0.00015	0.00041	0.00007	0.00013	0.00037
Sour cream	0.00001	0.00002	0.00010	0.00002	0.00003	0.00013	0.00001	0.00002	0.00006	0.00001	0.00001	0.00006
Cheese	0.00000	0.00002	0.00002	0.00000	0.00002	0.00002	0.00000	0.00001	0.00001	0.00000	0.00001	0.00001
Pasteurized and UHT milk	0.00032	0.00034	0.00076	0.00038	0.00039	0.00089	0.00018	0.00019	0.00043	0.00017	0.00018	0.00040
Whey liquid							0.00030	0.00034	0.00039			
Total	**0.00066**	**0.00093**	**0.00276**	**0.00071**	**0.00098**	**0.00286**	**0.00069**	**0.00089**	**0.00166**	**0.00037**	**0.00037**	**0.00158**

Potency estimates of 0.0017 (mean) per 100.000 person-years per ng kg^{-1} bw per day^{-1} were calculated for HBsAg-negative individuals. For HBsAg-positive individuals. potency estimates of 0.0269 (mean) per 100.000 person-years per ng/kg bw per day were calculated [31]. The risk of liver cancer was estimated as new cancer cases/year/100.000 population by multiplying the AFM1 EDI by the average HCC potency 0.001952 (mean) based on 1% prevalence of HBV infection in Serbia.

Table 7. Cancer risk estimates were calculated from the chronic dietary exposure to AFM1. Scenario 2 (95% upper bound (UB)

Food group	**Liver cancer risk (case/100.000 persons)**	
	Toddlers, 1-3 y	**Children, 3-9 y**

 doi:10.20944/preprints202111.0014.v1

	Male			Female			Male			Female		
	LB	**UB**	**P95**	**LB**	**UB**	**P95**	**LB**	**UB**	**P95**	**LB**	**UB**	**P95**
Infant formula	0.00002	0.00007	0.00016	0.00002	0.00007	0.00016						
Fermented milk products	0.00049	0.00070	0.00294	0.00046	0.00065	0.00275	0.00033	0.00047	0.00197	0.00033	0.00046	0.00194
Butter	0.00001	0.00002	0.00009	0.00001	0.00002	0.00010	0.00001	0.00002	0.00008	0.00001	0.00002	0.00009
Milk beverages	0.00038	0.00073	0.00205	0.00037	0.00073	0.00204	0.00021	0.00041	0.00115	0.00019	0.00037	0.00104
Sour cream	0.00004	0.00006	0.00028	0.00005	0.00008	0.00035	0.00003	0.00004	0.00018	0.00002	0.00004	0.00016
Cheese	0.00001	0.00005	0.00004	0.00001	0.00006	0.00005	0.00001	0.00004	0.00003	0.00001	0.00004	0.00003
Pasteurized and UHT milk	0.00089	0.00093	0.00211	0.00104	0.00109	0.00247	0.00050	0.00053	0.00119	0.00047	0.00049	0.00112
Whey liquid							0.00084	0.00096	0.00108			
Total	**0.00184**	**0.00257**	**0.00766**	**0.00197**	**0.00271**	**0.00792**	**0.00109**	**0.00150**	**0.00460**	**0.00103**	**0.00142**	**0.00437**

Potency estimates of 0.0049 (95% upper bound (UB)) per 100.000 person-years per ng kg^{-1} bw per day^{-1} were calculated for HBsAg-negative individuals. For HBsAg-positive individuals. potency estimates of 0.0562 (95% UB) per 100.000 person-years per ng/kg bw per day were calculated [31]. The risk of liver cancer was estimated as new cancer cases/year/100.000 population by multiplying the AFM1 EDI by the average HCC potency 0.005413 (UB) based on 1% prevalence of HBV infection in Serbia.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 November 2021 doi:10.20944/preprints202111.0014.v1

