



Dietary exposure assessment to mycotoxins from traditional meat products in Croatia

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ABSTRACT

Mycotoxins may be present in traditional meat products (TMPs) due to production of moulds overgrowing their surface during ripening or due to contaminated spices or meat used in their production. The aim of this study was to evaluate the human exposure to the mycotoxins, including aflatoxins (AFs) B₁, B₂, G₁ and G₂ (AFB₁, AFB₂, AFG₁, AFG₂), ochratoxin A (OTA), cyclopiazonic acid (CPA), citrinin (CIT) and sterigmatocystin (STC), which may be present in TMPs, using 288 samples available on the market in relation to the consumption data of Croatian citizens. AFs and CIT weren't detected in any sample, so the exposure to these mycotoxins in TMP can be considered negligible. The determined exposure to STC was 0.011–0.020 ng/kg bw/day far below the exposure of low health concern set by the European Food Safety Authority (EFSA). The determined exposure to CPA was up to 5.75 ng/kg bw/day, but there isn't yet a health-based guideline value (HBGV) for the consumer risk associated with CPA in food. The determined exposure to OTA was 0.043–0.121 ng/kg bw/day, and according to the margin of exposure (MoE) approach, in the case of the upper bound (UB) scenario, for neoplastic effects a low concern was identified in 0.12% of consumers, while for non-neoplastic effects a potential health concern was identified in 4.15% of consumers. Among the TMP categories, dry-fermented sausages contributed the most to consumer exposure for all three mycotoxins detected. Results indicate that mycotoxins presence should be monitored over a longer period of time where different data can be expected in order to observe the trend of human exposure.

1. Introduction

Autochthonous dry-cured meat products are an important part of regional culture and tradition in many European countries, attracting consumers and therefore the subject of research into their nutritional value and safety. In terms of safety, various authors have conducted studies on possible mycotoxin contamination (Bailly & Guerre, 2009; Markov et al., 2013; Peromingo et al., 2018; Perrone et al., 2019; Pleadin, Malenica Staver, et al., 2015; Vulić et al., 2016).

Mycotoxins are toxic secondary mould metabolites that are recognised worldwide as a food safety hazard and are responsible for acute and chronic toxicity (Hussein & Brasel, 2001). Mycotoxins can be found in traditional meat products (TMP) directly, as a result of mould

production that overgrows their surface during maturation, from contaminated spices used in their production, or from the meat of domestic animals that have consumed feed contaminated with mycotoxins (Bertuzzi et al., 2013; Pleadin et al., 2013; Zdravec et al., 2023).

Ochratoxin A (OTA) and cyclopiazonic acid (CPA) are shown to be the most abundant mycotoxins (Lešić et al., 2022; Peromingo et al., 2018; Perrone et al., 2019; Pleadin, Malenica Staver, et al., 2015; Vulić et al., 2020), while OTA and AFB₁ are the mycotoxins with the highest toxicity in meat products. OTA is classified by the International Agency for Research on Cancer (IARC) as a Group 2 B possible human carcinogen (IARC, 1993), AFB₁ belongs to IARC Group 1 of proven carcinogens (IARC, 2002). CPA has not yet been classified by IARC as the carcinogenicity studies are not sufficiently conclusive, but it has been shown

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that the digestive organs, muscles, liver and spleen are most sensitive to the toxic effects of CPA (Ostry et al., 2018.). In food, CPA often co-occurs with aflatoxins or other mycotoxins, which is why the EFSA Panel on Contaminants in the Food Chain (CONTAM) has emphasised the importance of assessing simultaneous exposure to CPA and AFB₁ (EFSA, 2020a).

Moulds found on the surface of TMP can produce mycotoxins such as sterigmatocystin (STC) - a precursor of AFB₁ - as well as other aflatoxins from the same biosynthetic pathway: B₂, G₁ and G₂, or mycotoxin citrinin (CIT). For CIT, a co-occurrence with OTA is described in the literature, which is mainly due to the same producers such as *Penicillium verrucosum* (Bailey & Guerre, 2009; EFSA, 2012). The toxic effects of STC are similar to those of AFB₁, with its carcinogenic, mutagenic and teratogenic properties, but the acute toxicity of STC is still 10 or more times lower than that of AFB₁. The IARC classified STC as a possible human carcinogen in Group 2 B (IARC, 1987). Most of the available toxicity data on aflatoxins relate to AFB₁; information on the other aflatoxins is sparse and only mentioned when available. There is evidence of differences in the carcinogenicity of AFB₁ and AFG₁, with AFB₁ being more potent in the liver and AFG₁ in the kidney. AFB₂ and AFG₂ did not induce genotoxicity in three human cell lines tested (EFSA, 2020a). The IARC classified CIT as Group 3, which is not classifiable as to its carcinogenicity to humans (EFSA, 2012).

