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Review paper

Current status of mycotoxin contamination of food and feeds and associated public health risk in Serbia

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A b s t r a c t: Mycotoxins are chemical hazards of microbiological origin, produced mainly by filamentous fungi during their secondary metabolism. The role of mycotoxins has been recognized in the aetiology of a number of diseases, particularly cancers that belong to non-communicable diseases (NCDs). The NCDs have a leading and growing contribution to preventable deaths and disability across the globe. The NCDs are known as chronic diseases, tend to be of long duration and are the result of a combination of genetic, physiological, environmental and behavioural factors. Following the increased interest in health effects caused by synergisms between natural and synthetic contaminants along the food chain, mycotoxin contamination will continue to be an area of concern for producers, manufacturers, regulatory agencies, researchers and consumers in the future. Considering that their presence in food depends strongly on climatic conditions, in Serbia, recent drought and then flooding confirmed that mycotoxins are one of the foodborne hazards most susceptible to climate change. In this article, we review key aspects of mycotoxin contamination of the food supply chain and attempt to highlight the latest trends and projections for mycotoxin reduction from a Serbian perspective.

Keywords: *mycotoxin, occurrence, public health, SWOT-analysis.*

Introduction

Food security and safety is one of the major problems currently in protecting human health and the economic development of countries around the world and constitute some of the main challenges of the 2030 Agenda for Sustainable Development. According to data from the World Health Organization (WHO), contaminated food and water cause over 200 human diseases, with up to 30% of the world population suffering from certain types of food- and water-related diseases each year. Among them, 2.2 million people face fatal outcomes, with 1.9 million children dying from these diseases (*WHO,* 2015). Due to continual growth and population migration, an increasing trend in the number of food-borne outbreaks and cases of diseases can be expected. In this context, the responsibility of competent authorities is directed towards the establishment of an effective food safety system, which includes an integrated and well-coordinated longitudinal system from field to table (*Akkerman et al.,* 2010).

Based on data belonging to the Rapid Alert System on Food and Feed (RASFF), in the last ten years, mycotoxins, particularly aflatoxins, were the most commonly reported type of hazard. Data show that 93% of the overall mycotoxin notifications referred to aflatoxins (*RASFF,* 2019). Also, on the basis of data from other agencies, in regard to occurrence, nutritional-health disorders and economic impact, mycotoxins are a very serious problem in the food supply system, especially in developing countries (*WHO,* 2018). Mycotoxins are defined as a structurally diverse group of secondary metabolites, often low molecular-weight compounds, produced by a large number of species of different fungal genera, which can contaminate food commodities along the food chain (*Marin et al.,* 2013). Production of

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secondary metabolites is not directly essential for normal fungal growth, but allows moulds to rapidly colonize the environment and compete with other organisms or inhibit competitor growth and reproduction and, therefore, gives moulds a competitive advantage within complex ecosystems (*Raffa and Keller*, 2019). Although 400 secondary metabolites with toxigenic potential have been identified to date, only about 50 of them have been studied in detail due to their important roles in food safety. The

Table 1. Toxicological aspects of the main mycotoxins

Mycotoxins	Most susceptible crops	Producing fungi	Primary mechanism of action/Health effects	IARC (Group)	Health guidance value	Ref.
AF′s	tree nuts, ground nuts, dried fruits, spices, maize	*Aspergillus flavus, A. parasiticus, A. section Flavi*	Binds to guanine (dnaadduct)/ Carcinogenic, Mutagenic, Teratogenic, Hepatotoxic, Nephrotoxic, Immunosuppressive	1	(ALARA principle) $BMDL_{10}$ 170 ng kg^{-1} bw day^{-1}	*EFSA,* 2007, *IARC,* 2012
OTA	cereal grains, coffee, beer, wine, dried fruits, spices, meat products,	*A. ochraceus, P. verrucosum, A. niger A. carbonarius*	Blocks protein synthesis/Mutagenic, teratogenic, neurotoxic, hepatotoxic, Nephrotoxic, immunotoxic	2B	TWI = 120 ng kg^{-1} bw	*IARC,* 1993, *EFSA*, 2006
FUM′s	maize, maize-based food	*Fusarium proliferatum, F. verticillioides*	Inhibit ceramide synthase/ Esophageal and liver carcinogens, neurotoxic, neural tube defects, genotoxic		TDI = 1 µg kg^{-1} bw day^{-1}	*IARC,* 1993, 2002, *EF*SA, 2018a
STG	coffee beans, spices, nuts and beer	*A. versicolor, A. flavus, A. parasiticus, A. nidulans*	Genotoxicity, carcinogenicity, Liver and kidneys		PTWI is not established	*IARC*, 2006, *EFSA*, 2013
DON and sum 3-Ac-DON, 15-Ac-DON, DON-3--glucoside	cereal crops, processed grains	*F. graminearum, F. culmorum*	Inhibition of protein synthesis/Gastrointestinal haemorrhagiae, immuno-suppression, dermatosis	3	TDI = 1 µg kg^{-1} bw day^{-1}	*IARC,* 1993, *EFSA*, 2014a
T-2, HT-2 toxin		*F. sporotrichioides, F. Poae, F. langsethiae*	DNA damage/Immun depressants mutagenic Gastrointestinal haemorrhaging, neurotoxic		TDI (T-2+HT-2) = = 0.1 µg kg^{-1} bw day^{-1}	*IARC,* 1993, *EFSA,* 2014b
NIV		*F. graminearum, F. crookwellense, F. nivale*	Inhibit protein and DNA synthesis, immunosuppresive		TDI = 1.2 µg kg^{-1} bw day^{-1}	*IARC,* 1993, *EFSA*, 2014b
ZEA	maize, cereal crops	*F. graminearum, F. culmorum, F. equiseti, F. cerealis, F. verticillioides,*	Binds to mammalian estrogen receptor/ Hyperestrogenism, Reproductive disorders, infertility, early pubertal changes		TDI = 0.25 µg kg^{-1} bw day^{-1}	*IARC,* 1993, *EFSA*, 2014a
PAT	apples and apple-derived foods	*Penicillim expansum, Bysochlamis nívea, A. clavatus*	DNA and RNA synthesis inhibition/ gastrointestinal symptoms, neurotoxic, immunosuppressive, mutagenic		TDI = 0.4 µg kg^{-1} bw day^{-1}	*IA*RC, 1993, *EFSA*, 2006

Legend: AF′s-Aflatoxin (B1,B2,G1,G2,M1); OTA-Ochratoxin A; FUM′s-Fumonisin (B1, B2); STG-Sterigmatocystin; DON-deoxynivalenol; NIV-nivalenol; ZEA- Zearalenone; PAT-patulin; Group 1, carcinogenic to humans; Group 2A, probably carcinogenic to humans; Group 2B, possibly carcinogenic to humans; Group 3, not classifiable as to its carcinogenicity to humans. ALARA principle- As low as reasonably achievable, TDI: tolerable daily intake, PTWI, provisional tolerable weekly intake, Reference Point

most important agro-economic and public health classes of mycotoxins are aflatoxins, ochratoxin A (OTA), zearalenone (ZEA), trichothecenes (TCTs), fumonisins (FUMs), patulin (PT), *Alternaria* toxins and ergot toxins. They are produced by species of *Fusarium, Penicillium, Aspergillus, Claviceps* and *Alternaria* (*Liew and Mohd-Redzwan,* 2018; *Rai et al.,* 2019). Some of them can produce more than one mycotoxin, and some mycotoxins are produced by more than one fungal species (*Marin et al.,* 2013) (Table 1). Their common co-occurrence at low levels in food and feed presents a threat to human and animal health, inducing major economic losses for farmers, industry, international trade and society.

Besides these, some other mycotoxins such as "modified mycotoxins", "masked mycotoxins", and new, emerging mycotoxins (moniliformin, enniatins, beauvericin and fusaproliferin) are also the subjects of notable attention in on-going investigations (*Jajic et al.,* 2019). The term *modified mycotoxins* refers to any mycotoxin with a structure that has been changed in the course of some chemical/biochemical reaction by plants, animals, fungi or by processing (*Rychlik et al.,* 2014). *Masked mycotoxins* are a group of mycotoxins produced during some detoxication reactions implemented by plants in an attempt to neutralize native mycotoxins (*Khaneghah et al.,* 2018). Considering that modified mycotoxins are usually not detected by commonly used analytical methods, only limited data are available on their occurrence in crops. Thus, their impact on food safety may be even more relevant than it currently seems.

Most mycotoxins are relatively heat-stable in the conventional food processing temperature range (cooking, baking, frying, roasting), and thus, they remain in the final product (*Udovicki et al*., 2018; *Carballo et al.,* 2019). Therefore, human contamination with mycotoxins can occur directly through the consumption of foods containing mycotoxins or indirectly (carry-over) through consumption of mycotoxins and/or their metabolites from animal tissues, milk and eggs (*Zadravec et al.,* 2020: *Milicevic et al.,* 2014) Ingestion of mycotoxin-contaminated food/feed results in a disease (mainly subclinical) known as mycotoxicosis. Depending on the mycotoxin's toxicity, its concentrations in food, the duration of exposure, and the age and nutritional status of the at-risk individual, mycotoxin health-related risks range from acute to chronic (mutagenic, teratogenic, carcinogenic) manifestations in both animals and humans (*Datsugwai et al.,* 2013; *Richard,* 2007). In 1993, the International Agency for Research on Cancer (*IARC*, 1993) evaluated the carcinogenic potential of aflatoxins OTA, TCT, ZEA and FUMs and in 2012 re-evaluated aflatoxin M1 (AFM1) and carcinogenicity (*IARC,* 2012; *Ostry et al.,* 2017). Naturally-occurring aflatoxins (AFB1, AFB2, AFG1, AFG2 and AFM1) were classified as carcinogenic to humans (Group 1), while OTA and FUMs were classified as possible carcinogens (Group 2B). TCT, ZEA and PT, however, were not classified as human carcinogens (Group 3) (Table 1).

The economic losses and health hazards posed by mycotoxin contamination of food and feed are a huge challenge, and this is especially severe in developing countries (*Milicevic et al.,* 2019a). The economic impact of mycotoxin contamination includes loss of human and animal life, increased health care and veterinary care costs, reduced livestock production, disposal of contaminated foods and feeds, and investment in research and applications to reduce the severity of the mycotoxin problem (*Zaki et al.,* 2012). However, these losses represent only part of the economic losses. The most information on economic impact is available for the United States, where the cost of mycotoxin contamination to the U.S. economy was estimated to be between $2 billion and $3 billion per year, depending on the year (*Sassi et al*., 2018). Accordingly, in order to protect consumer health and to reduce economic losses, surveillance and monitoring of mycotoxins in food and feed, and implemented in public health programs, have become major objectives for producers, regulatory authorities and researchers in Serbia (*Milicevic et al.,* 2016).

Mycotoxin Studies in Serbia

Since the discovery of aflatoxins in 1960, a number of studies on the occurrence of mycotoxins in Serbia have been conducted (*Ozegivic and Aganovic*, 1963; *Popovic et al.,* 1968; *Kordic,* 1986). These older surveys mainly followed diseases in domestic animals, leading to further research to answer questions related to the human health relevance. In general, from 2006 to date, more sensitive analytical techniques and research concepts were employed, leading to great progress in mycotoxin research. This resulted in large data pools and new insights into mycotoxin prevalences, concentrations, mitigation measures and risk assessments. Recently, several studies have assessed the effects of climate change on food safety, including the occurrence of mycotoxin-producing fungi and mycotoxins in foods/feeds. These data clearly show that mycotoxin

contamination is becoming a serious problem in Serbia because of the negative public health effects, but particularly because of the negative effects on the economy and trade.

Therefore, the aim of this review is to overview studies published between 2011 and 2019 about the incidence of mycotoxins in Serbia, and we attempt to highlight the latest trends and projections for mycotoxin reduction from the Serbian perspective.

Impact of climate factors in Serbia on mycotoxin production

Fungal colonization and/or mycotoxin production is influenced by various ecological and environmental factors (*Tola and Kebede,* 2016). *D'Mello and MacDonald* (1997) categorized these factors as physical (moisture, RH, temperature and mechanical damage), chemical (carbon dioxide, oxygen, composition of substrate, pesticide and fungicides), and biological (plant variety, stress, insects and spore load). The biological factors have been further subcategorized to include intrinsic factors (including fungal species, strain specificity, strain variation and stability of toxigenic properties). Moreover, temperature (t), relative humidity (RH), rainfall (R) and grain water activity (a_w) are the most important ecological factors that modulate fungal growth and mycotoxin production pre-harvest or during storage (*Palumbo et al.,* 2020). Therefore, drought (and the RH related to it) is a modulator of mycotoxin contamination that is expected to be more frequent in the future, depending on geography. Climate change seems to be another important factor affecting mycotoxin contamination of foods and feedstuffs (*Milicevic et al.,* 2019b).

Traditionally, the Serbian climate is considered as a warm-humid continental or humid subtropical climate, with more or less pronounced local characteristics. Recent decades have seen an increasing occurrence of extreme weather events. In the 2012 production year in the major part of the country, the hottest and driest period coincided with the most important generation phases of spring crops. This climatic event had not occurred previously in Serbia, and it caused substantial damage and losses in agricultural crop production. In addition, a high average frequency of *Aspergillus spp*., particularly *Aspergillus flavus* (which is xerophilic) and *Aspergillus niger*, on analyzed grain (95.3%) (*Levic et al.,* 2013), followed by the high incidence of aflatoxins in maize and consequently in feed and milk, were also attributed to the hottest and driest period (*Milicevic et al.,* 2017, 2019b). Therefore, several RASFF notifications related to aflatoxin levels above the MPLs in maize from countries from South-East Europe were issued at the end of 2012 and continued on in the first months of 2013 (*RASFF,* 2013).