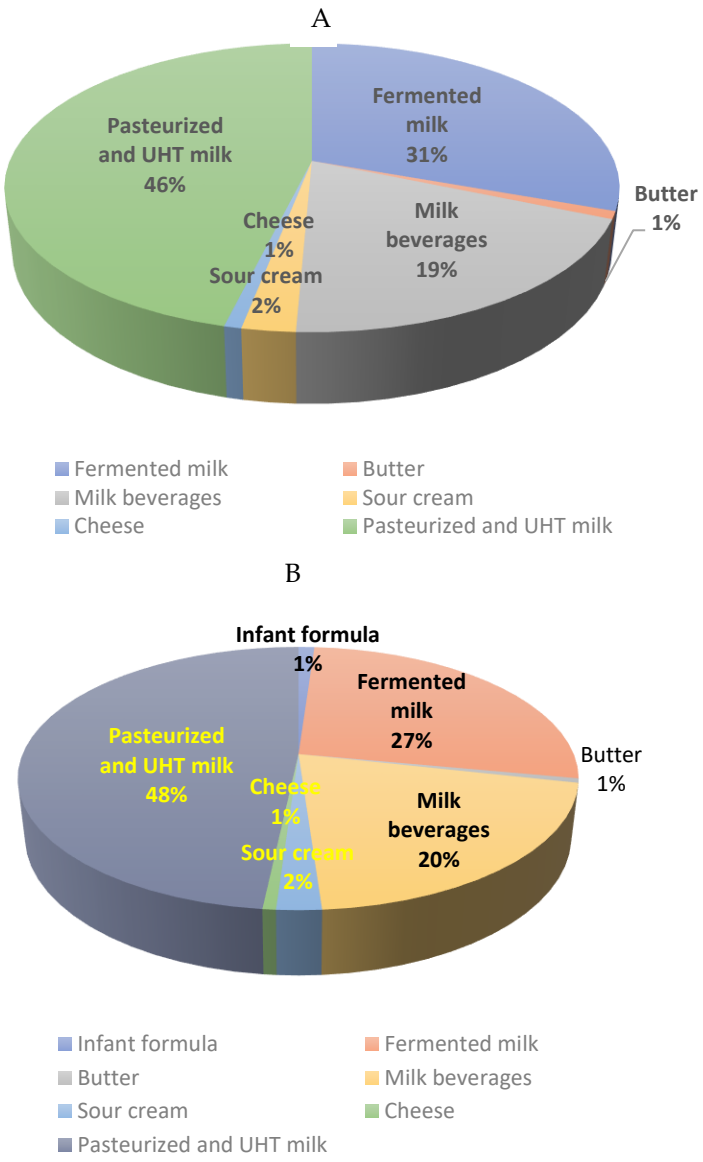

Figure 1. Contribution (%) of the most important food groups to the long-term dietary exposure of children aged 2 to 6 years (A) and toddlers. 1-3 years (B) to AFM1

4. Conclusions

Considering the present evidence on the negative health effects of AFM1, this study through the MOE approach and the population risk method suggests that milk and dairy products had negligible health risk to children population to the AFM1 exposure. Despite, current AFM1 concentrations are not high enough to elicit toxic effects, risk data should be interpreted carefully due to the present study investigating only the consumption of milk and dairy products. Thus, the focus of future studies should be on exposure from complete diets commonly consumed by Serbian children to estimate cumulative exposure from all sources of aflatoxins. Also, further research is advisable, in particular related to an association of liver cancer with AF intake and hepatitis B infection. Since the contamination of feedstuffs with AFB1 plays a major role in the contamination of milk, the government and all stakeholders involved in the milk supply chain should pay more attention to implementing an integrated food safety management system to prevent the production of mycotoxins in dairy cattle feed and reduce AFM1 residues in milk.

Author Contributions: Conceptualization, D.M. and Mirjana G.; methodology, D.M.; Mirjana G.; and S.J.; validation, S.J.; formal analysis, J.Đ.S.; J.M.; investigation, M.Z.; writing—original draft preparation, D.M..; writing—review and editing, D.M.; supervision, Maria G.; All authors have read and agreed to the published version of the manuscript.

Funding: Acknowledgement

This research was funded by the Ministry of Education, Science and Technological Development, Republic of Serbia, Grant No. 451-03-9/2021-14/200050 and Grant No. 451-03-9/2021-14/200015 and European Food Safety Authority (EFSA).

Institutional Review Board Statement: The study was conducted in accordance with the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Institute for Medical Research Ethics Committee in Serbia on December 8th, 2017 (EO 123/2017).

Informed Consent Statement: Not applicable.

Data Availability Statement: Results attained in this study are included in the manuscript. Individual data are not publicly available due to ethical restrictions.