Exposure to mycotoxins is a global problem. To ensure public health, it is essential to characterise the risk associated with these toxins. The assessment of human dietary exposure to mycotoxins is an important part of food safety strategies. The aim of this study was to assess human exposure to the mycotoxins AFB₁, AFB₂, AFG₁, AFG₂, OTA, CPA, CIT and STC that may be present in meat products through the consumption of Croatian TMPs available on the market. With the exception of OTA and AFB₁, there is very little data on the toxicity of these mycotoxins and their presence in meat products. These data are important to assess consumer exposure to mycotoxins and the risks to human health and to set the maximum levels (ML) for mycotoxins in meat products, which are not yet established in the European Union (EFSA, 2012; EFSA 2013; EU, 2023). Carballo et al. (2019) concluded in their study on the assessment of dietary exposure to mycotoxins that special attention should be paid to meat and cereal products due to the high percentage of consumers. There are studies assessing exposure to OTA and AFB₁ in other countries and in other foods (Carballo et al., 2019; Cressey & Reeve, 2013; Duarte et al., 2010; Elzupir et al., 2020; Meerpoel et al., 2021), but meat products are hardly considered, and accordingly exposure data on other mycotoxins that are less studied, such as CIT, STC and CPA, are even rarer or missing altogether. As far as we know, there are only two studies that relate exclusively to OTA exposure from meat products in Croatia (Vulić et al., 2014, 2016).

2. Materials and methods

2.1. Samples of traditional meat products

The study was conducted on 288 samples of Croatian traditional, non-thermally processed dry-cured meat products. They were produced in the traditional way, under uncontrolled conditions and without the addition of additives or starter cultures in Croatian family farms. The meat products were sampled in the period 2019–2021 in a quantity of 1.5–2.0 kg each. The analysed category of samples included six groups: dry-fermented sausages with 129 samples of various traditional Croatian sausages; *Kulen*, a special type of Croatian sausage, with 17 samples; *Pečenica/ombolo/kare* with 29 samples; prosciuttos/dry ham with 41 samples; bacon/pancetta with 52 samples and dry rack with 20 samples. The family farms from which the samples came are located in five Croatian regions (the eastern region of Slavonia and Baranja, the northern region of Međimurje, the central region of Zagorje, the western region of Istria and the southern region of Dalmatia), with five different farms from each region providing the samples.

After taking samples of market-ready meat products from family farms, the samples were stored in a mobile refrigerator and then transferred to a room refrigerator at +4 °C for a maximum of 72 h, which is the typical temperature for storing dry-cured products or fermented sausages, before the meat products were chopped into small pieces and homogenised using a Grindomix GM 200 (Retsch, Germany). After homogenization, the homogenates were stored in a refrigerator at –18 °C until the determination of mycotoxins. Dry-fermented sausages are products consisting mainly of pork meat, fatty tissue and ingredients such as salt and spices that, undergo a drying, fermentation and curing process with or without smoking after being minced and filled into casings (MA, 2018). *Kulen* is a special type of sausage with some specific parameters that should be satisfied during production and in final product such as raw material, condition and ripening time and final protein and water content (Kovačević, 2014; MA, 2018).

Dry-cured meat products such as prosciutto/dry ham, *pečenica/ombolo/kare*, bacon/pancetta and dry rack are produced from pork meat with or without belonging bones, subcutaneous fat and skin by salting, drying and ripening with or without process of smoking. Length of their production varies from 2 months for bacon/pancetta to up to more than 12 months for prosciutto/hams (MA, 2018). The production technology and recipes of these products under study are in more detailed described in research Kudumija et al. (2024). The pH values of all meat samples were below 6.2, with the sausage and *kulen* groups having significantly lower values (5.42 ± 0.38 – 5.56 ± 0.51) than the other meat product groups (6.01 ± 0.36 – 6.23 ± 0.38). The moisture content ranged from $25.90 \pm 10.31\%$ for the bacon/pancetta group to $45.80 \pm 7.78\%$ for the *pečenica/ombolo/kare* group (Kudumija et al., 2024).

2.2. Mycotoxin analysis

Eight mycotoxins that can be possibly found in TMP: AFB₁, AFB₂, AFG₁, AFG₂, STC, OTA, CIT and CPA were analysed by the liquid chromatography-tandem mass spectrometry (LC-MS/MS) method with use of high-performance liquid chromatograph (1260 Infinity, Agilent Technologies, Santa Clara, CA, USA) coupled with a triple quadrupole mass spectrometer (6410 QQQ, Agilent Technologies, Santa Clara, CA, USA) on a 150×4.6 mm, 5 μ m-particle size C18 Gemini analytical column (Phenomenex, Torrance, CA, USA) coupled with a SecurityGuard™ Cartridges Gemini C18, 4×3.0 mm ID pre-column (Phenomenex, Torrance, CA, USA). Sample preparation for mycotoxins other than CPA involved the use of highly specific immunoaffinity columns (R-Biopharm Rhône Ltd., Glasgow, Scotland) and for CPA the use of rOQ QuEChERS extraction packages (Phenomenex, Torrance, CA, USA). AFB₁ and OTA analysis employed AFLAOCHRAPREP® columns, while CIT analysis made use of Easi-extract CITRININ® and STC Easi-extract STERIGMATOCYSTIN® columns. The performance and validation of the method was previously described in detail by Kudumija et al. (2020) and Lešić et al. (2022). The obtained LOD values (limit of detection) were: OTA 0.18 μ g/kg, STC 0.02 μ g/kg, CPA 2.17 μ g/kg, CIT 0.60 μ g/kg, AFB₁ 0.03 μ g/kg, AFB₂ 0.03 μ g/kg, AFG₁ 0.04 μ g/kg, AFG₂ 0.05 μ g/kg.

2.3. Food consumption data

The data on food consumption in the Republic of Croatia come from a survey conducted by the Croatian Food Agency in 2011 and 2012 among a representative sample by age, gender and region (HAH, 2011–2012, unpublished data). The survey included 2002 respondents aged between 18 and 64. A “face-to-face” interview and a 24-h recall method was used, which lasted three days (two weekdays and one weekend day), and data on the frequency of consumption of certain foods was collected using the Questionnaire on the frequency of consumption. Data on food consumption are expressed in grams per day (g/day).