Before 2008, *Aspergillus spp*. in Serbian grain occurred mostly at low frequency with an incidence of 3% to 16% (*Levic et al.,* 2013), but the very high temperatures and extreme drought in 2012 caused an outbreak of aflatoxins in epidemic proportions. These findings indicate that changes in environmental temperature influence the expression levels of regulatory genes (*aflR* and *aflS*) and aflatoxin production in *A. flavus* and *Aspergillus parasiticus* (*Schmidt-Heydt et al.,* 2010). *Gallo et al*., (2016) reported that regulatory genes *aflR* and *aflS* were highly expressed at 28°C, while the lowest expression was observed at 20 and 37°C. Generally, the optimum conditions for AFB1 production were 30–35°C at 0.95 a_w, and 25–30°C at 0.99 a_w, while no fungal growth or AFB1 production was reported when the temperature fell below 20°C (a_w 0.90 and 0.93) or when the temperature was higher than 40°C (*Liu et al.,* 2017). However, the study revealed that low levels of OTA-producing *Aspergillus* were present on Serbian feed/food compared to levels in the tropical countries.

In contrast, 2014 weather conditions were significantly different to those in 2013 and 2015. Furthermore, 2014 was characterized by a significantly higher number of rainy days than normal, and thus, in most of Serbia, precipitation was at a historical maximum during the 2014/2015 production year. During the vegetation period (April-September) in 2014, an average of 700 mm (400–1200 mm) of rain was recorded in Serbia, which was the worst season in the last 45 years. This consequently induced a high average moisture content in harvested maize and wheat kernels (>12% w/w), followed by co-occurrence of multiple mycotoxin-producing moulds in cereals, mainly *Fusarium* and *Penicillium* (*Jajic et al.,* 2017; *Kos et al.,* 2020). Recently, a report on the occurrence of mycotoxins in maize harvested in Northern Serbia in the period that included seasons with extreme drought (2012), hot and dry conditions (2013 and 2015) and extreme precipitation (2014) revealed significant differences in the incidences of AF, OTA, ZEA and FUM in the different investigation years (*Kos et al.,* 2020). Results showed that FUMs were detected annually with very high prevalences (found in from 76% to 100% of maize). AFB1 was detected in 94% and 90% of maize from 2012 and 2015, respectively, while during the 2014

Table 2. Cereal production (yields) in Serbia during 2014–2019 (SYRS, 2019)

Year	Maize		Wheat		Barley		Oats	
	Total tons*	t/ha	Total tons*	t/ha	Total tons*	t/ha	Total tons*	t/ha
2014	7.95	7.5	2.38	3.9	3.23	3.6	7.49	2.4
2015	5.45	5.4	2.43	4.1	3.62	3.8	8.82	2.7
2016	7.37	7.3	2.88	4.8	3.95	4.3	8.13	3.0
2017	4.02	4.0	2.27	4.1	3.05	3.3	6.95	2.4
2018	6.96	7.7	2.94	4.6	4.10	3.9	7.47	2.9
2019	7.34	7.6	2.53	4.4	3.73	3.7	5.62	2.5

Legend: *–10^6

production year, DON, ZEA and their derivatives were detected in 100% of the maize studied. OTA was the most predominant in maize (25% of maize contained this mycotoxin) from 2012. In this context, weather conditions that occurred in the four-year period had a significant influence on the occurrence of the examined mycotoxins in maize (*Kos et al.,* 2020). Also, a similar feed survey performed during the same four-year period (2012–2015) highlighted the problem of high levels of co-contamination with a number of different mycotoxin-producing species (*Krnjaja et al.,* 2017). Most of the maize sampled (and based on average values) contained *Fusarium* (92.2%), followed by species of the genera *Aspergillus* (80.8%) and *Penicillium* (48.7%) (*Krnjaja et al.,* 2017). Analysis of publications in recent years indicated that weather conditions in 2014 and 2016 were much more favourable for some *Fusarium* species than for *Aspergillus* species and aflatoxin synthesis (*Kos et al.,* 2017). In 2017, agro-meteorological conditions were unfavourable for many agricultural crops. During the production part of the year, the maximum daily temperatures (>35°C) were above the annual average. Furthermore, drought was the predominant factor that caused the greatest damage, especially to maize, and when compared with 2016, the total 2017 maize production was decreased by 45.5% (Table 2). The next two years (2018 and 2019), particularly 2019, was the hottest year in the history of meteorological measurements in Serbia. According to a report by the Republic Hydrometeorological Service of Serbia, 13 of the 15 hottest years in Serbia were registered after 2000 (the period considered was 1951 to 2019) (*RHSS*, 2020).

The co-occurrence of high air temperatures (up to 40°C) and heavy rainfall with high RH (up to 80%) within the same year is an emerging weather pattern in Serbia. These conditions before cereal harvest and during storage play an important role in mycotoxin occurrence. Under such changed climate conditions, the occurrence of toxigenic fungi and consequent co-contamination of cereals with mycotoxins is a considerable hazard we should bear in mind.

Incidence of mycotoxins in Serbia

Mycotoxicoses in humans or animals are characterized as food or feed related, noncontagious, nontransferable, noninfectious, nontraceable to microorganisms other than fungi, and do not show immunogenicity (*Zain*, 2011). The symptoms of mycotoxicosis depend on the type of mycotoxin, the chemical properties of the agents such as the ability to penetrate cell membranes, the intake route, the duration of exposure, the concentration, and the presence of other mycotoxins and pharmacologically active substances, as well as the health, exposure to infectious agents, age and sex of the exposed individual (*Williams et al.,* 2011). Clinical symptoms usually subside on removal of contaminated food or feed. Despite efforts to control fungal contamination, multi-mycotoxin contamination is of great concern and seems to be "the most important chronic dietary risk factor, higher than synthetic contaminants, plant toxins, food additives, or pesticide residues" (*Lee et al.,* 2017; *Williams et al.,* 2011). On a global level, 30% to 100% of food and feed samples are

contaminated by mycotoxins. According to results from the World Mycotoxin Report, FUMs are still abundant at high concentrations in raw commodities. Regional examples of mycotoxin incidence indicated that in Europe, the most prevalent mycotoxin is DON (65%), followed by FUMs (56%), ZEN (44%), aflatoxins (31%) and OTA (27%) (*Pinotti et al.,* 2016; *Vila-Donat et al.,* 2018). *Fusarium* mycotoxins (FB1, FB2, DON, ZEN) and OTA were mostly detected in the maize harvested in 2018 from European countries.

Extreme weather events in Serbia pose one of the greatest risks for contamination of cereals such as wheat, maize, barley and oats by various species of toxigenic fungi and their related mycotoxins (*Udovicki et al.,* 2018; *Udovicki et al.,* 2019a). Serbia has a largely agrarian economy, and thus, mycotoxin contamination of agricultural products has had a strong negative impact on Serbian trade, especially with the European Economic Community markets. Cereals, particularly maize, are one of the major feedingstuffs in the world because of their importance as a main source of energy and protein in animal feeding (*Jovanovic et al.,* 2018). In the last few years in Serbia, cereal production showed year-to-year variations depending on the climate conditions (Table 2). However, maize is one of the most important agricultural products in the country, both by its production and by the profit it generates in foreign trade (*SYRS*, 2019; *Index Mundi*, 2018). A high portion of this production is consumed locally (4.2 and 0.2 to 0.3 million tons for animal feed and for human consumption, respectively) and the remainder is exported to foreign countries (*Maslac,* 2017).

Data on the occurrence of mycotoxins are extremely important to determine the risk posed by mycotoxins both to humans and animals, and moreover, this is one part of risk assessment that contributes to new and effective regulations, improvement of laboratory facilities etc. This is particularly the case in vulnerable countries that are prone to mycotoxin contamination, such as Serbia. In Tables 3–5, an overview of updated information on mycotoxin occurrence in different commodities since the start of the aflatoxin crisis in Serbia is shown. Most of the recently published papers deal with aflatoxins, DON, FUMs, ZEA, TCT and OTA mycotoxins, on which there are more data. Also of great concern for risk assessments and as possible hazards for human health are the emerging mycotoxins such as moniliformin (MON), enniatins (ENs), beauvericin (BEA) and fusaproliferin (FUS), which are under consideration. We emphasize that the data presented in the tables were obtained using different methodologies, with distinct sensitivities and accuracies, as well as sampling methods and number of analyzed samples.

Aflatoxins

Aflatoxins are difuranocoumarin derivatives produced by a polyketide pathway by many strains of *A. flavus*, *A. parasiticus*, and the rare *Aspergillus nomius*, which contaminate agricultural commodities (*De Ruyck et al.,* 2015). To date, nearly 20 different types of aflatoxins have been identified, the six predominant ones being aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), AFM1 and aflatoxin M2 (AFM2) (based on their blue or green fluorescence under UV light). AFB1 is identified as the most potent naturally occurring carcinogen in this group, and can cause serious health issues such as growth retardation, genotoxic, carcinogenic, and teratogenic effects for both humans and animals (*Zhou et al.,* 2019), while the other compounds have lower toxicity. *IARC* (2012) classify AFB1, AFB2, AFG1, AFG2 and AFM1 as Group 1 carcinogens, emphasizing their explicit carcinogenicity to humans. The no observed adverse effect level is not applied to genotoxic carcinogens, and therefore, no threshold is assigned to AFB1 (*IARC,* 1993). Aflatoxins are rapidly absorbed and metabolized, primarily in the liver by cytochrome P450 enzymes to reactive epoxides, which can react with cellular targets (e.g., DNA, RNA and proteins), forming covalent bonds (*Carter et al.,* 2019). The rate of metabolism and the type of metabolic products determine differences in species susceptibility to aflatoxins. Most of the metabolic products, such as AFM1 and aflatoxin Q1 (AFQ1), are less toxic than the parent AFB1, but aflatoxin B1–8,9-expoxide (AFBO) is the most toxic metabolite form and is responsible for carcinogenic effects, especially in the liver (*WHO*, 2018). The hepatocarcinogenicity of aflatoxins is mainly due to lipid peroxidation that disrupts transcription and translation to DNA (*Zhang*, 2015). Thus, epoxidation is generally considered as metabolite activation, while hydroxylation, hydration, and demethylation are considered metabolic detoxications. Aflatoxin adducts in urine and blood are reliable biomarkers of aflatoxin exposure (*Al-Jaal et al.,* 2019).

AFM1 is a less mutagenic and carcinogenic (2 to 10%) hydroxylated metabolite of AFB1, and is excreted into the urine and milk of mammals after they ingest foods contaminated with AFB1 (*Kumar et al.,* 2017). The average conversion value was

2.5%, although a direct relationship between the carry-over rate and the milk yield, with a maximal 6.2% carry-over rate, was found (*Walte et al.,* 2016). In dairy cows, excretion of AFM1 occurs in as little as 12 to 24 h and up to 2 to 3 days in milk (*Peles et al.,* 2019). AFM1 clearance from cow milk depends on several factors, but mainly on the amount of AFB1 ingested and the duration of mycotoxin consumption, with excretion by cows occurring for a variable period of about 5 to 7 days from the cessation of AFB1 consumption (*Peles et al.,* 2019). Approximately 95% of AFB1 metabolites excreted in milk are in the form of AFM1, although AFM2, AFG1, and AFB2 are also reported (*Ostry et al.,* 2017). A recent review (*Sengling Cebin Coppa et al.,* 2019) of the occurrence of mycotoxins in breast milk, fruit products and cereal-based infant formula found that mycotoxins such as AFM1 and OTA have been reported in human breast milk and in infant formulae in different concentrations globally, while the reported levels in some European, African and Asian countries indicates high exposure levels, which can potentially result in adverse health effects in infants and present a serious public health problem.

Various investigations conducted in Serbia in the last decade have revealed a significant presence of aflatoxins in maize (*Kos et al.,* 2017; *Udovicki et al.,* 2018). The most recent research from the same authors (*Kos et al.,* 2020) was conducted to evaluate the incidence of the mycotoxins aflatoxin, ZEN, OTA, DON and their metabolites in maize between 2012 and 2015. This study confirmed that conditions of extreme drought in 2012 had a great influence on the presence of AFB1 (94% of maize was contaminated). In comparison to maize from 2012, the percentages of contaminated maize in 2013 (33%) and 2015 (90%) were lower, as were the mean aflatoxin levels detected. In 2014, extremely rainy conditions recorded during the maize growing season were unfavourable for the growth of some *Aspergillus* species and for aflatoxin synthesis. Although, AFB1 was detected in maize in 2015 with a high prevalence (90%), the mean concentration of AFB1 (8 μg kg^{-1}) was significantly lower than the mean concentration (44 μg kg^{-1}) detected in maize from 2012 (*Kos et al.,* 2020).

Due to the severity of the maize contamination, elevated concentrations of AFM1 were found in milk countrywide, with a large percentage of milk samples (68%) being non-compliant according to the European Union maximum residue limit (EU-MRL 0.05 μg kg^{-1}) (*Stefanovic*, 2014). As a relatively stable compound during pasteurization and sterilization (*Peng et al.,* 2018), AFM1 is more concentrated in curd and cheese than in the milk itself that was used for cheese-making. Furthermore, 2.5–3.3-fold and 3.9–5.8-fold higher concentrations of AFM1 were recorded in soft and hard cheeses, respectively, than the AFM1 concentrations found in milk from which cheeses were made (*Filazi and Sireli*, 2013). As infants depend on milk as a basic food, it is extremely important to control the level of aflatoxins in milk. The results obtained from a systematic review (2015–2018) showed year-to-year variations of AFM1 prevalence, and average contamination levels in the analyzed milks were significantly different ($P < 0.001$) (*Milicevic et al.,* 2017; 2019b). Likewise, AFM1 incidence has shown an interesting periodic fluctuation over the survey period. Results of this study are consistent with reports from other countries stating that the prevalence of AFM1 in raw milk was significantly higher ($P < 0.05$) during spring than in summer and autumn. According to the report (*Milicevic et al.,* 2017; 2019b), improper storage conditions, particularly during winter and spring, combined with the initial fungal contamination in the fields had a great impact on AFB1 levels in cattle feed, and consequently on AFM1 occurrence in milk. Moreover, the findings of this study showed the increase in AFM1 occurrence was significantly positively correlated with air temperature and annual RH (*Milicevic et al.,* 2017; 2019b). Although the incidence of AFM1 is currently tending to decrease year by year in Serbia, it is evident that the incidence could increase with a rise in global temperatures. Overall, AFM1 has proved to be a great public health concern in Serbia and should be considered as a high priority for risk management actions.