Acknowledgments: Authors of this manuscript are grateful to Agnes Kadvan for IT support and data analyses of the EU Menu National Food Consumption Survey.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Medina. A.; González. J.M.; Sainz. M.J. Impact of global warming on mycotoxins. *Curr Opin Food Sci* **2017**, *18*, 76–81.
2. Raffa, N.; & Keller, N. P. A call to arms: Mustering secondary metabolites for success and survival of an opportunistic pathogen. *PLOS Pathogens* **2019**, *15(4),* e1007606. https://doi.org/10.1371/journal.ppat.1007606
3. Udovicki, B.; Audenaert, K.; De Saeger, S.; & Rajkovic, A. Overview on the Mycotoxins Incidence in Serbia in the Period 2004–2016. *Toxins* **2018**, *10(7),* 279. https://doi.org/10.3390/toxins10070279
4. Milićević, D.; Škrinjar, M.; and Baltić, T. Real and Perceived Risks for Mycotoxin Contamination in Foods and Feeds: Challenges for Food Safety Control. *Toxins* **2010**, *2(4),* 572-592; doi:10.3390/toxins2040572.
5. Milićević, D.; Spirić, D.; Radičević, T.;. Velebit, B.; Stefanović, S.; Milojević, L.; & Janković, S. A review of the current situation of aflatoxin M 1 in cow's milk in Serbia: Risk assessment and regulatory aspects. *Food Addit Contam A* **2017a,** *34(9),* 1617–1631. https://doi.org/10.1080/19440049.2017.1363414
6. Milićević, D.; Nastasijevic, I.; & Petrovic, Z. Mycotoxin in the food supply chain—Implications for public health program. *J Environ Sci Health C* **2016**, 34(4), 293–319. https://doi.org/10.1080/10590501.2016.1236607
7. Pleadin, J.; Frece, J.; Markov, K. Mycotoxins in food and feed. *Adv Food Nutr Res* **2019**, *89,* pp. 297-345
8. Milicević, D.; Udovički, B.; Petrović, Z.; Janković, S.; Radulović, S.; Gurinović, M.; Rajković, A. Current status of mycotoxin contamination of food and feeds and associated public health risk in Serbia. *Meat Technology* **2020,** *61 (1),* 1–36.
9. International Agency for Research on Cancer (IARC), In: Wild, C.P., Miller, J.D., Groopman, J.D. (Eds.), ARC Working Group Reports, 9; Mycotoxin Control in Low and Middle-income Countries. WHO. Geneva. Switzerland **2015**.
10. De Ruyck. K,; De Boevre, M.; Huybrechts, I.; & De Saeger, S. Dietary mycotoxins. co-exposure. and carcinogenesis in humans: Short review. *Mutat Res Rev Mutat Res* **2015,** *766*. 32–41. https://doi.org/10.1016/j.mrrev.2015.07.003
11. IARC (International Agency for Research on Cancer). Chemical Agents and Related Occupations; WHO: Geneva. Switzerland. **2012**, pp. 225–248.
12. EU Comission Regulation (EU) No 165/2010 of 26 February 2010 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins. *Off J Eur Union* **2010**, *L50/8.*
13. Serbian Regulation Maximum allowed contents of contaminants in food and feed. *Off Bull Rep Serbia***. 2019,** *22/2018. 90/2018. 76/2019 i 81/2019.*
14. Ferlay, J.; Soerjomataram, I.; Dikshit, R.; Eser, S.; Mathers, C.; Rebelo, M.; Parkin, D.M.; Forman, D.; Bray, F. Cancer incidence and mortality worldwide: sources. methods and major patterns in GLOBOCAN 2012. *Int J Cancer* **2015**, *136,* 359-386. [DOI: 10.1002/ijc.29210]
15. Wong, M.C.S.; Jiang, J.Y.; Goggins, W.B.; Liang, M.; Fang, Y.; Fung, F.D.H.; Leung, C.; Wang, H.H.X.; Wong, G.L.H.; Wong, V.W.S.; Chan, H.L.Y. International incidence and mortality trends of liver cancer: a global profile. *Sci Rep* **2017,** *7,* 45846. [DOI: 10.1038/srep45846]
16. Tesfamariam, K.; De Boevre, M.; Kolsteren, P.; Belachew, T.; Mesfin, A.; De Saeger, S.; & Lachat, C. Dietary mycotoxins exposure and child growth. immune system. morbidity. and mortality: A systematic literature review. *Crit Rev Food Sci Nutr* **2020,** *60,(19),* 3321-3341. doi: 10.1080/10408398.2019.1685455
17. Raiola, A.; Tenore, G.C.; Manyes, L.; Meca, G.; Ritieni, A. Risk analysis of main mycotoxins occurring in food for children: An overview. *Food Chem Toxicol* **2015,** *84*, 169–180