2.4. Exposure assessment

For the calculation of exposure, the average result for the occurrence of mycotoxins was used and linked to the individual result of product intake for each TMP taken from the database of adult food consumption in Croatia. The assessment of human exposure to mycotoxins from TMPs is calculated as estimated daily intake (EDI) in $\mu\text{g}/\text{kg}$ (bw) per day according to the following formula:

$$\text{EDI } (\mu\text{g}/\text{kg body weight}/\text{day}) = \text{individual food consumption data (g/day)} \times \text{average mycotoxin contamination from TMP } (\mu\text{g}/\text{kg}) / \text{body weight (kg)}$$

The average result for mycotoxin contamination was calculated using the “lower bound” (LB), “middle bound” (MB) and “upper bound” (UB) scenarios, as there were cases where the result was below the LOD (EFSA, 2010). Thus is in order to obtain the most accurate calculations possible and to explain the uncertainty of the results obtained, considering that a certain number of samples were taken into account in which the mycotoxin concentrations determined were below the LOD. The calculation was based on the following three scenarios: UB approach (“upper bound”; higher value), when a value equal to the LOD value was used for the calculation, in samples where the concentration was below the LOD; MB approach (“middle bound”; mean value), if half of the LOD value was used for the calculation, in samples where the concentration was below the LOD; LB approach (“lower bound”; lower value), if zero (0) was used for the calculation in samples where the concentration was below the LOD.

2.5. Health-based guidance values (HBGVs) for risk assessment

The EFSA CONTAM Panel selected a benchmark dose lower confidence limit (BMDL) of $0.4 \mu\text{g}/\text{kg}$ bw/day for AFB₁ exposure to be used in a MoE approach. The calculated MoE values below 10,000 for AFB₁ give rise to health concerns. The estimated cancer risks in humans after exposure to AFB₁ and AFM₁ are consistent with the conclusions from the MoEs. The conclusions also apply to the combined exposure to all five aflatoxins (AFB₁, AFB₂, AFG₁, AFG₂, AFM₁) (EFSA, 2020a).

For STC, despite the evidence of genotoxicity and carcinogenicity, only a limited tumorigenicity database was available for the dose-response assessment. A benchmark dose for a response with 10% additional risk (BMD₁₀) of 0.36 and a lower 95% confidence limit for a benchmark response with 10% additional risk (BMDL₁₀) of 0.16 mg STC/kg bw/day were calculated. A comparison of the BMD₁₀ of STC for the occurrence of hemangiosarcoma with that of AFB₁ for the occurrence of HCC indicates that the carcinogenic potency of STC is about three orders of magnitude lower than that of AFB₁. The EFSA CONTAM Panel concluded that the available data on the occurrence of STC are too limited to perform a reliable dietary and food exposure assessment for humans and animals (EFSA, 2013). For potentially genotoxic substances, EFSA recommends the use of a threshold of toxicological concern (TTC) value of $0.0025 \mu\text{g}/\text{kg}$ bw/day ($0.15 \mu\text{g}/\text{person}/\text{day}$) and EFSA has updated the term “genotoxic substances” to “potentially DNA-reactive mutagens and/or carcinogens” to reflect the importance of mode of action (EFSA, 2019).

The EFSA CONTAM Panel has updated its opinion on OTA from 2006. Although the mechanisms are not clear, OTA causes renal toxicity in various animal species and renal tumors in rodents, and it is genotoxic both in vitro and in vivo. A HBGV and a MoE approach were applied. For the MoE approach and characterization of non-neoplastic effects, a BMDL₁₀ of $4.73 \mu\text{g}/\text{kg}$ bw/day was used in calculation and for the characterization of neoplastic effects, a BMDL₁₀ of $14.5 \mu\text{g}/\text{kg}$ bw/day was used in calculation (EFSA, 2020b). The MoE was calculated as the quotient of the BMDL₁₀ values specified by the EFSA and the OTA exposure values determined in this study. A MoE of less than 10,000 indicates a potential health hazard if the genotoxicity is direct. However,

EFSA stated that the uncertainty in this assessment is high and the risk may be overestimated (EFSA, 2020b).

CIT is nephrotoxic and a NOAEL (no-observed-adverse-effect level) of $20 \mu\text{g}/\text{kg}$ bw/day was determined. Due to the limitations and uncertainties in the database, the derivation of a HBGV value was not considered appropriate, but a NOAEL for nephrotoxicity of $0.2 \mu\text{g}/\text{kg}$ bw/day was established. Based on the available data, a concern for genotoxicity and carcinogenicity could not be excluded at the safety level for nephrotoxicity (EFSA, 2012).

The IARC has not yet published its assessment of CPA, and due to insufficient data, the EFSA and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) have not yet carried out assessments of exposure and uptake of CPA in the organism (Ostry et al., 2018).

The EFSA CONTAM Panel concluded that the available occurrence data are too limited to make a reliable assessment of dietary exposure of humans and animals and that methods with a limit of quantification of less than $1.5 \mu\text{g}/\text{kg}$ should be used for food (EFSA, 2013; EFSA, 2019).

