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Risk ranking of mycotoxins in plant-based meat and dairy alternatives under protein transition scenarios

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ABSTRACT

While reducing the consumption of animal-source foods is recommended for planetary and human health, potential emerging food safety risks associated with the transition to dietary patterns featuring plant-based meat (PBMA) and dairy alternatives (PBDA) remain unexplored. We assessed the exposure to mycotoxins and ranked the associated health risks related to the consumption of PBMA and PBDA. We simulated diets by replacing animal-source proteins with their plant-based alternatives. A risk ratio method, based on the hazard quotient (HQ), was applied to rank mycotoxin-related food safety risks. An aggregated dataset containing contamination data of 45 mycotoxins distributed over 182 PBDA and 131 PBMA samples, collected and analyzed in Europe, representing a convenience sample, was used as input in our risk assessment. The highest risk mycotoxins identified for PBMA in the lower bound scenario (LB) were the sum of aflatoxins (sum AF), alternariol monomethyl ether (AME), aflatoxin B1 (AFB1) and alternariol (AOH), with HQs for children of 121.3, 66.8, 24.0 and 4.3, respectively. In the milk substitution model (LB), HQs of 4.9 (sum AF) and 1.4 (AFB1) were calculated for children, indicating a potential food safety risk. Further analysis demonstrated that soy-based meat alternatives are the highest risk food products, in particular for sum AF, AME, AOH and AFB1. With regard to PBDA, highest HQs were found for sum AF in almond and oat drink, and AFB1 in oat drink. This research presents an approach to overcome the lack of data in the assessment of emerging risks associated with the shift to more sustainable dietary patterns.

1. Introduction

Public health recommendations increasingly consider the replacement of animal protein with plant-based sources in the transition to more sustainable food systems (Duluins & Baret, 2024; Willett et al., 2019). The global food system has been identified as a major driver of climate change and environmental degradation, including biodiversity loss and depletion of natural resources, which in large part can be attributed to the (over)production of animal proteins (Poore & Nemecek, 2018; Springmann et al., 2018). In the past decades, a global shift towards unhealthy diets, characterized by higher caloric intakes and increased consumption of heavily-processed and animal-source foods, has been associated with an increased incidence of diet-related non-communicable diseases and overall poor health (Tilman & Clark, 2014; Willett et al., 2019). The consumption of red and processed meat in particular has been associated with an increased risk of chronic diseases, such as colorectal cancer, type 2 diabetes mellitus and cardiovascular disease (Bradbury et al., 2020; Richi et al., 2015; Schwingshackl et al., 2017; Zhong et al., 2020).

Food-based dietary guidelines are now increasingly emphasizing on the replacement of dietary proteins (Dooren et al., 2024; Duluins & Baret, 2024). For instance, the recently updated German food-based dietary guidelines suggest a diet that comprises more than 75% plant-based foods, supplemented with limited foods of animal origin, echoing the EAT-Lancet Commission's planetary health diet (DGE, 2024). These concerns have also contributed to the increased interest of consumers towards plant-based products and diets, and the development of plant-based food products (Aschemann-Witzel et al., 2020).

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Plant-based meat alternatives (PBMA) are composed of functionalized and reassembled proteins extracted from plant-based protein sources, e.g. cereal grains, next to other plant-derived macro- and micronutrients. The most commonly used plant protein sources include legumes (e.g. soybeans, peas, beans and chickpeas), grains (e.g. wheat), pseudocereals (e.g. quinoa), and nuts and seeds (e.g. cashew nuts, flaxseed, chia seeds and sunflower seeds) (Langyan et al., 2022; McClements et al., 2019; Sha & Xiong, 2020; Toh et al., 2022). Plant-based dairy alternatives (PBDA), on the other hand, are complex colloidal dispersions of small particles in aqueous media, usually derived from soybeans, almonds, oats, rice or coconut (McClements et al., 2019; Paul et al., 2020).