18. Peraica, M.; Richter, D.; Rašić, D. Mycotoxicoses in children. *Arch Ind Hyg Toxicol* **2014,** *65,* 347–363.
19. Assuncao. R.; Vasco, E.; Nunes, B.; Loureiro, S.; Martins, C.; & Alvito, P. Single-compound and cumulative risk assessment of mycotoxins present in breakfast cereals consumed by children from Lisbon region. *Food Chem Toxicol* **2015**, *86*, 274–281.
20. Hulin, M.; Bemrah, N.; Nougadere, A.; Volatier, J. L.; Sirot. V.; & Leblanc. J. C. Assessment of infant exposure to food chemicals: The French total diet study design. *Food Addit Contam A* **2014**, *31(7),* 1, 226–1.239
21. Juan, C.; Raiola, A.; Mañes. J.; Ritieni. A. Presence of mycotoxin in commercial infant formulas and baby foods from Italian market. *Food Control* **2014,** *39,* 227–236.
22. Pereira, P.C. Milk nutritional composition and its role in human health. *Nutrition* **2014,** *30(6),* 619-27.
23. Zekovic, M.; Gurinovic, M.; Milesevic, J.; Glibetic, M. National Food Consumption Survey among children from 1 – 9 years in Serbia– final delivverable. *EFSA supporting publication* **2021**, *20YY:EN-NNNN*. 26pp.doi:10.2903/sp.efsa.20YY.EN-NNNN
24. EFSA Contam Panel. Schrenk, D.; Bignami, M.; Bodin, L.; Chipman, J.K.; del Mazo, J.; Grasl-Kraupp, B.; et al (EFSA Panel on Contaminants in the Food Chain) Scientific opinio – risk assessment of aflatoxins in food. *EFSA J***, 2020**, *18*, (6040), 112.
25. Milićević, D.; Spirić, D.; Janković, S.; Velebit, B.; Radičević, T.; Petrović, Z.; and Stefanović, S. Aflatoxin M1 in processed milk: Occurrence and seasonal variation with an emphasis on risk assessment of human exposure in Serbia. The 59th International Meat Industry Conference MEATCON2017, Zlatibor, Serbia, 1–4 October 2017; *IOP Conf. Series: Earth and Environmental Science 85, 2017, 012040. IOP Publishing* doi:10.1088/1755-1315/85/1/012040.
26. Milićević, D.; Petronijević, R.; Petrović, Z.; Đjinović-Stojanović, J.; Jovanović, J.; Baltić, T.; Janković, S. Impact of climate change on aflatoxin M1 contamination of raw milk with special focus on climate conditions in Serbia. *J Sci Food Agric* .**2019**, *30,* 99(11), 5202-5210. doi: 10.1002/jsfa.9768. https://doi.org/10.1002/jsfa.9768
27. Nikolic, M.; Milesevic, J.; Zekovic, M.; Gurinovic, M.; Glibetic, M. The development and validation of food atlas for portion size estimation in the Balkan region. *Front Nutr* **2018**, *5*, 78. https://doi.org/10.3389/FNUT.2018.00078
28. Gurinović, M.; Milešević, J.; Kadvan, A.; Nikolić, M.; Zeković, M.; Djekić-Ivanković, M.; Glibetić, M. Development, features and application of DIET ASSESS & PLAN (DAP) software in supporting public health nutrition research in Central Eastern European Countries (CEEC). *Food Chemistry*, **2018**, *238,* 186–194. https://doi.org/10.1016/J.FOODCHEM.2016.09.114
29. JECFA - Joint FAO/WHO Expert Committee on Food Additives Evaluation of certain contaminants in food: eighty-third report of the joint FAO/WHO expert committee on food additives. *WHO Technical Report Series 1002*, **2017,** *1–166.* https:// apps. who. int/ iris/handle/ 10665/ 254893
30. EFSA. Management of left-censored data in dietary exposure assessment of chemical substances. *EFSA J.* **2010**, *8,* 1557.
31. FAO/WHO/UN; Safety evaluation of certain contaminants in food: prepared by the eighty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Geneva: World Health Organization and Food and Agriculture Organization of the United Nations; 2018 (WHO Food Additives Series, No. 74; FAO JECFA Monographs 19 bis).
32. Kos, J.; Lević, J.; Đuragić, O.; Kokić, B.; & Miladinović, I. Occurrence and estimation of aflatoxin M1 exposure in milk in Serbia. *Food Control* **2014,** *38*, 41–46.