2.6. Statistical analysis

Descriptive statistic and exposure assessment was calculated using the program Microsoft® Excel® for Microsoft 365 MSO (version 2401 64-bit version 16.0.17231.20236).

3. Results and discussion

3.1. Food consumption data

Descriptive statistics on the average consumption of consumers in Croatia for the TMP groups, expressed in g/day, are presented in Table 1. Out of a total 2002 consumers, most of them reported consuming bacon/pancetta, followed by the dry-fermented sausages group, while the lowest number of consumers reported the *pečenica/ombolo/kare* group. The highest average consumption value was reported for the dry rack group ($40.81 \text{ g}/\text{day}$), followed by the sausage ($33.25 \text{ g}/\text{day}$) and *kulen* ($33.99 \text{ g}/\text{day}$) groups, while the lowest value was reported for the bacon/pancetta group ($20.77 \text{ g}/\text{day}$).

3.2. Occurrence of mycotoxins

Table 2 shows the distribution of samples with detected mycotoxin concentrations (above LOD) by TMP group. The results show that the concentrations of one of the analysed mycotoxins were above the LOD in a total of 29 % of the analysed samples, i.e. at least one of the analysed mycotoxins was detected in 84 out of 288 samples (CPA, OTA and STC). The mycotoxins AFB₁ and CIT were not detected in a single sample. The

Table 1

Descriptive statistics on consumer consumption in Croatia for groups of traditional meat products.

TMP group	Consumers number ^a (n = 2002)	Min g/day	Mean	Max	P95	SD
Dry-fermented sausages	423	0.03	33.25	230	100	34.65
<i>Kulen</i>	78	0.46	33.99	200	150	46.95
<i>Pečenica/ombolo/kare</i>	13	0.25	25.56	143.5	81.96	36.09
Prosciutto/Dry ham	155	0.04	27.59	200	94.19	32.83
Bacon/Pancetta	464	0.03	20.77	600	72	36.91
Dry rack	48	0.09	40.81	200	111	44.64

n = total number of consumers surveyed.

^a Number of consumers reporting the consumption of traditional meat products (TMP) group; Min = minimum, Max = maximum, P95 = 95 percentile, SD = standard deviation.

Table 2

Distribution of samples with detected mycotoxin concentrations by groups of traditional meat products.

TMP group	N	OTA		STC		CPA	
		N*	%	N*	%	N*	%
Dry-fermented sausages sausagesausages	129	23	18	7	5	20	16
<i>Kulen</i>	17	3	18	1	6	3	18
<i>Pečenica/ombolo/kare</i>	29	3	10	2	7	5	17
Prosciutto/Dry ham	41	1	2	0	0	6	15
Bacon/Pancetta	52	1	2	1	2	4	8
Dry rack	20	1	5	1	5	2	10
In total	288	32	11	12	4	40	14

N = total number of samples per traditional meat product (TMP) group; N* = number of samples with detected mycotoxin concentrations (>LOD); % = percentage of samples with detected mycotoxin concentrations.

total number of samples with detected concentrations > LOD was highest for CPA (14 %), then OTA (11 %) and lowest for STC (4 %). Looking at each group of meat products individually, it can be seen that no STC was detected in the ham/prosciutto group., The highest number of detected mycotoxins (CPA and OTA and STC) was found in the sausage group, but also the highest number of analysed samples. CPA was most frequently found in the *kulen* group (18 %), OTA in the sausage (18 %) and *kulen* (18 %) groups and STC in the *pečenica/ombolo/kare* group (7 %). The product types differ in their production technology and physico-chemical properties, which can influence the occurrence of mycotoxins. The effect of pH on the formation of certain mycotoxins still needs to be investigated for each species individually, but it is known that in most cases more acidic conditions promote the formation of mycotoxins. For example, a pH of 4.0 is suitable for the formation of AFB₁, and in this case the lower the pH, the greater the synthesis. A similar observation is made for OTA, whose concentration is much higher when *A. ochraceus* grows in lower pH ranges (Daou et al., 2021). Dry-fermented sausages with a higher occurrence of mycotoxins have a more acidic pH compared to other meat product groups (Kudumija et al., 2024). The higher frequency of sausages and *kulen* contamination found could also be due to the use of contaminated spices, such as red pepper and garlic, in the filling of these product types compared to other products. For example, Pleadin, Kovačević, and Persi (2015) determined OTA concentrations of up to 8.11 µg/kg in red pepper as spice used in the production of Slavonian kulen, while OTA could not be detected in raw meat.

Table 3 shows the average and P95 values of mycotoxin contamination depending on the TMP group for all three scenarios: UB, MB and LB. The highest average concentrations for OTA were found in the dry rack group (0.133–0.304 µg/kg), for the STC group of sausages and *kulen* with the same values (0.039–0.058 µg/kg) and for the CPA group *pečenica/ombolo/kare* (5.214–7.010 µg/kg). There are no previous studies on STC concentrations in different meat products, while other studies conducted so far on the occurrence of OTA and CPA in traditional meat products reported the maximum concentrations of OTA and especially CPA in cured meat products in comparison to dry-fermented sausages (Peromingo et al., 2018; Perrone et al., 2019). This could be due to the longer maturation process, especially in prosciuttos/hams, or to the presence of casings in dry-fermented sausages, which may act as a protective system of the sausage to prevent the mycotoxin from migrating into the filling (Roncada et al., 2020). In this study, as well as in the study by Vulić et al. (2016), the highest consumption and occurrence of OTA was reported in home-made dry-fermented sausages, with the maximum OTA concentration found in dry-cured ham (4.81 µg/kg and 9.95 µg/kg, respectively). The highest concentrations of CPA among detected mycotoxins could be a consequence of accumulation in meat products during storage, as it has been shown to be stable compared to OTA and CIT (Bailey et al., 2005).