In the past years, there has been a significant expansion of the European plant-based food sector, with a growth of 49% from 2018 to 2020 and an increase in sales values from €2.4 billion to €3.6 billion (SmartProtein, 2020; EUVEPRO, 2019). Consumers have expressed their willingness to reduce their meat consumption, which is signified by the number of consumers identifying themselves as flexitarian (or semi-vegetarian), i.e. a loosely defined term referring to predominantly vegetarian diets that include moderate amounts of meat, fish, eggs, and dairy (Herpich et al., 2022; HGR, 2021; WHO, 2021). For instance, in a monitoring study of the protein intake in Flanders, 43.4% of Flemish respondents were identified as flexitarian (i.e. they don't consume meat or fish at least one day a week), while only 1.3% and 0.4% were categorized as vegetarian and vegan, respectively (Rubens et al., 2024).

Emerging food safety risks may arise as a result of changing dietary patterns, and subsequent exposure to certain food safety hazards (EFSA, 2024b). In the transition to dietary patterns that feature PBMA and PBDA, food safety implications have remained mostly unexplored. As the raw materials from which these food products are derived are susceptible to contamination with natural toxins, in particular mycotoxins, it can be expected that the exposure to these chemical hazards will change significantly in the transition to more plant-based diets (Banach et al., 2022; FAO, 2022; Fleury et al., 2017; Penczynski et al., 2022; Wells et al., 2017). Mycotoxins are toxic fungal secondary metabolites that commonly occur in food commodities, such as cereal grains, nuts, spices, and dried fruits, and cause adverse health effects in humans and animals. Mycotoxin-producing fungi generally belong to the *Aspergillus, Alternaria, Fusarium* and *Penicillium* genera (EFSA, 2024b; WHO & FAO, 2018). Numerous mycotoxins have been identified to date, of which aflatoxins, especially aflatoxin B1 (AFB1), ochratoxins (e.g. ochratoxin A, OTA), trichothecenes (e.g. deoxynivalenol, DON), fumonisins (e.g. fumonisin B1, FB1), zearalenone (ZEN) and patulin (PAT) are the most frequently observed and pathologically-significant. They exhibit a wide spectrum of toxic effects including cytotoxicity, genotoxicity, immune system modulation, hepatotoxicity and carcinogenicity (Peraica et al., 1999; Ueno, 1985; WHO & FAO, 2018).

In a risk–benefit study, Mihalache et al. (2022) evaluated the mycotoxin-related health impacts of switching from current (meat-based) diets to an alternative soy-based scenario. They found that, in a pessimistic scenario, cancer risks due to AFB1 and OTA exposure could outweigh the elimination of the colorectal cancer risk factor of processed meat, i.e. a net health loss, emphasizing the potential significance of mycotoxin-related exposures in soy-based meat alternatives. In a biomonitoring study, Penczynski et al. (2022) found significantly higher serum levels of OTA in vegans than in omnivores, and found that this was moderately correlated to the consumption of 'vegan products' and 'pasta and rice'. While mycotoxins can be present in animal-derived food products mostly through the carry-over from contaminated feed to animal tissue and other by-products, the prevalence and concentration levels are generally lower than in plant-derived foods (EFSA, 2024a). Aflatoxin M1 in milk and dairy products and OTA in meat by-products have been identified as food – mycotoxin combinations of highest concern in relation to foods of animal origin (Tolosa et al., 2021). Overall, the evidence regarding mycotoxin exposure due to the consumption of processed plant-based products, such as meat and dairy alternatives, remains scattered and inconclusive, with an overall lack of understanding of which mycotoxins should be prioritized by risk managers in the transition to more sustainable dietary patterns. As such, the objective of the current study was threefold:

(1) To compose an aggregated dataset containing mycotoxin levels in PBMA and PBDA, based on a literature review, supplemented by research projects, to overcome the issue of fragmented and limited occurrence data; (2) To assess the exposure to mycotoxins using a substitution modeling approach, to deal with the lack of up-to-date consumption data in the protein transition, by modeling the replacement of animal-source proteins with PBMA and PBDA; (3) To rank the associated health risks, considering the simulated exposure and toxicity of mycotoxins, to evaluate potential emerging risks. In general, an approach to overcome the lack of data in the assessment of emerging food safety risks is presented, and demonstrated for the case of mycotoxins in plant-based meat and milk alternatives.