33. Škrbić, B.; Živančev, J.; Antić, I.; & Godula, M. Levels of aflatoxin M1 in different types of milk collected in Serbia: Assessment of human and animal exposure. *Food Control* **2014**, *40,* 113–119.
34. Tomašević, I.; Petrović, J.; Jovetić, M.; Raičcević, S.; Milojević, M.; Miočinović, J. Two year survey on the occurrence and seasonal variation of aflatoxin M1 in milk and milk products in Serbia. *Food Control* **2015,** *56,* 64–70.
35. Walte, H. G.; Schwake-Anduschus, C.; Geisen, R.; and Fritsche, J. Aflatoxin: food chain transfer from feed to milk. *J Consum Protect Food Safety* **2016,** *11,* 295-297 DOI 10.1007/s00003-016-1059-8
36. Kos, J.; Janić Hajnal, E.; Šarić, B.; Jovanov, P.; Mandić, A.; Đuragić, O.; & Kokić, B**.** Aflatoxins in maize harvested in the Republic of Serbia over the period 2012–2016. *Food Additiv Contam* B, **2018**, *11(4),* 246-255. DOI: 10.1080/19393210.2018.1499675.
37. Škrbić, B.; Antić, I.; and Živančev, J. Presence of aflatoxin M1 in white and hard cheese samples from Serbia. *Food Control* **2015**, *50*, 111**–***7*.
38. Milicevic, D.; Jankovic, S.; Muric, K,; Petrovic, Z.; Petronijevic, R.; Raseta, M; and Djinovic-Stojanovic, J. Safety of milk and whey from Zlatibor region in relation to aflatoxin M1 contamination: a seasonal study. The 60th International Meat Industry Conference MEATCON2019, Kopaonik, Serbia, September 22-25, 2019; *IOP Conf. Series: Earth and Environmental Science 333,* 2019, 012077, IOP Publishing doi:10.1088/1755-1315/333/1/012077.
39. Costamagna, D.; Gaggiotti, M.; Chiericatti, C. A.; Costabel, L.; Audero, G. M. L.; Taverna, M., et al. Quantification of aflatoxin M1 carry-over rate from feed to soft cheese. *Toxicol Rep* **2019**, *6,* 782–787. https://doi.org/10.1016/j. toxrep.2019.07.00
40. Torović, Lj.; Popović, N.; Živkov-Baloš, M.; Jakšić, S. Risk estimates of hepatocellular carcinoma in Vojvodina (Serbia) related to aflatoxin M1 contaminated cheese. *J Food Compos Anal* **2021**, *103,* 104122.
41. Iqbal, S.Z.; &Asi, M.R. Assessment of aflatoxin M1 in milk and milk products from Punjab, Pakistan. *Food Control* **2013**, *30(1),* 235–239.
42. Turnaa, N. S.; Wu, F. Aflatoxin M1 in milk: A global occurrence, intake, & exposure assessment. *Trends Food Sci Tech* **2021**, *110,* 183–192.
43. Kuiper-Goodman T. Uncertainties in the risk assessment of three mycotoxins: aflatoxin, ochratoxin, and zearalenone. *Canad J Physiol Pharmacol* **1990**, *68,* 1017-24.
44. Pitt, J.I.I.; Wild, C.P.; Baan, R.A.; Gelderblom, W.C.A.; Miller, J.D.; Riley, R.T.; Wu, F. Improving Public Health Through Mycotoxin Control; IARC WHO, Lyon, France, **2012,** pp. 225–248. ISBN 978-92-832-2158-6.

45. Makori, N.; Matemu, A.; Kimanya, M.; & Kassim, N. Inadequate management of complementary foods contributes to the risk of aflatoxin exposure and low nutrition status among children. *W Mycotoxin J* **2019,** *12*, 67–76. https://doi.org/10.3920/WMJ2018.2354
46. Ismail, A.; Naeem, I.; Gong, Y. Y.; Routledge, M. N..; Akhtar, S.; Riaz, M.; Ramalho, L. N. Z.; de Oliveira C. A. F.; Ismail, Z. Early life exposure to dietary aflatoxins, health impact and control perspectives: A review. *Trends Food Sci Tech* **2021**, *112*, 212–224.
47. Liu, Y.; & Wu, F. Global burden of aflatoxin-induced hepatocellular carcinoma: A risk assessment. *Environ Health Perspect* **2010,** *118*, 818–824. https://doi.org/10.1289/ehp.0901388
48. Institute of public health of Serbia „Dr Milan Jovanovic Batut" The Report on infectious diseases in the Republic of Serbia for 2019. **2020**. https://www.batut.org.rs/download/izvestaji/Godisnji%20izvestaj%20o%20zaraznim%20bolestima%202019.pdf [Accesed 26. October 2021].