Table 3

Results of the average and 95 percentile values of mycotoxin contamination depending on the group of traditional meat products.

TMP group	Ochratoxin A (OTA)			Sterigmatocystin (STC)			Cyclopiiazonic acid (CPA)								
	Mean (µg/kg)			Mean (µg/kg)			Mean (µg/kg)								
	UB	MB	LB	UB	MB	LB	UB	MB	LB						
Dry-fermented sausages	0.267	0.193	0.119	0.664	0.058	0.048	0.039	0.068	0.060	6.796	5.879	4.962	22.69	22.69	22.69
<i>Kulen</i>	0.274	0.200	0.126	0.880	0.058	0.048	0.039	0.148	0.132	3.940	3.046	2.153	14.17	14.17	14.17
<i>Pečenica/ombolo/kare</i>	0.201	0.117	0.033	0.198	0.029	0.019	0.010	0.086	0.078	7.010	6.112	5.214	37.37	37.37	37.37
Prosciutto/Dry ham	0.293	0.205	0.117	0	0.020	0.010	0.000	0.020	0	6.149	5.223	4.296	20.90	20.90	20.90
Bacon/Pancetta	0.190	0.101	0.013	0	0.029	0.019	0.009	0.020	0	2.639	1.637	0.636	4.980	4.980	4.980
Dry rack	0.304	0.219	0.133	0.133	0.025	0.016	0.006	0.025	0.016	2.596	1.619	0.643	6.130	6.130	6.130

UB UP = upper bound; MB = middle bound; LB = lower bound; LOD = limit of detection; OTA LOD = 0.18 µg/kg, STC LOD = 0.02 µg/kg, CPA LOD = 2.17 µg/kg; TMP = traditional meat product; P95 = 95 percentile.

3.3. Exposure assessment

No exposure assessment was performed for AFs (B₁, B₂, G₁ and G₂) and CIT, as these mycotoxins were not detected in any TMP sample, so it can be concluded that exposure to these mycotoxins from TMP is negligible.

The results of a study on the risk assessment of dietary exposure to AFB₁ in neighboring Serbia indicate an increased health risk for all population groups, especially children and adolescents, with corn-based products accounting for the largest share of total exposure (Udovicki et al., 2021). Other food categories included in the study were peanuts and other nuts, rice and dried ground red bell pepper, but no meat or meat products were included. The main contributors to the intake of mycotoxins depend on both the dietary habits population of the respective country and the occurrence during the study period (Udovicki et al., 2021).

Studies from other countries, e.g. in Catalonia, Spain, reported peanuts as the main cause of AFs intake, in Brazil rice, in New Zealand spices and nuts, in Cameroon maize and cassava but again no meat or meat products were included in the study although authors stated that cow and beef meat, poultry meat, pork meat, fish and eggs are most frequently consumed food of animal origin (Andrade & Caldas, 2015; Bouelet Ntsama et al., 2023; Cano-Sancho et al., 2013; Cressey & Reeve, 2013). The mean exposures of adults and children to AFs determined in the aforementioned studies were: 0.09–0.11 ng/kg bw/day and 0.32–0.39 ng/kg bw/day in New Zealand; 0.39–0.76 ng/kg bw/day and 0.79–1.66 ng/kg bw/day in Serbia; 0.072–0.098 ng/kg bw/day and 0.033–0.105 ng/kg bw/day in Spain, respectively (Cano-Sancho et al., 2013; Cressey & Reeve, 2013; Udovicki et al., 2021). One study investigated the health risks associated with AFs in meat, i.e., processed poultry and beef products sold in Riyadh, Kingdom of Saudi Arabia, e.g. meatballs, hot dogs, burgers, grilled chicken and minced meat. Processed meat products showed significant AFs contamination, with 38% of samples contaminated with AFB₁, AFG₁ or AFB₂, while AFG₂ was not detected in any sample. The maximum exposure limit (MOE) for genotoxic and carcinogenic substances was reported to be 175 and 311 for total AFs in processed beef and poultry meat products, respectively (Elzupir & Abdulkhair, 2020).

To date, there is no data on the occurrence of CIT and consequently no risk assessment for CIT and for CIT and OTA in combination due to their possible co-occurrence in different foods (Meerpoel et al., 2021). The EFSA CONTAM Panel concluded that the combined effects of CIT and OTA are at most additives (EFSA, 2012). In this study, no CIT was detected in pork products, but in fresh Belgian chicken meat, CIT was detected above the LOQ in 7 samples and when included in the exposure assessment, levels between 0.082 ng/kg bw/day and 0.849 ng/kg bw/day were found for high consumers, but no health risk was found for CIT in Belgian food in general (Meerpoel et al., 2021). Another study on CIT exposure in Germany using urine biomarkers showed a mean exposure of adults of 0.013 µg/kg bw/day and a mean exposure of children of 0.05 µg/kg bw/day, which suggests a non-negligible CIT exposure, although the source of CIT exposure in food must be identified (Degen et al., 2023). The exposure assessment for the detected mycotoxins OTA, STC and CPA in TMP for all three scenarios is shown in Table 4.