2. Materials & methods

2.1. Mycotoxin concentration data from scientific literature

A literature review was performed to obtain analytical concentration data on both regulated and non-regulated mycotoxins in processed plant-based/vegetarian products, including PBMAs and PBDA from scientific literature. Three databases (Web of Science, Scopus and PubMed) were searched using the following search string: ("soy*" OR "s*food" OR "seitan" OR "tempeh" OR "plant-based" OR "meat analogue" OR "vegetarian" OR "patty" OR "burger") AND ("mycotoxin*" OR "aflatoxin*" OR "total aflatoxin*" OR "deoxynivalenol" OR "nivalenol" OR "ochratoxin A" OR {OTA} OR "zearalenone" OR "fumonisin*" OR "citrinin" OR "alternariol" OR "alternariol monomethyl ether" OR "tentoxin" OR "tenuazonic acid" OR "trichothecene*" OR "enniatin*" OR "emerging mycotoxin*" OR "ergot" OR "Alternaria toxin*" OR "beauvericin" OR "moniliformin" OR "diacetoxyscirpenol" OR "sterigmatocystin" OR "neosolaniol" OR "fusarenon X") AND ("contamin*" OR "occurr*" OR "co-occurr*" OR "preval*" OR "concentration*" OR {risk assessment} OR "chemical hazard" OR "dietary exposure") in titles, abstracts and keywords in the timeframe of January 2010 – December 2023. The search was performed in December 2023.

Only full-text downloadable articles written in English, published in peer-reviewed journals were retained. In addition to this, the analytical method, including limit of detection (LOD) or limit of quantification (LOQ), must be provided, and mycotoxins must have been analyzed in plant-based meat alternative samples (also referred to as meat substitutes, analogues or imitates) or plant-based dairy alternative samples (also 'milks', drinks or beverages) obtained from the EU market. The review process was performed with the aid of the Rayyan software (Rayyan, 2021).

From the included publications the type of product (PBMA or PBDA), the protein source (if mentioned), LOD and/or LOQ (μg/kg), the analytical method and the mycotoxin concentration (μg/kg) was recorded and included in a dataset setup in Microsoft Excel. For the plant-based dairy alternatives (drinks) a density of 1 kg/L was assumed, meaning that data expressed in μg/L were recorded in μg/kg in the dataset, and used as such in the exposure calculations. If only a mean concentration was provided, a weighted average was calculated based on the number of samples analyzed and the number of positive samples, and included as such in the dataset.

2.2. Combining mycotoxin occurrence data in plant-based meat and dairy alternatives into an aggregated dataset

In addition to the data from scientific literature, concentration data of multiple mycotoxins, analyzed with an in-house validated method, in 53 processed plant-based meat alternative products sampled from the Belgian market in the period of March 2022 – August 2023, were obtained. Data were collected in the context of a Belgian research project

(Pavicich et al., 2024c). Furthermore, we obtained mycotoxin level data in PBMA products (n = 17) from the Belgian consumer organization (Testaankoop, 2024). Samples were collected from the Belgian market in 2023 and analyzed by an ISO17025 accredited lab. In the dataset, the mycotoxin concentrations, the unit, the LOD and/or LOQ, the product type (PBMA or PBDA), the protein source (if reported) and number of samples, in the case of pooled samples, were recorded. Based on this final dataset, summary statistics of mycotoxins in PBMA and PBDA were calculated in R version 4.3.2 (R Core Team, 2023).