Sterigmatocystin

Sterigmatocystin (STC) is a polyketide mycotoxin structurally related to AFB1, and is produced by several fungal species, including *A. flavus, A. parasiticus, Aspergillus versicolor* and *Aspergillus nidulans*, of which *A. versicolor* is the most common source. During the last 30 to 40 years, only a limited number of surveys on the occurrence of STC in different foods and feed were carried out. Therefore, data are too limited to conduct a reliable human dietary exposure assessment. The toxin has been reported in grains, nuts, green coffee beans, spices, beer and cheese. The carry-over of STC and/or its metabolites from feed to animal products such as meat and eggs, leads to an exposure of low health

concern (*EFSA,* 2013). Liver and kidneys are the target organs of acute toxicity. STC is hepatotoxic in poultry and pigs, and nephrotoxic in poultry. The carcinogenic potency of STC is approximately three orders of magnitude lower than that of AFB1. Due to the absence of exposure data for the European population, the margin of exposure (MOE) approach for substances that are genotoxic and carcinogenic cannot be applied for STC, and, therefore, the European Food Safety Authority's CONTAM Panel could not characterize the risk STC has for human health (*EFSA,* 2013). Furthermore, *IARC* (1987) concluded that STC is possibly carcinogenic to humans (in the group 2B carcinogens).

Ochratoxin A

Ochratoxin (OTA) is produced by fungi of the genera *Aspergillus* and *Penicillium* contaminating a wide range of commodities, including staple food crops and beverages such as beer and wine. OTA comprises a dihydrocoumarin moiety linked to a molecule of L-β-phenylalanine via an amide bond. OTA is one of the most relevant mycotoxins, with great public health and agro-economic significance due to the toxin's confirmed nephrotoxic, genotoxic, neurotoxic, immunotoxic, embryotoxic and teratogenic effects, and its suspected carcinogenicity (*Pfohl-Leszkowicz*, 2012; *IARC*, 2014; *Malir et al.,* 2016; *Koszegi and Poor,* 2016).

Hence, OTA has been studied more often than other mycotoxins in our region. In temperate countries like Serbia, OTA is produced by *Penicillium verrucosum* and associated with contamination of several foodstuffs, such as cereals, wine, eggs, pork meat and some herbs. A recent study detected OTA in 13 (25%), 1 (2%) and 9 (18%) maize samples from 2012, 2013 and 2015, respectively (*Kos et al.,* 2020). The highest OTA prevalence detected (25% of maize examined contained OTA) was from 2012, which means the prolonged drought provided the most favourable conditions for the growth of some *Aspergillus* species and synthesis of OTA (*Kos et al.,* 2020). These findings are in line with trends of OTA occurrence in Serbian cereal grains or different types of flour as observed in similar survey (*Torovic et al.,* 2018a). Currently in Serbia, research is not focused on OTA, since research attention is oriented toward aflatoxins and fusariotoxins. However, under climate conditions with elevated temperatures, there is an important question regarding which fungal species is responsible for OTA contamination of cereals. Not surprisingly, low levels of OTA-producing *Aspergillus* are present in agricultural products. OTA is a heat-stable compound (stable at temperatures >250°C) that is not destroyed by common processing treatments (*Marin et al.,* 2013). Animal feeds are usually contaminated by OTA due to improper storage conditions during production and transportation (*Krnjaja et al.,* 2014; *Radulovic et al.,* 2013).

Unlike the other monitored mycotoxins, OTA has the potential to bioaccumulate in animals' bodies, and thus, contamination of animal feeds by OTA results in the presence of residues in edible tissues (kidneys and liver, in particular), which are often used in the meat industry (*Milicevic et al.,* 2014). OTA levels in cow milk are low due to efficient degradation of OTA in the rumen. The most important contributors to chronic dietary exposure to OTA were processed meat, cheese and grains and grain-based products. Historically, consumption of pork has been a significant source of human exposure to OTA in Serbia. A recent OTA survey of pig kidneys originating from different regions of Vojvodina (Serbia's northern province) revealed that 14 of 95 (14.7%) kidneys were contaminated with OTA at levels between 0.10–3.97 μg kg^{-1} (average 1.36 μg kg^{-1}) (*Polovinski et al.,* 2019). Considering the differences in the occurrence of OTA in edible tissues reported in previous research (*Milicevic et al.,* 2011), and in spite of the fact that levels of OTA in edible tissues did not pose immediate hazards for human health, it seems that stored feed should be regularly monitored to detect unexpected OTA residues. This is because of the bioaccumulation of OTA in humans.

The target organ for OTA toxicity is the kidneys, and initial interest in this group of toxins was as a causative agent of porcine nephropathy (*Milicevic et al.,* 2009a). OTA has been hypothesized to cause oxidative damage to DNA, leading to mutagenesis and potential carcinogenesis. Subsequently, OTA has been associated with human disorders, chronic interstitial nephropathy and Balkan endemic nephropathy in the former Yugoslavia, associated with urothelial cancer *(Pavlovic*, 2013). OTA is classified as a possible human carcinogen (group 2B) by IARC (1993) on the basis of sufficient evidence of carcinogenicity in animal models, but insufficient evidence from human studies. Based on the last assessment by the Scientific Committee on Food (SCF), a tolerable weekly intake (TWI) of 120 ng kg^{-1} body weight (bw) was derived for OTA (*EFSA,* 2010).

Fumonisins

These compounds (FUMs) are predominantly produced in maize and maize products by *Fusarium proliferatum* and *Fusarium verticillioides* (formerly *Fusarium moniliforme*) (*Uegaki et al.,* 2012). Although 28 FUM analogues have been identified, fumonisin B1 (FB1) is the most studied and most toxic of the metabolites, which have a long chain hydrocarbon unit (similar to that of sphingosine and sphinganine) playing a role in their toxicity. The toxicity of FUMs largely reflects their ability to disrupt sphingolipid metabolism by inhibiting the enzyme ceramide synthase, an enzyme responsible for the acylation of sphinganine and sphingosine. These lipids play an important role at the cellular level, maintaining cell membrane structure, and enhancing cell interaction and extracellular interaction (*Wan et al.,* 2013) According to results from the World Mycotoxin Survey, FUMs are still abundant at high concentrations in raw commodities. Results from a global survey indicated that FUMs are the most common mycotoxins, found in 64% of all analyzed maize samples (*Biomin*, 2019).

As demonstrated in two studies on FUM contamination of maize in Serbia, the percentage of contamination varied from 51% to 100%, depending on the harvesting season (2005 to 2014), as did the mean FUM concentration in positive maize (from 0.227 to 35.760 mg kg^{-1}) (*Krnjaja et al.,* 2015; *Jaksic et al.,* 2019). A similar regional pattern of contamination was confirmed by a recent report, which concluded that FUMs were the most prevalent mycotoxins found in contaminated maize during 2012 to 2015 (*Kos et al.,* 2020). These results can be explained by the heavy total rainfall during the maize harvest in 2014 and mild winter during 2015, as well as uncontrolled temperature and RH in the warehouses, which caused the intensive development of toxigenic mould and particularly increased the content of TCT mycotoxins in stored kernels.

Consumption of FUMs has been associated with elevated human oesophageal cancer incidence in high-risk populations (*Waskiewicz et al.,* 2012; *Chen et al.,* 2018). Because FB1 reduces uptake of folate in different cell lines, FUM consumption has been implicated in neural tube defects in human babies. FUMs can also induce hepatotoxicity, nephrotoxicity and renal carcinogenesis (*Kamle et al.,* 2019). In animals, FUMs cause equine leukoencephalomalacia (ELEM) a brain disease in horses, porcine pulmonary oedema in swine and liver and kidney cancer in multiple rodent species and strains. Hence, FB1 is listed as a Group 2B carcinogen (*IARC*, 1993), and a recent evaluation by *EFSA* (2018) established a group tolerable daily intake (TDI) of 1μg kg^{-1} bw day^{-1} based on increased incidence of megalocytic hepatocytes found in a chronic study with mice.

Zearalenone

Zearalenone (ZEA) is one of the most prevalent nonsteroidal oestrogenic mycotoxins produced by *Fusarium* genera such as *Fusarium graminearum, Fusarium culmorum, Fusarium crookwellense, Fusarium semitectum* and *Fusarium equiseti*, *w*hich are distributed worldwide (*Rai et al.,* 2019). ZEA and its major alcohol metabolites, α-zearalenol (α-ZOL), β-zearalenol (β-ZOL), α-zearalanol (α-ZAL, zeranol) and β-zearalanol (β-ZAL, tarelanol) share structural similarity with the sex hormone, 17β-estradiol, and can be found naturally or as result of metabolism of humans and animals (*Danicke,* 2015). Therefore, ZEA and its metabolites with their binding affinities for hepatic, uterine, mammary, and hypothalamic oestrogen receptors play an important role in reproductive disorders in human and animals (*Poor et al.,* 2015). Swine are the most sensitive domestic animal species, followed by ruminants, while birds are the most resistant species. In the agroecological conditions in Serbia, *Fusarium*, particularly *F. graminearum*, has relatively high potential for the synthesis of ZEA (*Stepanic et al.,* 2011), and hence, ZEA is one of the most common contaminants of cereals and their products (*Milicevic et al.,* 2009b; *Torovic,* 2018b). Moreover, rainy conditions during the maize growing season, besides being favourable for production of DON and its derivatives, were also favourable for synthesis of other fusariotoxins, ZEA, α-ZOL, β-ZOL and ZEA-S (*Kos et al.,* 2020). ZEA and ZEA-S were detected in 100% of examined maize samples in 2014, while β-ZOL was detected in 96% and α-ZOL in 61% of maize. In 2012, 2013 and 2015, ZEA was detected in 12%, 37% and 53%, respectively, of the maize examined, with significantly lower mean concentrations than occurred in 2014 (*Kos et al.,* 2020). A number of studies (described in *Nesic,* 2015) suggest that *Fusarium* toxicoses are one of the major threats to farmers in Serbia. Consumption of contaminated feed by dairy cows did not result in carry over of ZEA or its metabolites in milk at levels hazardous to human health (*Flores-Flores et al.,* 2015).

Taking into account its prevalence and heat stability (up to 160°C), ZEA cannot be completely removed from the food chain. IARC found limited evidence of ZEA carcinogenicity in animal models,

classifying it together with DON in Group 3 carcinogens. In 2000, the SCF established a TDI of 0.2 μg kg^{-1} bw day^{-1} for ZEA. However, in 2011 the SFC concluded that a TDI of 0.25 μg kg^{-1} bw day^{-1} should be established based on recent data in the most sensitive animal species (*EFSA,* 2014a).

Trichothecenes

Several fungal genera are capable of producing trichothecenes (TCT), but most of them are produced by *Fusarium spp*. The TCT mycotoxins comprise a vast group of more than 100 fungal metabolites with the same basic structure, affecting several major cereal crops including oats, barley, maize and wheat. Examples of type A TCT include T-2 toxin (T-2), HT-2 toxin (HT-2) and diacetoxyscirpenol (DAS) (*Escriva et al.,* 2015). Fusarenone-X (FUX), DON, and nivalenol (NIV) are some of the common naturally occurring type B TCTs.

T-2 toxin has received particular attention due to its specific effects on humans and animals, including cardiotoxicity, hepatotoxicity, digestive toxicity, neurotoxicity, and other multisystemic toxicities (*Escriva et al.,* 2015). However, reproductive disorders are the principal deleterious effects of T-2 toxin (*Schuhmacher-Wolz et al.,* 2010). At the cellular level, the main toxic effect of TCT mycotoxins appears to be a primary inhibition of protein synthesis, interfering in the initiation, elongation and termination steps, and secondary destruction of DNA and RNA synthesis. The toxins bind to peptidyl transferase, which is an integral part of the 60S ribosomal subunit. However, studies on cellular dysfunctions and mitochondrial fusion/fission suggest T-2 toxin could inhibit mitochondrial dysfunction and promote mitochondria fragmentation accompanied by deficiency of ATP supply and oxidative stress, which could greatly contribute to the disorder of mitochondrial dynamic balance (*Yang et al.,* 2020). Furthermore, exposure to TCNs can lead to multiple adverse health effects such as vomiting, anorexia, headache, intestinal haemorrhage and oxidative stress (*Alshannaq and Yu,* 2017). Pigs and horses are among the animals that are most sensitive to T-2, the major effects of which are immunological and haematological in nature (*Fang et al.,* 2019).

Although the group of TCTs has been thoroughly studied worldwide, in Serbia, more intensive studies on DON were initiated after 2005. Recently, a very comprehensive study was conducted in a four year period (2012–2015) in order to follow annual climate conditions and impact on mycotoxin incidences (*Kos et al.,* 2020). The prevalence of DON and its derivatives in maize from the 2014 growing season, which was described as extremely rainy and wet, was very high, since DON, DON-3G and 15-ADON were detected in 100%, 100% and 98% of analyzed maize, respectively. Contrary to this, in the hot and dry conditions recorded in 2012, 2013 and 2015, DON was detected in 63%, 35% and 63% of examined maize, respectively. Moreover, mean concentrations in examined maize from 2012, 2013 and 2015 were significantly lower than the mean detected concentration in 2014 (*Kos et al.,* 2020). Similarly, *Jajic et al.* (2017) investigated DON in maize from two harvest seasons in Serbia (2014 and 2015), and found the presence of DON in maize was related to the different weather conditions that prevailed during the two harvest seasons (*Jajic et al.,* 2017). It is evident high temperatures and high air humidity during the vegetation period of cereals promote *Fusarium* producers of DON and the consequent high contamination levels of cereals in Serbia. Although the maximum permitted level of DON in food is specified by regulation in Serbia, and consumption of maize and wheat has increased, there are still insufficient data about the daily intake of *Fusarium* toxins by consumers in Serbia.