EFSA (2013) reported on the genotoxicity and carcinogenicity of STC in its scientific opinion on STC in food, but as no exposure data were available, the MoE approach for genotoxic and carcinogenic substances could not be applied to STC, and thus the characterization of risk to human health could not be performed. Nevertheless, for potential DNA-reactive mutagens and/or carcinogens, which previously were named potentially genotoxic substances TTC value of 2.5 ng/kg bw/day is used (EFSA, 2019). The results of this study showed significantly lower average values (0.011–0.020 ng/kg bw/day) compared to the value given by the EFSA. The maximum assessed exposure to STC was 0.29 ng/kg bw/day, 8 times lower than the TTC value. As far as we

Table 4

Exposure assessment for detected mycotoxins in traditional meat products.

Mycotoxin	Scenario	Min	Mean	Max	P95	SD
		ng/kg bw/day				
OTA	LB	0.000004	0.043	0.502	0.180	0.063
	MB	0.000028	0.082	1.001	0.290	0.110
	UB	0.000052	0.121	1.701	0.410	0.160
STC	LB	0.000000	0.011	0.122	0.046	0.017
	MB	0.000005	0.016	0.201	0.060	0.022
	UB	0.000008	0.020	0.292	0.074	0.028
CPA	LB	0.000018	1.520	15.832	5.957	2.123
	MB	0.000450	1.975	20.040	7.474	2.627
	UB	0.000730	2.429	28.371	8.905	3.164

OTA Ochratoxin A; STC Sterigmatocystin; CPA Cyclopiiazonic acid; UB = upper bound; MB = middle bound; LB = lower bound; Min = minimum, Max = maximum, P95 = 95 percentile, SD = standard deviation.

know, there are only risk assessment studies for STC in Korea and Japan, in which the exposure to STC from the average consumption of food (agricultural products and processed food) was estimated to be 0.09 ng/kg bw/day for the Korean population. It was concluded that STC exposure from consumption of food distributed in Korea is unlikely to cause human health problems (Kang et al., 2022). In a study of Japanese staple foods, including rice and wheat, AFLs were not detected in all samples, while STCs were detected in 22.4% of the samples analysed. In their study, dietary exposure to STC ranged from 0.11 to 0.67 ng/kg bw/day, values that were 10–30 times higher than in our study because more different foods were included, but based on the MOE approach, STC in staple foods posed a low risk to the Japanese population (Yoshinari et al., 2024).

To date, EFSA has not issued a scientific opinion on the consumer risk associated with CPA in food, nor is there any information on CPA listed by IARC, as there are no data on their carcinogenicity, i.e. on the toxicity of CPA in general. De Waal (2002) proposed a tolerable daily intake (TDI) of 0.1 µg/kg bw/day CPA, based on a no observed effect level (NOEL) of 0.1 mg/kg bw/day derived from the 90-day study in dogs. Overall, Ostry et al. (2018) estimate that the data from relevant sub-chronic studies on CPA in experimental animals are insufficient to determine a TDI. The average exposure to CPA in this study was 1.52–2.42 ng/kg bw/day with a maximum exposure of 28.37 ng/kg bw/day. The French Agency for Food, Environmental and Occupational Health and Safety (ANSES) reported in 2006 on the risk assessment of mycotoxins in the human and animal food chain and concluded that it is unlikely that CPA poses a major health risk to humans and that the risk of exposure arises mainly from the consumption of contaminated cereals (ANSES, 2006). Furthermore, its presence as a co-contaminant with AFB₁ in North America is not thought to have adverse consequences, as it is thought to reduce the risk of AFL by contributing to its metabolic inhibition. On the other hand, Ostry et al. (2018) reported that the effects of the combination of AFL and CPA in toxicology studies in chickens were additive in most cases. Undoubtedly, the results of chronic toxicity and carcinogenicity studies and current data on the occurrence of CPA in food are needed for the evaluation of toxicity and the estimation of human dietary exposure and health risk.

The contribution of each individual TMP group to mycotoxin exposure from this study is expressed as a percentage (contribution) of the total intake and is shown in Fig. 1. For all three mycotoxins, the TMP group that contributes the most was group of sausages with 46–58%, 59–73% and 58–68%, for OTA, STC and CPA, respectively. This result was to be expected because the second largest number of consumers reported the consumption sausage (more reported only bacon), and the average daily intake of sausage is significantly higher than that of bacon (33.25 g and 20.77 g, respectively; Table 1). The sausage group had the highest number of samples analysed, the highest average STC concentration and the second highest CPA concentration (Table 3), with the highest number of detected concentrations above the LOD for OTA