In 2003, IARC designated DON, NIV, T-2 and HT-2 toxins as Group 3 (not classifiable) human carcinogens due to inadequate evidence of animal carcinogenicity, and lack of investigation in humans. TDIs of 1 μg kg^{-1} bw day^{-1} and 1.2 μg kg^{-1} bw day^{-1} were established for DON and NIV, respectively (*EFSA,* 2014a; 2014b). Recently, the SCF concluded that a full TDI of 0.1 μg kg^{-1} bw day^{-1} for the sum of T-2 and HT-2 toxins be established (*EFSA*, 2014b).

Patulin

Patulin (PAT) is a mycotoxin produced by a wide range of fungal species of the *Penicillium, Aspergillus*, and *Byssochlamys* genera (*Frisvad*, 2018; *Vidal et al.,* 2019). Chemically, PAT belongs to a group of compounds commonly known as toxic lactones (4-hydroxy-4H-furo(3,2-c) pyran-2(6H)-one). PAT is regarded as the most dangerous mycotoxin in injured fruits stored under improper environmental conditions (post-harvest). PAT is commonly investigated in apples and apple-derived foods (*Torovic et al.,* 2017), and it has been reported that approximately 50% of the apple juice samples analyzed worldwide contained relatively high detectable PAT levels (*Ying et al.,* 2018). Moreover, organic apples have higher PAT contamination than do

Table 3. The incidence of mycotoxins in foodstuffs in Serbia (2012–2019)

Mycotoxins	Commodity	Method of analysis	N (%)	Range (μg kg^{-1})	Mean (μg kg^{-1})	Ref.
AF-s	Maize	ELISA	13/29 (44.83)		13.95	*Krnjaja et al., 2013a*
			16/29 (55.17)		>40	
			12/12 (100)	0.33–2.40	1.39	*Krnjaja et al., 2013b*
			137 (68.5%)	1.01–86.1	36.3	*Kos et al., 2013a*
				Up to 560	33,21	*Stefanovic, 2014*
			20/20 (100)	2.31–3.34 (harvested)	2.77	*Krnjaja et al., 2015*
			20/20 (100)	1.03–4.11 (stored)	2.16	
		ELISA		1.98–7.01	1,33	*Jaksic et al., 2015*
		HPLC-FLD	103/180 (57.2)	1.3–91.4	12.7	*Janic Hajnal et al., 2017*
		ELISA	5	2.28–4.31	3.22	*Kos et al., 2017a*
			5–72.3	1.0–111.2	3.1–37.4	*Kos et al., 2018*
			(50–87.5)	1.3–1.9		*Krnjaja et al., 2018*
			36/37 (97.3)	0–491.7	60.3	*Obradovic et al., 2018*
			12/90 (18.9)	0–27.9	1.3	
	Flours of various cereals	HPLC	5.2	1.59–4.76	2,13	*Torovic, 2018a*
	Maize flour		48.2	max. 9.14	0.55	
FUM	Wheat	ELISA	35/41 (85.4)	750–2465	882.7	*Stepanic et al., 2011*
	Maize	ELISA	29/29 (100)		3590.00	*Krnjaja et al., 2013a*
			12/12 (100)	880–2950	1610.83	*Krnjaja et al., 2013b*
			90/90 (100)	520–5800	1730	*Kos et al., 2014*
		LC-MS/MS	2/10 (20)	75–561 (organic)		*Vukovic et al., 2014*
			4/9 (44)	10–230 (conventional)		
		ELISA	20/20 (100)	1519–9780 (harvested)	3700.84	*Krnjaja et al., 2015*
			20/20 (100)	760–35760 (stored)	5976.50	
		HPLC−FLD	88–98	Up to 20340	672–2290	*Jaksic et al., 2015*
		ELISA	74	540.1–5076	2750	*Kos, 2017a*
		ELISA	29/37 (78.4)	0–10790	1300	*Obradovic et al., 2018*
			30/90 (33.3)	0–10860	2800	
			37 (75.7)	830–10.790	1406	*Udovicki et al., 2019*
			90 (34.4)	930–10.860	1905	
			33 (93.9)	1050–3790	580	
			98 (100)	890–34.480	4310	
	Corn flours	HPLC-UV	51/56 (96.4)	Max.1468.5	205.5	*Torovic et al., 2018b*
	Corn flake		11/15 (73.3)	Max. 579.4	87.3	

Mycotoxins	Commodity	Method of analysis	N (%)	Range (μg kg^{-1})	Mean (μg kg^{-1})	Ref.
TCT	Cereal	LC	15 (68.2)	68–19.520	537	*Jajic et al., 2011*
	Wheat	ELISA	30/41 (73.2)	50–5000	1988.1	*Stepanic et al., 2011*
			37/41 (90.2)	25–135.6	24.2	
	Wheat flour	UHPLC	13 (86.7)	17.5–976	325	*Skrbic et al., 2012*
			4 (26.7)	9.8–26.9	4.1	
	Crop maize	ELISA	26/50 (52.0%)	25.3–200	154.1	*Janic Hajnal et al., 2013*
	Maize	ELISA	22/29 (75.86)		235	*Krnjaja et al., 2013a*
			12/12 (100)	41–226	128.17	*Krnjaja et al., 2013b*
			48/90	25.09–209.0	50.93	*Kos et al., 2014*
			2/90	600.0–700.0	650.0	
		ELISA	20/20 (100)	42–238 (harvested)	117.83	*Krnjaja et al., 2015*
			20/20 (100)	380–10.684	2034.4	
			52	275.2–882.1	541	*Kos et al., 2017a*
		ELISA	2.5	260.1- 1388	642.3	*Kos et al., 2017b*
			96.0	260.4–9050	3063.3	
			15.5	252.3–6280.0	921.1	
		HPLC	221/245 (54.3)		1806	*Jajic et al., 2017*
		ELISA	(22.2–100)	445–1977		*Krnjaja et al., 2018*
	White wheat flour		23/45 (51)	99–440	142	*Jaukovic et al., 2017*
	Corn flour	HPLC-UV	24/56 (42,9)	Max. 931.8	101.3	*Torovic et al., 2018b*
	Corn flake		6/15 (40)	Max. 878.6	255.1	
Alternaria toxins, TeA	Wheat	HPLC	63/92 (68.5%)	0.75- 48.9	18.6	*Janic Hajnal et al., 2015*
AOH			11/92 (12.0%	0.49–70.2	39.0	
AME			6/92 (6.5%)	2.5- 2676	92.4	
PAT	apple-based food	HPLC	32/114 (28.1	1–8.3	3.5	*Torovic, 2017*
ZEA	Wheat	ELISA	37/41 (90.2)	10–1000	442.6	*Stepanic et al., 2011*
	Wheat flour	UHPLC	5 (33.3)	1.9–21.1	4.6	*Škrbic et al., 2012*
	Maize	ELISA	35.0	1.81−3.32	2.67	*Jakšić et al., 2011*
			12/12 (100)	15.44–188.05	71.79	*Krnjaja et al., 2013b*
		ELISA	15	35.6-183.5	83.3	*Kos et al., 2017a*
			(88.89–100)	16.82-26.97		*Krnjaja et al., 2018*
	Corn flour	HPLC	37/56 (66,1)	max. 242.1	15	*Torović et al., 2018b*
	Corn flake		13/15 (86,7)	max. 121.6	13.6	
OTA	Flours of various cereals	HPLC	7/58 (29.3)	0.07–23.04	2.04	*Torović et al., 2018a*
	Maize		21/56 (37.5)	Max. 7.16	1.21	
	Pig kidney	HPLC	14/95 (14.74)	0.10–3.97	1.36	*Polovinski Horvatovic et al., 2019*
	Maize	LC–MS/MS	13 (25)	2–318	53	*Kos et al., 2020*
			9(18)	0.5–27	6	

conventional apples, leading to consequent risk, particularly for infants and preschool children (*Marin et al.,* 2013). During the fermentation of apple juice to cider, PAT is completely destroyed.

Clinical signs usually include gastrointestinal symptoms (nausea, vomiting, gastric ulcers, intestinal haemorrhages, and lesions in the duodenum) that are accompanied by kidney damage. Chronic symptoms include genotoxic, neurotoxic, immunosuppressive and teratogenic effects (*Vidal et al.,* 2019). Furthermore, at the cellular level, PAT has been reported to lead to cell (plasma) membrane rupture, inhibition of protein synthesis, and inhibition of DNA and RNA synthesis. Regarding its carcinogenicity to humans, the IARC included PAT in category 3, as not classifiable (*Saleh and Goktepe,* 2019). The JECFA established a provisional maximum TDI (PMTDI) for PAT of 0.4 μg kg^{-1} bw day^{-1} (*JECFA,* 1995).

Emerging mycotoxins

Emerging mycotoxins are defined as mycotoxins that are neither routinely determined, nor legislatively regulated (*Gruber-Dorninger et. al.,* 2016). However, the evidence of their incidence is rapidly increasing (*Gruber-Dorninger et. al.,* 2016). The most relevant and frequently occurring emerging mycotoxins are *Fusarium* toxins. *Fusarium* emerging mycotoxins include enniatins (ENNs), beauvericin (BEA), moniliformin (MON) and fusaproliferin (FUS). Carcinogenicity, immunotoxicity and neurotoxicity are the main toxicological effects of emerging mycotoxins (*Cimbalo et al.,* 2020). Emerging fusariotoxins have mostly been investigated in Mediterranean countries. Moreover, their presence has been reported recently in maize from Serbia (*Jajic et al*., 2019; *Janic Hajnal, et al*., 2020). The authors found that MON, BEA and FUS had the highest presence among the emerging mycotoxins and were present in maize from all the investigated regions in the country (*Jajic et al*., 2019; *Janic Hajnal, et al*., 2020).

Also, emerging mycotoxins include citreoviridin, gliotoxin, griseofulvin, mycophenolic acid, β-nitropropionic acid, kojic acid, tremorgenic mycotoxins (penitrems, janthitrems, lolitrems, and the paspalitrems), penicillic acid, viomellein, vioxantin, xanthomegnin and walleminols. Their carcinogenicity to humans is not classifiable by IARC. Due to the lack of research showing direct human and animal

Table 4. The incidence of AFM1 in milk and dairy products in Serbia (2012–2019)

Mycotoxins	Commodity	Method of analysis	N (%)	Range (μg kg^{-1})	Mean (μg kg^{-1})	exceed EU MRL n (%)	Ref.
AFM1	Pasteurized and sterilized milk	HPLC	54/60 (90)	0.003–0.104	0.026	6 (10.0)	*Torovic,* 2015
	Raw cow milk	ELISA	540/678 (79.7)	0.026–>1	0.282	14 (21)	*Tomasevic et al.,* 2015
	Milk products		322/184 (57)		0.268	122 (37.8)	
	Heat treated milk		438/317 (72.4)	0.026–0.50	0.090	3 (3.3)	
	Raw cow milk	HPLC	32/38 (84)	0.006–0.864	0.273	24 (63,16)	*Polovinski--Horvatovic et al.,* 2016
	Raw cow milk	ELISA	503/1207 (41.7)	0.026–0.263	0.037	353 (29.25%)	*Miocinovic et al.,* 2017
	Dairy products		236/997 (23.7)	0.026–0.320	0.019	42 (4.21%)	
	Raw cow milk		4078/ 5054 (80.7)	0.005–1.26	0.071	1557 (30.1)	*Milicevic et al.,* 2017a
	Heat-treated milk		1117/1233 (90.6)	0.005–0.280	0.035	214 (17.3)	
	Dairy products		61/501 (12)	0.005–0.147	0.021	3 (0,6)	*Milicevic et al.,* 2017b
	Raw cow milk		12268/ 15178 (81)	0.005–1.09	0.055–0.074	3608 (23.7)	*Milicevic et al.,* 2019

Table 5. The incidence of mycotoxins in feed samples in Serbia (2012–2019)

Mycotoxins	Commodity	Method of analysis	N (%)	Range (μg kg^{-1})	Mean (μg kg^{-1})	Ref.
AFB1	Maize	ELISA	59/70 (84.3)	2.17–88.85	18.15	*Ljubojevic et al.,* 2013
	Cattle feedstuffs		66/67 (98.5)	0.3–8.8	1.6–7.9	*Krnjaja et al.,* 2013c
	Chicken feed		12/14 (85.71)	1.79–16.01	4.47	*Krnjaja et al.,* 2019
	Hen feed		16/16 (100)	1.34–18.29	4.56	
OTA	Maize		11/28 (39.3)	5.03- 11.99	8.05	*Ljubojevic et al.,* 2013
	Chicken feed		100	19.04–51.30	34.40	*Krnjaja et al.,* 2014
	Hen feed		100	28.34–65.30	43.89	
DON	Cattle feedstuffs		62–67 (92.5)	2.0–1149	11–694.2	*Krnjaja et al.,* 2013
	Maize		7/28 (25)	82.0–792	239	*Ljubojevic et al.,* 2013
T-2			11/29 (37.9)	54.7–374.0	113.4	
ZEA			10/28 (35.7)	25.84- 130	73.34	
	Cattle feedstuffs		67/67 (100)	29.2–2477.5	64.9–2477.5	*Krnjaja et al.,* 2013
FUMs	Pig feed		28/30 (83)	218–3540	893	*Jaksic et al.,* 2018
	Horse feeds	HPLC		1680–6050	7,73	Jovanovic *et al.,* 2016
	Pig feed		3/3 (100)	12–775		*Milicevic et al.,* 2016
Mycophenolic acid			3/3 (100)	97–142		
DON			3/3 (100)	930–8220		
3-DON			1/3 (33)	<0.05–570		
15-DON			3/3 (100)	58–451		

health effects, there are no current regulations implemented regarding the presence of these emergent mycotoxins in food or feed.

The first preliminary study of the natural occurrence of *Alternaria* toxins (alternariol (AOH), alternariol monomethyl ether (AME) and tenuazonic acid (TeA)) in wheat from different wheat-growing areas from Vojvodina, Serbia, was conducted during 2011–2013. Among 92 analyzed wheat samples, 63 (68.5%) were contaminated with TeA, 11 (12.0%) with AOH and 6 (6.5%) with AME (*Janic Hajnal et al.,* 2015). This study also revealed the incidence of *Alternaria* toxin in wheat shows considerable variations from year to year, depending on weather conditions. Humid conditions (above 75% RH) and higher temperatures in the period from flowering to wheat harvesting could lead to increased fungal growth and mycotoxin production (*Janic Hajnal et al.,* 2015).

Mycotoxin Exposure and Associated Human Health — Risk Assessment

A report from the Foodborne Diseases Burden Epidemiology Reference Group of the World Health Organization used global estimates of incidence to calculate illnesses, deaths, and disease attributable life years lost (DALYs), and revealed the highest global DALYs (636,869) were due to liver cancer attributed to aflatoxin (*Gibb et al.,* 2015). Among the four chemicals examined, aflatoxin was associated with the greatest number of DALYs. In the risk assessment context, chronic consumption of mycotoxin-contaminated foods has been linked to very broad effects, comprising essentially (*De Ruyck et al.,* 2015):

- carcinogenic effects, e.g., in lung, liver, kidney;
- genotoxic and/or mutagenic effects;
- toxic effects specifically in target organs such as kidney, liver, and the nervous and reproductive systems;
- teratogenic effects; and
- immunosuppressive effects.

Although mycotoxins have been clearly implicated in these health symptoms, many interacting factors in the pathogenesis of a mycotoxicosis make clinical signs and diagnosis complex and diverse. One of the most important health burdens associated with mycotoxin exposure is the development of cancers. In order to prevent cancer risks stemming from exposure to mycotoxins, the IARC has performed

the carcinogenic hazard assessment of some mycotoxins in humans, on the basis of epidemiological data, studies of cancer in experimental animals and mechanistic studies (*IARC,* 2015).

Group 1 (Carcinogenic to humans)

Among mycotoxicosis described in humans, aflatoxicosis is of the greatest concern. The IARC classified AFB1 in group 1, i.e., *carcinogenic to humans*. The symptoms of acute aflatoxicosis include oedema, haemorrhagic necrosis of the liver and profound lethargy (*Williams et al.,* 2004). Since 2004, multiple aflatoxicosis outbreaks have been reported worldwide, resulting in 500 acute illness and 200 deaths. Most of these outbreaks have been reported from rural areas and occurred because of consumption of home grown maize contaminated with moulds (*Wild et al.,* 2015). The occurrence of acute aflatoxicosis in humans after aflatoxin consumption has been reported in India, Malaysia and Kenya (*Richard,* 2007). Reports that evaluated outbreaks of aflatoxicosis have estimated acutely toxic and potentially lethal AFB1 doses in humans to be between 20 and 120 μg kg^{-1} bw day^{-1} when consumed over a period of 1 to 3 weeks (*Groopman et al.,* 2014).

Regarding chronic effects of aflatoxin exposure, almost 600,000 people globally die annually due to hepatocellular carcinoma (*Yang et al.,* 2019). This makes hepatocellular carcinoma the second most common cause of cancer deaths, following lung cancer, worldwide (*Dietrich et al.,* 2019). Of the more than 700,000 new hepatocellular carcinoma cases worldwide each year, about 25,200–155,000 can be attributable to aflatoxin exposure. Moreover, aflatoxin (when there is no involvement of Hepatitis B virus) could play a causative role in 4.6–28.2% of all global hepatocellular carcinoma cases (*Liu et al.,* 2012). A much larger public health concern is likely to be associated with the influence of mycotoxins in childhood health disorders. The associated mechanism, as previously discussed, involves metabolism of AFB1 in the liver to a highly reactive species capable of forming mutagenic DNA adducts. The association of Hepatitis B virus and aflatoxins with hepatocellular carcinoma is well established in less developed and developing countries (common in China and Africa), where 83% of new cases were registered in 2012 (*WHO,* 2015). A multinational study estimated the incidence of hepatocellular carcinoma could be decreased in high-risk areas by up to 23% if dietary aflatoxin exposure is thoroughly controlled and reduced below detectable levels (*Liu et al.,* 2012). Aflatoxins have been found in the tissues of children suffering from Kwashiorkor or Reye's syndromes (*Peraica et al.,* 2001), and the toxins were thought to be a contributing factor to these diseases. Reye's syndrome, which is characterized by encephalopathy and visceral deterioration, results in liver and kidney enlargement and cerebral oedema (*Wittenstein et al.,* 2004) Moreover, in children, aflatoxins cause reduced immunization efficiency that leads to enhanced risk of infections (*Kumar et al.,* 2017). Aflatoxins also have been reported to cause morphological changes in the testes, impaired sperm viability, poor foetal growth during pregnancy and stillbirth (*Smith et al.,* 2017).

In recent years in Serbia, most health concerns around mycotoxins have been related to aflatoxin exposure due to unusual contamination of maize and, consequently, of milk. A recent study conducted by *Udovicki et al.* (2019a) to asses aflatoxin exposure revealed a high average dietary intake (5.59 ng kg^{-1} bw) through maize consumption in Serbia. Taking into account the contamination level of AFM1 in milk (*Milicevic et al.,* 2017), the highest estimated daily intake (EDI) values for AFM1 in raw milk were calculated for infants (1–4 years old) (2.257 and 2.206 ng kg^{-1} bw day^{-1} for males and females, respectively). The EDI values for AFM1 were found to decrease with increasing age; thus, the lowest values were recorded for adult females (age 16–25 years; 0.144 ng kg^{-1} bw day^{-1}) and males (age >25 years; 0.168 ng kg^{-1} bw day^{-1}). The estimated intakes of AFM1 in this study (*Milicevic et al.,* 2017) show lower levels of exposure of the Serbian adult population during 2015 and 2016 in comparison with the estimate of AFM1 intake reported in previous studies (*Torovic et al.,* 2015; Skrbic et al*.,* 2014; *Kos et al*., 2014). However, different study settings and methods were used, which do not allow results to be easily compared. Besides infants and young children, groups that are commonly recognized as populations vulnerable to AFM1 exposure, *Udovicki et al.* (2019b) identified the student population of Serbia as a group particularly vulnerable to AFM1 exposure in recent years, due to outbreaks of aflatoxin contamination. The model estimation was performed via probabilistic Monte Carlo simulation, deriving a mean AFM1 EDI for the student population in the range of 1.238 to 2.674 ng kg^{-1} bw day^{-1} (depending on the number of intake days considered). Similarly, EFSA has concluded that highest estimated chronic dietary exposure to AFB1 was in the young population groups, and the highest estimated chronic dietary exposure to AFM1 was in

infants and toddlers, which can be explained by their specific consumption patterns that are mostly based on milk and milk products (*EFSA*, 2019).

Biomarkers of exposure (in serum, urine and milk) are useful tools to complete the information about exposure, metabolism, toxicology and the carry-over rates of the different parent compounds or metabolites in the context of human (particularly infant) or animal exposure to mycotoxin hazards (*De Ruyck et al.,* 2020). Due to the long half-life of albumin in humans, the measurement of aflatoxin albumin adducts and their derivatives in blood are strongly preferred, and indicate an exposure extent over 1–2 months (*Leong et al.,* 2012).

Group 2B (Possibly carcinogenic to humans)

Group 2B applies to agents for which there is some evidence from human, experimental animals and/or mechanistic data that they can cause cancer in humans, but the data are still far from being conclusive. Consumption of FUM-contaminated foods by humans has been correlated with increased incidence of oesophageal cancer, hepatotoxicity (hepatocellular carcinoma), and nephrotoxicity in populations in various parts of the world (*Waskiewicz et al.,* 2012). Similar observations have been reported from China (*Xue et al.,* 2019), Italy (*Franceschi et al*., 1990) and Brazil (*Van Der Westhuizen et al.,* 2003). The ratio of sphinganine/sphingosine in serum, plasma or urine has been used as a biomarker of exposure to FUMs (*Solfrizzo et al.,* 2011). Recently, interest has emerged in the link between FUM exposure and child growth impairment. Based on the study by *Chen et al.* (2018) and several other studies, a link was found between FUM exposure and child growth impairment. The prevalences of child growth stunting are highest in sub-Saharan Africa, South Asia, and Central America. The incidence of neural tube defects was linked with high FUM intake by women along the Mexico-Texas border (*Voss and Riley,* 2013). However, in a second evaluation in 2002, there was inadequate evidence in humans for the carcinogenicity of FB1, which confirmed the classification of FB1 as possibly carcinogenic to humans (Group 2B) (*IARC,* 2002).

The kidney is considered to be the major target organ for OTA effects. OTA, which has been classified by the IARC as a group 2B carcinogen, is the most potent renal carcinogen in all mammalian species reported to date (*IARC,* 2014). OTA has been associated with human disorders, chronic interstitial nephropathy and Balkan endemic nephropathy in the former Yugoslavia, associated with urothelial cancer (*Pavlovic*, 2013). Human diets that exceed OTA levels of 70 μg kg^{-1} day^{-1} can result in renal tumours (*Reddy and Bhoola*, 2010). It can cross the placenta and accumulate in foetal tissue, causing various morphological anomalies. Early life exposure to OTA can cause testicular cancer in men (*Bayman and Baker*, 2006). Biomarkers for dietary exposure are reflected in OTA levels in plasma, serum and urine, and in breast milk particularly in an assessment of the risk for infants (*Valetta et al.,* 2018). Recently, *Zhang et al.* (2009) reported induction of apoptosis in neuronal cells that might be a contributing factor to the pathogenesis of neurodegenerative diseases like Alzheimer's and Parkinson's diseases. At present, new information regarding the genotoxicity of OTA (formation of OTA-DNA adducts), its role in oxidative stress and the identification of epigenetic factors involved in OTA carcinogenesis could lead to classification in Group 2B (Possibly carcinogenic to humans) (*Ostry et al.,* 2017).

Group 3 (Not classifiable as to its carcinogenicity to humans)

Other mycotoxins, i.e., PAT, citrinin (CIT), ZEA, TCTs (in particular T-2 toxin), NIV and DON, are considered by IARC as "*not classifiable as to its carcinogenicity to humans*" (Group 3). Group 3 applies to agents for which there are too limited, inadequate, or no data to allow classification. DON has been reported as the causative agent of Kashin-Beck disease (KBD), an endemic, chronic and deformed osteoarthropathic disease, which mostly occurs from north-eastern to south-western China, south-eastern Siberia and North Korea (*Li et al.,* 2016). Previously, it was reported that DON induced gastrointestinal poisoning and was a suspected aetiologic agent of gastroenteritis in children (*CDCP*, 1999). Prior to the discovery and implementation of reliable methods for the analysis of mycotoxins, *Fusarium* species were implicated in several human outbreaks of mycotoxicoses. Alimentary toxic aleukia in Russia from 1932 to 1947 was correlated with cereal grains contaminated with *Fusarium sporotrichioides* and *Fusarium poae*. Symptoms included mucous membrane hyperaemia, oesophageal pain, laryngitis, asphyxiation, gastroenteritis, and vertigo. With regard to the relatively recent studies considering risk assessment (EDI) of PAT intake through apple-based products by infants and children in Serbia, the results revealed no health risk for Serbian infants and preschool children through apple-based foods

(*Torovic et al.,* 2017). In contrast to the situation for aflatoxins (*Udovicki et al.,* 2019a; 2019b), in Serbia, DON and ZEA intake by adults was 0.262 and 0.05 μg kg^{-1} bw day^{-1}, respectively, through consumption of wheat-based food. Only 3.96% and 2.25% of the population exceeded established TDI values for DON and ZEA, respectively (Djekic et al., 2019).

CIT, a secondary metabolite of *Penicillium citrinum*, has been associated with yellowed rice disease in Japan. Considering its co-occurrence with OTA, CIT is responsible for nephropathy in pigs and other animal species (*Rasic et al.,* 2015). According to a recent EFSA report, occurrence data are lacking for a correct risk assessment of citrinin. Associated health risks of ergot mycotoxins are not of much significance today, and human ergotism is extremely rare (*Peraica et al.,* 1999), probably due to two reasons: primarily, recent improvements in grain cleaning and milling processes that are able to remove most of the ergots, leaving very low levels of the alkaloids in the flour, and; secondly, these alkaloids might be relatively unstable and can be destroyed easily by conventional processing (baking, cooking, milling).

Current methods in risk assessment of genotoxic and non-genotoxic compounds

Additional to the principles described by the *IARC* (2015), methodologies for human and animal risk assessments have been improved further, with a special emphasis on animal exposure assessment. EFSA guidance includes new approaches to risk assessment, such as the use of the Margin of Exposure (MOE). The MOE is a tool used by risk assessors to characterize the risk from exposure to genotoxic and carcinogenic substances that can be found in food or feed. The MOE is the ratio between the benchmark dose level (BMDL) that causes a 10% increase in cancer incidence in animals ($BMDL_{10}$) and the total intake ($MOE=BMDL_{10}/EDI$). For substances that are both genotoxic and carcinogenic, the EFSA Scientific Committee (*EFSA*, 2012) stated that an MOE of 10,000 or higher, if based on a $BMDL_{10}$ from an animal carcinogenicity study, would be of low health concern. However, it provides an indication of the level of safety concern about a substance's presence in food but does not quantify the risk. The EFSA CONTAM Panel concluded that calculated BMD_{10} values through carcinogenicity data in animal experiments were 170 ng kg^{-1} bw per day^{-1} and 21.0 μg kg^{-1} bw day^{-1} for AFB1 and OTA, respectively (*EFSA,* 2006; *EFSA,* 2007).