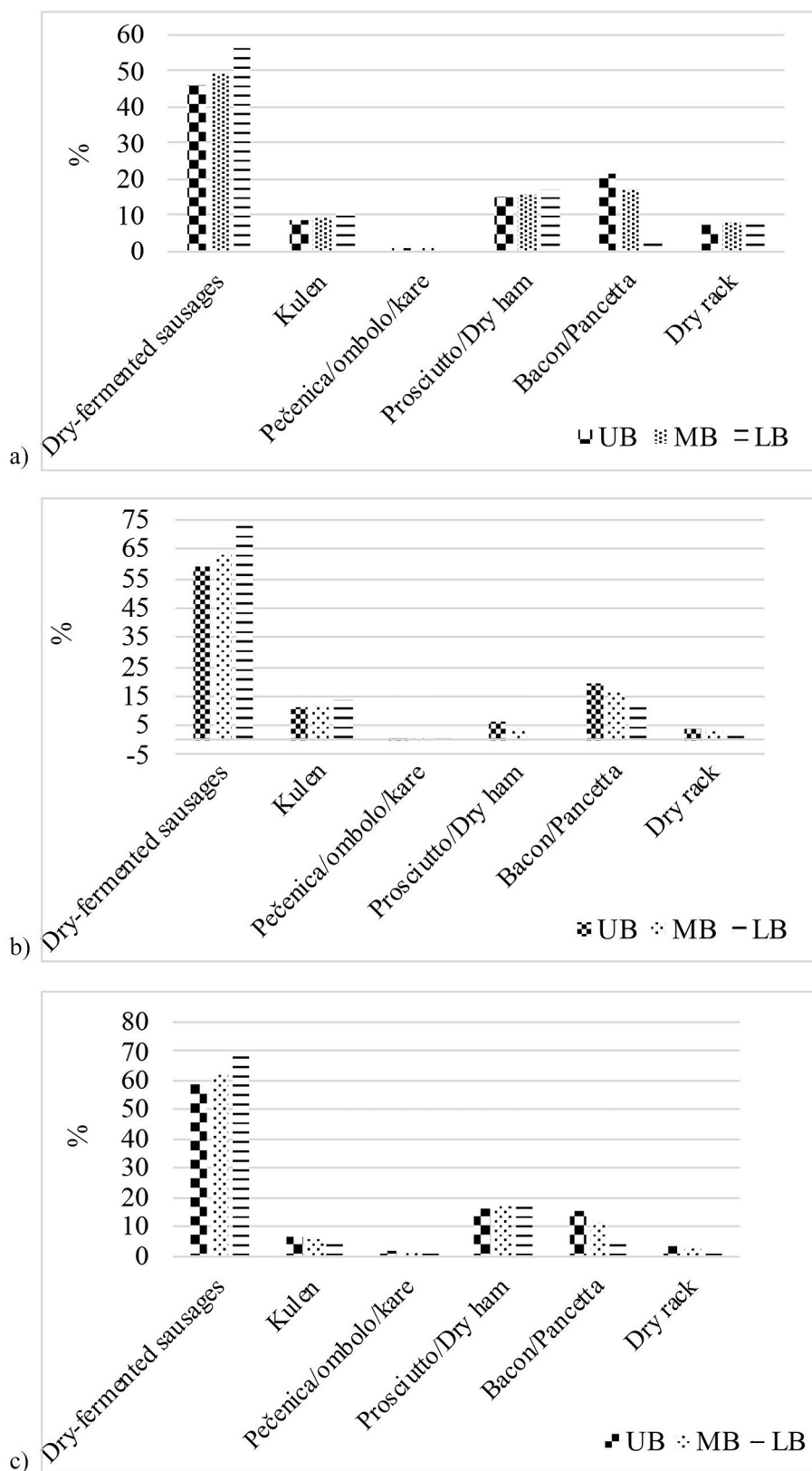


Fig. 1. The relative contribution of traditional meat product groups to mycotoxin contamination: a) Ochratoxin A b) Sterigmatocystin c) Cyclopiazonic acid
 UB = upper bound; MB = middle bound; LB = lower bound.

(Table 2).

In a European assessment of the contribution of individual foods to the average total intake of OTA, cereals and products derived from them were considered the main source of human exposure to OTA, accounting for half of all contributions (50%) (Duarte et al., 2010). This was also confirmed in individual Dutch and Croatian studies (Bakker & Pieters, 2002, HAPIH, 2023). In the Netherlands, the contribution of meat was in third place (8%) after coffee (9%) (Bakker & Pieters, 2002), while in Croatia alcoholic beverages were in second place after cereals and meat products in fifth place (3%) (HAPIH, 2023). A serious drawback in estimating the exposure and risk of mycotoxins in food is the small amount of available data on food contamination and the fact that the available data comes from different analytical methods.

EFSA gave a TWI value of 120 ng/kg bw in its scientific opinion on OTA (EFSA, 2006) and in this study no consumer was exposed to OTA concentrations above the TWI value (Table 5). In 2020, EFSA presented a new risk assessment opinion for OTA in food, in which it considered its carcinogenicity and genotoxicity using the MoE approach and set a BMDL₁₀ of 4.73 µg/kg bw/day as the toxicological reference point (RP) for non-neoplastic effects and a BMDL₁₀ of 14.5 µg/kg bw/day for neoplastic effects (EFSA, 2020b). The interpretation of the results for OTA via the MoE portal is shown in Table 5. For the UB scenario only, a health concern for neoplastic effects was identified in 0.12% of consumers, while a potential health concern for non-neoplastic effects was identified in 4.15% of consumers.

When interpreting these results, the aforementioned uncertainty must be taken into account, which is related to the lower number of samples with detected concentrations above the LOD, but also to the fact that consumers are also exposed to OTA through other foods in addition to TMP. So far, there are two studies in the Croatian population conducted by the same group of authors Vulić et al. dealing with human exposure to OTA from dry-cured meat products. Vulić et al. (2014) reported a low probability of human exposure to OTA concentrations above the TWI and estimated that only when eating pancetta, 0.8% of the population is exposed to OTA above the TWI. Vulić et al. (2016) showed that the weekly OTA intake of 90% of male consumers of raw ham was a maximum of 51.9 ng/kg bw, well below the TWI value, while the OTA intake from the consumption of dry-fermented sausages and the other products studied was even lower (0.1–42.1 ng/kg bw/week) suggesting that the health risk from OTA from the consumption of dry-cured meat products is negligible. The mean exposure to OTA in this study was even lower and was in the range of 0.043–0.121 ng/kg bw/day with a maximum value of 1.701 ng/kg bw/day. The results show that consumers in Croatia are nevertheless frequently exposed to OTA, albeit not at levels associated with a health risk.