In the case of non-genotoxic compounds, EFSA have determined hazardous quotients for the TDI and TWI of several food contaminants. TDI is used for food contaminants, while acceptable daily intake (ADI) is used for food additives. For the purpose of risk characterization, the hazard index (HI) should be used. The HI is the ratio between EDI and TDI. A value higher than 1 indicates a risk for consumers. Table 1 includes TDI and PTWI for the major mycotoxins previously described.

Cancer incidence in Serbia

Chronic non-communicable diseases, comprising cardiovascular diseases, malignant tumours, diabetes, obstructive lung disease, injury and poisoning, mental health disorders and other chronic diseases have dominated Serbia's national disease pathology for decades. In fact, they constitute the major contributor to the burden of disease in terms of disability-adjusted life years (DALYs) or mortality. The cause of death for almost one in five persons who died (21.3%) was malignant tumour. Cancer incidence data in Serbia are collected and reported by the Institute of Public Health of Serbia (2019). According to data from Cancer Registry of Central Serbia, in 2015, 27,867 new cases of malignant tumours (14,582 men and 13285 women) were registered, and 15,224 people (8,790 males and 6,434 females) died of cancer. Furthermore, men were mostly diagnosed with and died of bronchus and lung cancer, colon and rectum cancer, and prostate cancer. In women, the most frequent sites of malignant tumours were breast, colon and rectum, and bronchus and lung.

The cancer incidence rate in males was 297.6 per 100,000 population, and in females 256.7 per 100,000 population. The highest cancer rates in males were registered in the City of Belgrade (349.7 per 100,000) and in the District of Pirot (347.0 per 100,000) and in females in the City of Belgrade (301.3 per 100,000) and in the District of Sumadija (295.3 per 100,000). The possible mechanisms of the genotoxicity of AFB1 and AFM1 and associated co-factors that act synergistically (both high and/or low doses) such as other mycotoxins, hepatitis viruses B and C and cyanotoxins, and their roles as hepatocarcinogens are still unclear in this Serbian case study. Considering the increasing number of malignant tumours in our country, further research into the relationship between the occurrence of mycotoxins and cancer incidence is required.

Impact of mycotoxins on animal health and productivity

In animals, mycotoxins produce a broad range of harmful effects to livestock health, production and welfare (*Pleadin,* 2015). The main effects include reduction in animal productivity, increased incidence of disease due to immuno-suppression, damage to vital organs accompanied by pathological change, interference with reproductive performance, little or no response to veterinary therapy, and in some extreme cases, death (*Yang et al.,* 2020). Because of their co-occurrence usually at low concentrations, mycotoxins can cause subclinical losses in production and increase the risk and incidence of other diseases. One of the first indications of a chronic mycotoxicosis is growth depression, which can result from reduced feed intake, impaired nutrient utilization and changes in feed quality or toxicity (*Bryden,* 2012). Among the animals, poultry, pigs and aquatic vertebrates are very sensitive to mycotoxins (*Oliveira and Vasconcelos*, 2020).

In animal studies, poultry are reported as the most sensitive domestic animals to aflatoxin toxicity. Ducks are the most sensitive to aflatoxins followed by turkeys, quail, broilers and layers. Aflatoxin toxicity in poultry causes fatty liver and kidney disorders, leading to numerous illnesses. OTA has been linked with porcine nephropathy in the Balkans. Lower concentrations of OTA in pigs are of major concern due to the mycotoxin's distinct toxicokinetic characteristics including long plasma elimination half-life and entero-hepatic and renal recirculation tissue accumulation (*Milicevic et al.,* 2008). Poultry are generally less prone to the effects of OTA due to birds excreting OTA faster than mammals, leading to more limited accumulation. The elimination half-life of OTA in broiler chickens is significantly shorter than that in pigs (4 h versus 150 h, respectively), leading to a lower systemic exposure of OTA in chickens *(Duarte et al.,* 2011). Differences in sensitivity between avian species are as follows: duck s>broiler>chickens>turkeys>Japanese quail (LD_{50} values for birds are 0.5, 3.3, 5.9 and 16.5 mg kg^{-1} bw^{-1}, respectively) (*Puvaca et al.,* 2012). Otherwise, turkeys seem to be more susceptible to several other mycotoxins than ducks, broiler chickens, or laying hens. These differences in sensitivity between avian species can be attributed to differences in toxicokinetics of the mycotoxin (*Guerre*, 2015). Impairment of feather cover could result in the carcass being downgraded due to blemishes and scratches on exposed skin. It has also been demonstrated that skin pigmentation is reduced during aflatoxicosis and ochratoxicosis, probably due to decreased absorption of dietary carotenoids.

Among animals, in almost all the species tested, FB1 has been shown to be hepatotoxic and nephrotoxic. In orally exposed animals, FUMs are in general poorly bioavailable, rapidly distributed mainly to liver and kidney, extensively biotransformed and rapidly excreted, mostly *via* the faecal route. Despite the fact that the toxicity of FUM is low, it has been linked with several diseases in domestic animals: equine leukoencephalomalacia (ELEM) in horses, recently recorded in Serbia (*Jovanovic et al.,* 2015), and porcine pulmonary oedema syndrome (PPE) in swine. PPE is observed in animals exposed to low levels of FUM (3–10 mg FB1 kg^{1} feed) (*Souto et al.,* 2015). Evaluations of outbreaks of ELEM in the USA showed that consumption of feed containing more than 10 mg FB1 kg^{-1} feed was associated with increased risk of ELEM, while no increased risk was found for feed containing less than 6 mg kg^{-1} feed (*Ross et al.,* 1990). The potential for FUM contamination in animal food products such as milk and eggs is of concern due to their widespread consumption and, especially for milk, the exposure potential of children (*Voss et al.,* 2007).

In domestic animals, ZEA poisoning has been associated with hyperoestrogenic or feminizing syndromes. Pigs are generally the most affected animal, in which it causes genital/urinary problems (*Zielonka et al.,* 2020). The major symptoms of ZEA poisoning include hyperaemia and oedematous swelling of the vulva in prepubertal gilts and in severe cases, prolapse of the vagina and rectum. In pregnant gilts and sows, ZEA can increase abortion, stillbirths and neonatal mortality. In male pigs, atrophy of the testes occurs with decreased libido and hypertrophy of the mammary glands (*Danicke et al.,* 2015). Poultry are the least affected of the livestock animals after ingestion of ZEA. Zeranol, a derivative of ZEA, is used in some countries as a growth promoter for sheep and cattle.

The clinical symptoms of TCT toxicosis vary from acute mortality to reduced growth and productivity. Group A TCTs (T-2 and HT-2 toxins) are of major concern as they are more toxic than the type B TCTs (deoxynivalenol and NIV). Group A TCTs induce necrotic changes in the mouth and gastrointestinal tract, emesis, diarrhoea, anorexia, haematological and immunological alterations and sometimes even a lethal outcome. Group B TCTs (at concentrations of 2–5 mg kg^{-1}) are associated with feed refusal, and concentrations > 20 mg kg^{-1} will induce

emesis, especially in pigs, the species most susceptible to this mycotoxin (*Haschek et al*., 2013). Consumption of low levels of these mycotoxins, especially in combination with the stress of commercial production, can result in chronic effects including impaired immunity and decreased resistance to infectious diseases. Consumption of vomitoxin-contaminated products has been correlated with reduced milk production in dairy cattle, vomiting in swine, and inhibition of reproductive performance and immune function in several animal species. T-2 toxin is rapidly metabolized and eliminated in different animal species, and therefore, there is no evidence for tissue accumulation or transfer into milk (*Li et al.,* 2011). In an outbreak of T-2 toxicosis, the rate of egg production decreased from 51% to 72%, while the incidence of cracked eggs increased from 3% to 15%. These clinical signs were accompanied by thinner and more fragile shells and would also have implications for hatchability.

Key information derived from animal exposure assessment to mycotoxins includes the amount and nature of residues of the parent compound and/or its biologically active metabolites, which can occur in animal-derived products, such as milk, meat and eggs. Thus, the prediction of potential residues and/or metabolites of contaminants is an important objective of human exposure assessment. There are significant differences among pig and poultry tissue deposition studies due to differences in absorption and metabolism of toxins and their metabolites. In general, residues of the mycotoxins ZEA, TCTs and FUMs are not considered to be of public health importance, as only very low levels of the toxins were found in the tissues of animals that had been fed very high levels of the toxins in experimental situations (*Fink-Gremmels et al.,* 2019).

Major techniques for the determination of mycotoxins in Serbia

For the purposes of risk assessment, confirming the diagnosis of a mycotoxicosis, and for monitoring mycotoxin mitigation strategies, it is important to use sensitive, specific, and reproducible methods for mycotoxin analysis in various food matrices. Mycotoxin analysis in food and feed is generally a multistep process comprised of sampling, sample preparation, toxin extraction from the matrix (usually with mixtures of water and polar organic solvents), extract clean-up and finally detection, and quantitative determination. A successful detection method should be robust, selective, sensitive and flexible regarding the expandability to other mycotoxins. Methods commonly used for mycotoxin detection and quantification in Serbia are presented in Tables 3–5. Besides conventional laboratory methods such as enzyme-linked immunosorbent assay (ELISA) and chromatographic methods (high performance liquid chromatography (HPLC), liquid

Table 6. Advantages and disadvantages of different mycotoxin analysis methods commonly used in Serbia

Method	Advantages	Disadvantages
HPLC	Good sensitivity, selectivity and repeatability, automated, short analysis times, official method of mycotoxin analysis.	Expensive equipment and analysis costs, requires trained and skilled personnel, destructive sample preparation, may require derivatization, time consuming, laboratory use only.
LC-MS	Multi-mycotoxin analysis, low LOD/LOQ, good sensitivity, selectivity and repeatability no derivatization required, automated, gold standard of mycotoxin analysis.	Very expensive equipment and analysis costs, requires highly trained personnel, sensitivity relies on ionization, matrix assisted calibration curve due to matrix interferences, time consuming, laboratory use only.
ELISA	Fast, relatively easy to use, simple sample preparation, inexpensive equipment, low limit of detection, simultaneous analysis of multiple samples, limited use of organic solvents, possible automatization, screening method.	High level of cross reactivity with related mycotoxins, possible false positives, matrix interference problems, narrow detection range, semiquantitative, laboratory use only.
LFIA	Fast, no clean-up, inexpensive equipment, easy to use, no specific training required, screening method, on-site analysis.	High level of cross-reactivity with related mycotoxins, validation required for additional matrices, semiquantitative.

chromatography coupled with mass spectrometry (LC-MS)), in recent years, several lateral flow immunoassays (LFIA) have become available on the Serbian market, providing the possibility of on-site mycotoxin screening. Major advantages and disadvantages of the mycotoxin analysis methods usually used in Serbia are presented in Table 6.

New developments in mycotoxin analysis focus on faster, multi-mycotoxin, environmentally friendly, cost-effective and fit-for-purpose methods in food, feed, biological tissue and body fluids. Today, the food industry clearly has a need for both rapid screening techniques, which could be also used outside the laboratory environment, and high sensitivity-precision methods for confirmatory purposes. Novel materials, methods and techniques for this purpose are developed daily. Among novel materials aptamers, molecular-imprinted polymers (MIPs) and various nano materials (nano metals, quantum dots etc.) have great potential for use in mycotoxin analysis. Sometimes termed chemical antibodies, aptamers are single-stranded oligonucleotides of DNA or RNA sequences (usually 25–80 bases long) that are produced by an *in vitro* selection process called systematic evolution of ligands by exponential enrichment (SELEX) and have high affinity and specificity target molecules. To date, several techniques for sample clean-up (based on SPE/IAC technology) and mycotoxin analyses have been developed (*Yang et al.,* 2013). MIPs, a robust alternative to natural recognition elements (antibodies and biological receptors), have also found use in sample clean-up and mycotoxin analysis (*Mueller and Appell,* 2016). Current trends in food analysis are focused on application of fast, easy to use, and cheap biosensor technologies (surface plasmon resonance, surface-enhanced Raman scattering, piezoelectric, fluorescence polarization) that are able to detect with high sensitivity and selectivity various compounds connected with food quality and safety (*Puiu et al.,* 2014; *Evtugyn et al.,* 2017). Following the success story of glucose biosensors, and with the use of novel materials, the development of biosensors for mycotoxin detection and quantification provides the perspective for cost-effective, small portable devices allowing precise and high-throughput on-site measurements; these should prove to be valuable tools in protecting human health. Concerning confirmation methods, there is a clear trend towards the use of multiple-analyte methods, mostly based on ultrahigh-performance liquid chromatography (UHPLC) coupled with mass spectrometry, with various mass analyzers allowing the use of streamlined sample preparation procedures that save time and labour and reduce the overall costs associated with mycotoxin testing (*Malachová et al.,* 2018).

Measuring equipment and measurement processes could produce incorrect results affecting the quality and validity of obtained results (*ISO*, 2013). Measurement errors and test uncertainties in the context of product (in this case product tested for mycotoxins) conformity assessment, in particular, highlight the increasing interest and enhanced insight into decision-making gained when extending classical, purely statistical treatment of consumer and producer risks. Samples of product are checked against a specification, but even if the mean mycotoxin concentration is under the specified limit, there is still a finite probability that mycotoxin concentration in the batch actually lies outside the limit (*Pendril,* 2006). This occurs because of non-zero measurement uncertainty and when the mycotoxin concentration is relatively close to the specified limit (*Pendril,* 2006). As a result of a recent ballot, *ILAC* has published guidelines to advise on this issue (*ILAC,* 2019). This publication was extensively revised by the ILAC Accreditation and Laboratory Committees to provide guidance to laboratories, assessors, regulators and customers in the use of decision rules when issuing statements of conformity to specifications or standards as required in the 2017 edition of ISO/IEC 17025 (*ILAC,* 2019). Here, the role of the ISO 17025 (*ISO*, 2017) accreditation process in order to ensure the overall quality of laboratory work and also to enforce confidence in any results obtained must be emphasized. However, the European Union Reference Laboratory (EURL) for mycotoxins has, together with its partners from the national reference laboratories, continuously monitored and also evaluated the performance of analytical methods with the aim of ensuring a reliable measurement capacity in Europe.

Mycotoxin legislation and regulations

Since it is impossible to fully eliminate the presence of undesirable substances and contaminants in food and feed, legislation and regulation are constantly evolving issues. Besides the adverse health impacts they have on both humans and animals, the presence of mycotoxins negatively influences food and feed trade. Thus, maximum concentrations of mycotoxins should be set at strict levels, which are reasonably achievable considering the risk analysis related to food consumption (Table 1). Risk analysis is a key discipline for reducing food-borne

Table 7. Maximum residue levels for mycotoxins in foodstuffs, according to the European Union and Serbian legislation

Mycotoxins	Foodstuffs	Maximum levels (μg kg^{-1})		
		EU[1–5], SRB[6,7]		
		B1	Sum of *AFs*	M1
AFB1[2]	Groundnuts (peanuts) and other oilseeds. Hazelnuts and Brazil nuts, to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs, with the exception of: — groundnuts (peanuts) and other oilseeds for crushing for refined vegetable oil production	8.0	15.0	
	Almonds, pistachios and apricot kernels to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs	12.0	15.0	
	Tree nuts. Dried fruit. Maize and rice to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs.	5.0	10.0	
	Groundnuts (peanuts) and other oilseeds and processed products thereof, intended for direct human consumption or use as an ingredient in foodstuffs, with the exception of: crude vegetable oils destined for refining, refined vegetable oils. Tree nuts, dried fruit and processed products thereof intended for direct human consumption or use as an ingredient in foodstuffs. All cereals and all products derived from cereals, including processed cereal products.	2.0	4.0	
	Almonds, pistachios and apricot kernels, intended for direct human consumption or use as an ingredient in foodstuffs	8.0	10.0	
	Hazelnuts and Brazil nuts, intended for direct human consumption or use as an ingredient in foodstuffs.	5.0	10.0	
	Raw milk, heat-treated milk and milk for the manufacture of milk-based products			0.05/ 0.25[6]
	Spices:	5.0	10.0	
	Processed cereal-based foods and baby foods for infants and young children	0.10		0.10[7]
	Dietary foods for special medical purposes intended specifically for infants			
	Infant formulae and follow-on formulae, including infant milk and follow-on milk			
OTA[1]	Unprocessed cereals Roasted coffee beans and ground roasted coffee, excluding soluble coffee	5.0		
	All products derived from unprocessed cereals, including processed cereal products and cereals intended for direct human consumption	3.0		
	Dried vine fruit (currants, raisins and sultanas), Soluble coffee (instant coffee)	10.0		
	Wine, grape juice, concentrated grape juice as reconstituted, grape nectar	2.0		
	Processed cereal-based foods and baby foods for infants and young children. Dietary foods for special medical purposes intended specifically for infants	0.50		
PT[1]	Fruit juices, concentrated fruit juices as reconstituted and fruit nectars. Spirit drinks, cider and other fermented drinks derived from apples or containing apple juice	50.0		
	Solid apple products, including apple compote, apple puree intended for direct consumption	25.0		
	Apple juice and solid apple products, including apple compote and apple puree, baby foods other than processed cereal-based foods for infants and young children	10.0		

Mycotoxins	Foodstuffs	Maximum levels (μg kg^{-1})		
		EU[1–5], SRB[6,7]		
		B1	Sum of *AFs*	M1
DON[1]	Unprocessed cereals other than durum wheat, oats and maize	1250		
	Unprocessed durum wheat, oats and maize	1750		
	Cereals intended for direct human consumption, cereal flour (including maize flour, maize meal and maize grits), bran as end product marketed for direct human consumption and germ. Pasta (dry)	750		
	Bread (including small bakery wares), pastries, biscuits, cereal snacks and breakfast cereals	500		
	Processed cereal-based foods and baby foods for infants and young children	200		
ZEN[1]	Unprocessed cereals other than maize	100		
	Unprocessed maize. Maize intended for direct human consumption, maize flour, maize meal, maize grits, maize germ and refined maize oil	200		
	Cereals intended for direct human consumption, cereal flour, bran as end product marketed for direct human consumption and germ.	75		
	Bread (including small bakery wares), pastries, biscuits, cereal snacks and breakfast cereals, excluding maize snacks and maize based breakfast cereals. Maize snacks and maize based breakfast cereals	50		
	Processed cereal-based foods (excluding processed maize-based foods) and baby foods for infants and young children. Processed maize-based foods for infants and young children.	20		
FUM-s (B1+B2)[1]	Unprocessed maize	2000		4000
	Maize flour, maize meal, maize grits, maize germ and refined maize oil	1000		1400–2000
	Maize based foods for direct human consumption	400		800–1000
	Processed maize-based foods and baby foods for infants and young children	200		200
T-2+HT-2[3]	Unprocessed cereals			
	▪ barley (including malting barley) and maize	200		–
	▪ oats (with husk)	1000		–
	▪ wheat, rye and other cereals	100		–
	Cereal grains and products for direct human consumption			
	Oats. Oat bran and flaked oats	200		–
	Maize. Cereal bran except oat bran, oat milling products other than oat bran and flaked oats, and maize milling products	100		–
	Other cereals and other cereal milling products.	50		–
	Breakfast cereals including formed cereal flakes	75		–
	Bread (including small bakery wares), pastries, biscuits, cereal snacks, pasta	25		–
	Cereal-based foods for infants and young children	15		–
Ergot sclerotia and ergot alkaloids[4]	Unprocessed cereals (18) with the exception of corn and rice	0.5 g kg^{-1}		0.5 g kg^{-1}
Citrinin[5]	Food supplements based on rice fermented with red yeast *Monascus purpureus*	100		

Legend: [1]-EC 1881/2006, [2]-EC165/2010; [3] EC 165/2013, [4]-EC 2015/1940; [5]-EC 2019/1901: [6]-Serbian Regulation 22/2018; 81/2019), 0.05 μg kg^{-1} (since 1 December 2020), Total AFs (sum of AFB1, AFB2, AFG1, and AFG2). [7]- Serbian Regulation (7/2017)

Table 8. Maximum permitted content and recommendations for mycotoxins in feedstuffs according to EU and Serbian legislation (mg kg^{-1})[1]

Mycotoxins	Products intended for animal feed	Maximum content (mg kg^{-1})	
		EU[2–4]	SRB[5]
AFB1[2]	All feed materials	0.02	0.03
	Complete feedingstuffs for cattle, sheep and goats with the exception of:		0.02
	▪ complete feedingstuffs for dairy animals	0.005	0.005
	▪ complete feedingstuffs for calves and lambs	0.01	0.005
	Complete feedingstuffs for pigs and poultry (except young animals)	0.02	0.02
	Other complete feedingstuffs	0.01	0.01
	Complementary feedingstuffs for cattle, sheep, goats, pigs and poultry (except complementary feedingstuffs for dairy animals, calves, lambs and young animals)	0.02	0.02
	Other complementary feedingstuffs	0.005	0.01
OTA[3]	Feed materials: cereals and cereal products	0.25	0.25
	Complete and complementary feedstuffs		
	▪ For pigs	0.05	0.1
	▪ For poultry	0.1	0.2
DON[3]	Feed materials:		
	cereals and cereal products, with the exception of maize by-products	8	8
	maize by-products	12	12
	Complementary and complete feedstuffs	5	5
	with the exception of:		
	▪ Complementary and complete feedstuffs for pigs	0.9	0.9
	▪ Complementary and complete feedstuffs for calves (< 4 months), lambs and kids	2	2
FB1, FB2[3]	Feed materials: maize and maize by-products	60	–
	Complementary and complete feedstuffs for:		–
	▪ pigs, horses (Equidae), rabbits and pet animals	5	–
	▪ fish	10	–
	▪ poultry, calves (< 4 months), lambs and kids	20	–
	▪ adults ruminants (> 4 months) and mink	50	–
ZEN[3]	Feed materials:		
	▪ cereals and cereal products, with the exception of maize by-products	2	4
	Maize by-products	3	6
	Complementary and complete feedstuffs:		
	▪ for piglets and gilts (young sows)	0.1	0.2
	▪ for sows and fattening pigs	0.25	0.5
	▪ for dairy cattle, sheep (including lambs) and goats (including kids)	0.5	1.0
T-2, HT-2[4]	Cereal products for feed and compound feed:		–
	oat milling products	0.25	–
	other cereal products	0.5	–
	compound feed (with the exception of feed for cats)	2	–
Rye ergot	All feedingstuffs containing unground cereals	1000	1000

Legend: [1]-Relative to a feedingstuff with a moisture content of 12%. [2]-Directive, 2002/32/EC, amended by Directive, 2003/100. [3]- 2006/576/EU (for DON, ZEN, FBs and OTA); [4]–2013/165/EU (for T-2 and HT-2). [5]- Serbian Regulation (39/2016).

illness and strengthening food safety systems based on scientific opinion. Factors influencing mycotoxin regulations include availability of toxicity data, availability of data on the occurrence in different commodities, survey analytical data, methods of sampling and analysis, and trade contacts with other countries (*van Egmond et al*., 2007). However, factors fundamental to a country's ability to protect its population from mycotoxins include the political will to address mycotoxin exposure and capability of testing food for contamination, which determines whether the requirements can be enforced.

In Serbia, in accordance with European Union regulation, maximum levels (MLs) are prescribed for 11 mycotoxins in food: AFB1 and AFM1 individually as well as the sum of aflatoxins (AFB1, B2, G1, and G2), FUMs (FB1, FB), OTA, patulin, DON, ZEA and ergot sclerotia (*Serbian Regulation,* 2019) (Table 7). Surprisingly, in Serbia the regulatory authorities have not established MLs for FUMs in feed, despite their widespread occurrence and their health hazards for animals (*Serbian Regulation*, 2016) (Table 8). An increasing number of residue studies suggest that EFSA's guidance for mycotoxin MRLs in feed and, consequently, residues in animal-derived products, results in food that presents minimal risk to human consumers (*Dongping et al.,* 2019). In order to ensure the safety of food, existing legislation in Serbia encourages producers and researchers to pay serious attention to food and feed production processes and to develop comprehensive quality policies and management systems to improve food safety. Monitoring and control systems as integral components of the food safety system has been established to obtain reliable information about the real exposure of human populations to mycotoxins and any risk for public health. The Ministry of Agriculture Forestry and Water Management has overall responsibly for food and feed monitoring. The Veterinary Directorate has been assigned overall responsibility for monitoring and controlling mycotoxins in animal-derived products, while the Plant Protection Directorate is responsible for the implementation of monitoring/official controls of mycotoxins in plants. The presence of selected mycotoxins (AFM1 in milk and OTA residues in kidney and liver of slaughtered animals) has been systematically monitored according to an annually planned monitoring program. Types, numbers of samples, and combinations of analyzed mycotoxins/groups of mycotoxins are planned annually, taking into account the number of slaughtered animals in previous years. The combinations of analyzed mycotoxins and matrices are chosen predominantly according to the Council Directive 96/23/EC. In addition, a monitoring program is conducted by the Institute of Meat Hygiene and Technology, Belgrade. Also, the official control of food and feed in Serbia covers samples from border inspection, samples that have been the subject of complaints or are derived from food/feed poisoning cases, and samples from any follow-up actions.

Integrated food safety management system/ risk management and control strategies

Several codes of practice, including the Code for the Prevention and Reduction of Mycotoxins in Cereal Grains and Grain-Derived Foods and Feeds, have been developed by Codex Alimentarius. These recommendations are divided into two parts: pre-harvest practices based on good agricultural practice (GAP) and post-harvest practices such as good manufacturing practice (GMP) and good hygiene practice (GHP) that are implemented in hazard analysis and critical control point (HACCP) systems. An organization dealing with feed production and/or grain storage will develop a formal food safety management system (FSMS) to ensure that feed it produces is safe for consumption. Organizations need to establish and implement suitable control measures that are appropriate for the specific hazards existing in the food/feed and the risk they pose to the final consumer. The Codex standard (*CAC,* 2003a) for HACCP uses a decision tree in order to determine whether the hazard should be controlled as a CCP or not. It does not attempt to assist the organizations in determining what type of control should be employed where "Not a CCP" is the outcome. This makes it limited for most modern food businesses seeking to develop a robust food safety plan (*Politis et al.,* 2017). Storage conditions are one of the critical stages for the post-harvest prevention of mycotoxins. Among many factors within a storage ecosystem, temperature and humidity are crucial for the fungal infection and mycotoxin contamination. Maintaining uniform grain temperatures throughout the grain mass is important to avoid moisture imbalance. This can be achieved by passing large volumes of ambient air (aeration) through the grain mass. Improved storage management (GMP, GHP), especially at the farmer and small trader levels, will prevent fungal growth and mycotoxin contamination in stockpiled grains (*Milicevic et al.,* 2019b). However, GAP associated with prediction models that integrate the most important field parameters and weather inputs are the best options to prevent fungal colonization

and mycotoxin production in the field. If mycotoxin has occurred, contaminated feed and food must be managed through post-harvest decontamination/detoxifying procedures to convert mycotoxins into non- or less toxic products (Figure 1).