Miličević et al. (2012) estimated the daily intake of OTA from pork meat in a 60 kg adult in neighboring Serbia to be 0.46–0.90 ng/kg bw, which is below the levels that pose a health risk to consumers, but even 10 times higher than the exposure levels in this study. The reason for this could be that the pork samples were liver, blood and kidney, where OTA

is normally found in higher concentrations than in muscle tissue of traditional products (Persi et al., 2014). The EDI of OTA by chicken gizzard was lower, 0.28–0.32 ng/kg bw/day, but still up to 6 times higher than the EDI of TMP in this study. When results from other studies are used for comparison, most of them consider dietary exposure to different foods and this study only considers TMP, which is the reason for the lower values in this case (Bakker & Pieters, 2002; Duarte et al., 2010). In the Netherlands, the calculated OTA intake was 1.0 ng/kg bw/day, which is 10 times higher than the values of our study, but leads to the conclusion that dietary intake of OTA does not pose a health risk, as the study included meat as one of the foods (Bakker & Pieters, 2002). In Portugal, the EDI was 0.19–3.35 ng/kg bw/day, and the one related to pork only was 0.018 ng/kg bw/day, 2–6 times lower than the values in this study for TMP made out of pork. The high consumption of pork in Portugal compared to poultry meat was compensated by the low OTA contamination levels (Duarte et al., 2010). A study conducted in Italy on cocoa and chocolate products, for example, found the highest weekly intake for children to be 0.10 ng/kg bw/day, with the conclusion for risk assessment that OTA exposure is not of particular concern; there, 60% of the samples tested were contaminated with OTA, compared to only 11% in this study (Brera et al., 2011). In a study by Meerpoel et al. (2021) for the Belgian population, a mean EDI for OTA of between 0.017 ng/kg bw/day in flour and 0.429 ng/kg bw/day in rice was determined for individual foods, whereby meat products were not included in the estimate. In the US, oat-based cereal products were the largest contributor to OTA exposure, followed by pork, and the mean consumer OTA exposure was highest in children aged 1–5 years at 0.16 ng/kg bw/day, while in adult consumers it was 0.07 ng/kg bw/day, in the middle of the range of values in our study, although pork consumption increases with age (Mitchell et al., 2017). However, as EFSA proposes a different approach in its scientific opinion on the risk assessment of OTA in food from 2020, the results of this study are presented differently and cannot be compared with the data presented in the manner used until then. The study by Meerpoel et al. (2021) followed the same approach as this study, in which several MoE values were below 10,000 in the maximum estimates, suggesting a potential health risk for neoplastic effects in various foods such as biscuits, rice, flour, spices, etc., but no meat products were analysed.

4. Conclusions

AFs and CIT weren't detected in any sample, so the exposure to these mycotoxins in TMP can be considered negligible. The determined exposure to STC was far below the exposure of low health concern set by the EFSA, while for CPA there is still no health-based guideline value to which the results can be compared for exposure and risk assessment. Although OTA was detected in a relatively small number of samples, it contributed to the exposure of TMP consumers. According to the MoE approach for OTA, a concern was identified in 0.12% of consumers for neoplastic effects in the case of the UB scenario, while a potential health risk was identified in 4.15% of consumers for non-neoplastic effects. Among the TMP categories, dry-fermented sausages contributed the most to consumer exposure for all three mycotoxins detected, which was to be expected as dry-fermented sausages were one of the most frequently reported TMP groups by consumers. Low levels of the detected mycotoxins were found in the study, but this type of food is not necessarily the only source of these mycotoxins in the diet, and their occurrence depends on various factors such as weather conditions, which is why these mycotoxins should be continuously monitored so that maximum levels can be set in order to protect consumer health.

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Table 5
Number of consumers exposed to ochratoxin A above toxicological value.

Scenario	^a TWI = 120 ng/kg bw		^b Neoplastic effect BMDL ₁₀ = 14.5 µg/kg bw/day		^b Non neoplastic effect BMDL ₁₀ = 4.73 µg/kg bw/day	
	N ^c	% ^c	N ^d	% ^d	N ^d	% ^d
LB	0	0	0	0	1	0.12
MB	0	0	0	0	14	1.61
UB	0	0	1	0.12	36	4.15

UB = upper bound; MB = middle bound; LB = lower bound.

^a EFSA 2006.

^b EFSA 2020.

^c Number and percentage of examinee above toxicological value.

^d Number and percentage of examinee in relation to health risk.

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CRedit authorship contribution statement

Tina Lešić: Writing – review & editing, Writing – original draft, Validation, Investigation, Formal analysis, Data curation, Conceptualization. **Brigita Hengl:** Software, Methodology, Data curation. **Ana Vulić:** Validation, Methodology, Investigation. **Nada Vahčić:** Resources. **Nina Kudumija:** Validation, Investigation, Formal analysis. **Ivica Kos:** Resources. **Dragan Milićević:** Supervision. **Jelka Pleadin:** Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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