Traditional detoxifying methods include physical, chemical and biological methods (*Wan et al.*, 2020). Mycotoxin decontamination by physical methods includes various procedures such as sorting and separation, immersing and washing, irradiation, filtering and adsorption. Novel processing technologies like microwave heating, gamma and electron beam irradiation, ultraviolet and pulsed light, electrolyzed water and cold plasma are also being continuously investigated. These practices, despite their various efficiencies, advantages and limitations, are applicable both in food and feed contaminated by various mycotoxins. Chemical treatments for mycotoxin decontamination involve bases, oxidizing agents, organic acids and other agents. Currently, application of chemical treatments for mycotoxin reduction in food or feed has many limitations due to consumers' health concerns. Also, losses in the nutritional value and the palatability of feeds and interactions with food components are disadvantageous factors of these methods (*Kolosova and Stroka*, 2011). Moreover, the use of chemical decontamination processes is not legal within the EU (*Directive,* 2002/32). Among them, only ammonia and ozone have been developed and utilized industrially. The main advantage of biological degradation of mycotoxins is that it works under mild, environmentally friendly conditions. Some microorganisms and/or enzymes can degrade mycotoxins into less toxic or non-toxic derivatives by transforming their toxicological properties. A wide range of bacteria (lactic acid bacteria), moulds and yeasts (*Saccharomyces cerevisiae*) have shown the ability to biodegrade mycotoxins. Moulds such as *Aspergillus, Rhizopus* and *Penicillium spp*. show effective abilities to detoxify mycotoxins (*Cheng et al.,* 2016). Recent research in Serbia indicates the biocontrol agent, a natively atoxigenic *A. flavus* strain, has high potential for reducing aflatoxin contamination in local environmental conditions (*Savic et al.,* 2020).

In 2009, the European Union approved the use of mycotoxin-detoxifying agents, by including a new group of feed additives defined as 'substances that can suppress or reduce the absorption, promote the excretion of mycotoxins or modify their

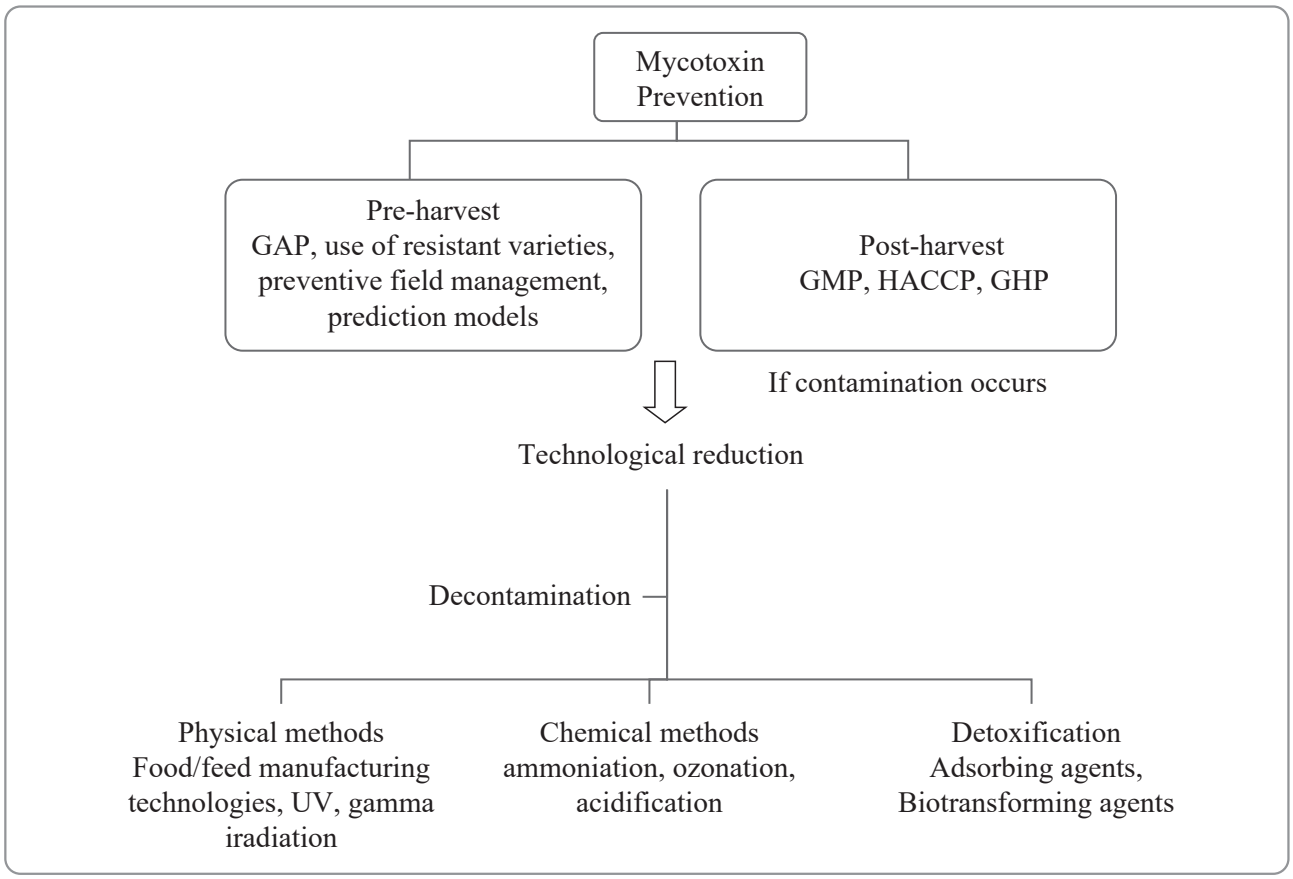

Figure 1. Strategies to prevent and reduce mycotoxins

Table 9. SWOT analysis for improving the national food safety system

Strengths	Weaknesses
▪ Harmonized national and EU legislation exist in the agri-food sector. ▪ Updated food laws, unified standards and conformity of assessment systems have all been addressed. ▪ National food contaminant monitoring programs to ensure prevention of potential risks are in place. ▪ The Consumer Protection Department has been established. The National Expert Council for Food Safety Risk Assessment has been established. ▪ Testing laboratories in the field of food safety are equipped with fit-for-purpose equipment, running validated methods and operating according to the requirements of ISO 17025. Food safety management is on the political agenda. ▪ The national action plan for public health highlights the necessity to perform research on food quality and safety. ▪ Availability of competent authorities to set national norms and standards. ▪ Food safety is based on food business operator responsibility. ▪ Mandatory HACCP/equivalent quality assurance system for producers.	▪ Dual authority in policy making. ▪ Risk assessment has not yet been implemented. In a number of cases, inspection is carried out only after a problem arises. Lack of effective coordinated effort and flow of information between all of the authorities involved in the food control system (One Health). ▪ No food quality and safety control agency has been established. ▪ Absence of local risk (assessment) data. Inadequate data collection, storage and analysis. ▪ Lack of a national database on population characteristics of diet and food composition. The National Reference Laboratory is not operational. ▪ The Rapid Alert and Alert System (RASFF) has not been developed and implemented.
Opportunities	**Threats**
▪ Adequate research infrastructures (RIs) in food, nutrition, and health domain are essential for nutrition epidemiology, innovative nutritional research, dietary exposure and food safety risk assessments and effective public health nutrition (PHN) strategies to address the diet-related diseases, malnutrition and foodborne diseases. ▪ Policymakers should be informed by timely, quality information on the severity of hazards, economic costs and the nutritional impacts, particularly in vulnerable groups. ▪ Harmonized and standardized food consumption data should be collected from national dietary surveys at individual level. ▪ The complex nature of food safety means a holistic and multidisciplinary approach needs to be developed. ▪ A regional mycotoxin risk assessment center must be established. ▪ The use of IoT, artificial and business intelligence, cloud systems, sensors and algorithms for generation, storage, interpretation and distribution of all relevant data for myctoxin management all require expert attention. ▪ Climate change aslo requires the development of predictive models to forecast and prevent mycotoxin contamination. ▪ Model developed in Serbia can be further disseminated and applied in other countries in the Balkans.	▪ Food imports are steadily increasing, predominantly from at-risk regions. A large number of laboratories carrying out food analysis are not at the appropriate technical and technological level of equipment. ▪ Lack of: risk analysis capabilities in regulatory bodies; governmental budget allocated for policy implementation; knowledge within professionals and policy makers; adequate research infrastructure for food nutrition and health (FNH-RI). ▪ Low purchase power of consumers does not encourage operators to invest into costlier quality measures. ▪ Price of food commodities is the major factor in the consumer decision. ▪ Impoverished consumers are not protected from eating commodities rejected by exporters due to high contamination levels. ▪ Limited number of inspectors and limited scope in monitoring programs. ▪ Insufficient technological readiness for climate change.

mode of action' (*EC*, 2009). These feed additives are known as detoxifying agents and were designed to reduce the potential adverse effects of mycotoxins after the feed is ingested by animals. These binding agents are roughly classified as 'inorganic' or 'organic'. The main mycotoxin-restricting mechanisms involved with these additives include: 1) physically binding the mycotoxins and, thus, decreasing the gastrointestinal absorption of mycotoxins and their distribution to blood and target organs; 2) inactivating mycotoxins, and; 3) degrading or transforming mycotoxins into less toxic metabolites (biotransformation) (*Peng et al.,* 2018). These feed additives also have some advantages and disadvantages. Due to their low costs and high efficacy, mycotoxin binders have been widely used by local farmers to reduce the potential adverse effects of mycotoxins. The main disadvantage is limited multi-toxin-binding efficacy, meaning even if a parent mycotoxin is deactivated, its metabolic products are not necessarily eliminated. With both low costs and low efficacy, these mycotoxin binders are sometimes added into feed in large amounts by farmers, which can decrease the total nutritional values of feed and result in nutritional imbalance in the animals (*Karlovsky*, 2011).

In Serbia, future *in vivo* research should include assaying naturally multi-contaminated feeds, which reflect real mycotoxin concentrations, taking into account EU-regulations and EFSA report endpoints. The use of mycotoxin-detoxifying feed additives, regarding their efficacy, safety and their potential for interactions with critical nutrients (vitamins and minerals) requires further study.

GHP and HACCP are the primary tools available to control chemical hazards in food operations. The basic idea of HACCP system is to manage food safety based on risk management principles and cover a range of biological, chemical and physical hazards (*Akkerman et al.,* 2010; *Maldonado-Siman et al.*, 2014). Historical and current thinking limits the scope of FSMS to the control and management of the aforementioned hazards, but does not included the wider consideration of prevention of NCDs, although it can be argued that NCDs could involve "conditions of food with the potential to cause an adverse health effect" (*Manning et al.,* 2019). It could be supposed that organizations need to consider how these developments will influence the categorization of food hazards and intoxication in the future (*Manning*, 2019) and the impact on management approaches to mycotoxin hazard control and management.

Recently, the food safety management approach has been completed and developed through the inclusion of other metrics like the Food Safety Objective (FSO) (*García-Cela et al.,* 2010). An FSO is defined in FSMSs as the maximum frequency and/or concentration of a microbiological hazard in a food at the time of consumption that provides the appropriate level of protection (*CAC,* 2003b). In practice, FSOs are achieved through the establishment and implementation of performance and process criteria (performance criterion (PC), process criterion (PcC) and product criterion (PdC)). In every step of the food chain, it is necessary to know the effect of every treatment ensure hazard levels never overtake safety levels before the time of consumption (performance objective (PO)).

Current and future outlook

Although the Serbian Food Safety Act is proactive and is based on CAC standards and on the principles of European Union legislation, there are some deficiencies in its development, implementation, control of the implementation and efficiency evaluation. These are required in order to further improve the country's food safety system. *FAO/WHO* (2003) documents, based on extensive experience in different systems, provide useful suggestions on how to effectively establish and maintain a food safety system. The use of key structural components of the FAO/WHO Guide (*FAO/WHO*, 2006) enables the identification of significant indicators and parameters of a food safety system, such as strength, weakness, potentialities and hazards, termed SWOT analysis (strengths, weaknesses, opportunities and threats). Using SWOT analysis as a basis, it is possible to make recommendations for improving the national food safety system (*Gurinovic*, 2016; *Gurinovic et al.,* 2018) (Table 9).

Aktuelna situacija kontaminacije hrane i hrane za životinje mikotoksinima sa osvrtom na javnozdravstveni rizik u Srbiji

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A p s t r a k t: Mikotoksini predstavljaju hemijski hazard mikorbiološkog porekla, proizvod sekundarnog metabolizma pretežno filamentoznih plesni. Značaj mikotoksina najčešće se vezuje za pojavu brojnih oboljenja kod ljudi i životinja, koja pripadaju grupi nezaraznih bolesti (eng. non-communicable diseases). Nezarazne bolesti, (npr. maligni tumori), vodeći su uzroci obolevanja, invalidnosti i prevremenog umiranja (pre 65. godine života) u svetu, a i u našoj sredini (eng. Disability Adjusted Life Years-DALY). Maligna oboljenja se karakterišu dužim vremenom trajanja i nastaju kao posledica interakcije mnogobrojnih faktora kao što su genetski, fiziološki status organizma, prirodno okruženje i biološkog odgovora čovekovog organizma. Sve veće interesovanje za sinergistički efekat sintetskih i prirodnih kontaminenata na zdravlje ljudi, ukazuje na to da kontaminacija mikotoksinima predstavlja idalje oblast od prioritetnog značaja za sve učesnike u lancu hrane. Uzimajući u obzir da kontaminacija hrane mikotoksinima prvenstveno zavisi od klimatskih faktora, ekstremne klimatske pojave kao što su suša i poplave poslednjih godina zabeležene u Srbiji, potvrđuju činjenicu da su mikotoksini jedan od hazarda u lancu hrane na koji klimatske promene imaju najveći uticaj. U ovom radu pokušali smo da analiziramo ključne faktore od značaja za kontaminaciju mikotoksinima, kao i da se ukaže na najnovije trendove i strategije u prevenciji štetnih efekata mikotoksina u lancu hrane, sagledavajući stanje i mogućnosti u Srbiji.

Ključne reči: *mikotoksini, zastuljenost, javno zdravlje, SWOT-analiza.*

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