2.3. Consumption data for the development of a substitution model

Due to the lack of accurate and up-to-date consumption data of PBMA and PBDA in Europe, it was decided to work with two substitution models, based on the replacement of meat or milk consumption in the diet. Belgian national representative consumption data from 2014 were retrieved from the Comprehensive European Food Consumption Database (EFSA, 2022). The median and 95th percentile of the 'consumers only' category of the 'Meat and Meat Products' (Level 1) and 'Milk' (Level 3) food categories in kg/kg body weight per day for chronic consumption were exported to Microsoft Excel. Consumption data covered three age groups, namely children (3–9 years), adolescents (10–17 years) and adults (18–64 years).

In addition to this, the consumption data obtained by Clarys et al. (2014) were used to cross-check our exposure assessment outcomes. In this study, a validated Food Frequency Questionnaire (FFQ) was used for the collection of consumption data from an adult population in Belgium for the evaluation and comparison of the nutritional quality of different plant-based diets, i.e. vegetarian, pescatarian, vegan and semi-vegetarian diets. The P50 and P95 of the consumption of the 'meat replacers (tofu, Quorn)' and 'grain- and soymilk' food groups were derived in kg/kg body weight/day for both the vegan and vegetarian diet.

2.4. Exposure calculations

The exposure calculations were performed in R. The exposure was calculated for both a (modified) lower bound and upper bound scenario. In the former, the concentrations below the LOD were set equal to zero, while concentrations below the LOQ were set equal to the LOD, while in the latter case concentrations below the LOD were adjusted to the LOD and concentrations below the LOQ were adapted to the LOQ. Where relevant, i.e. in the case of group health-based guidance values (HBGVs) for a family of mycotoxins, the summed mycotoxin concentration was calculated from the individual mycotoxin concentrations in a sample. This was the case for aflatoxins (sum AFB1, AFB2, AFG1, AFG2) (EFSA, 2020), enniatins (sum ENNA, ENNA1, ENNB, ENNB1) (EFSA, 2014), HT-2 and T-2 toxin (EFSA, 2017a), deoxynivalenol and modified forms (sum DON, 3-Ac-DON, 15-Ac-DON and DON-3-glucoside, when analyzed) (EFSA, 2017b), fumonisins (sum FB1, FB2, FB3 and FB4) (EFSA, 2018) and ergot alkaloids (sum of twelve) (EFSA, 2012). The exposure to the individual and grouped mycotoxins was based on the mean concentration (Eq. (1)).

$$Exposure = consumption\left(\frac{kg}{kg\ \ bw^{*}day}\right)^{*}average\ \ mycotoxin\ \ concentration\ \ \left(\frac{\mu g}{kg}\right) \quad (1)$$

The chronic exposure to the different mycotoxins was obtained by multiplying the mean mycotoxin concentrations for the two food categories, i.e. meat alternatives and dairy alternatives, with the 50th and 95th percentile of the chronic consumption of the 'Meat and Meat Products' and 'Milk', respectively, resembling the total substitution of some important animal-source proteins in Belgium for the median and high consumers.

2.5. Confidence intervals

In order to assess uncertainty in the sampling process of the mycotoxin concentrations, a nonparametric bootstrap with 10,000 replicates was used to generate a 95% confidence interval (CI) around the mean mycotoxin concentrations, using the boot and boot.ci function from the 'boot' package (Canty & Ripley, 1999). In the case of weighted average concentrations, a weighted bootstrap was performed. Since only point estimates were available for the consumption no bootstrap or similar method could be employed here. CIs were propagated to the final risk estimates, which thus only represent the sampling uncertainty in the concentration data, not consumption data.

2.6. Risk ranking

A risk ratio method, as described by Van Asselt et al. (2024a) was used for the risk ranking of mycotoxins for the different age groups and scenarios. To this end, a hazard quotient (HQ) was calculated from the exposure and the HBGV or reference point for health concern (RPHC) (Eq. (2)):

$$HQ = \frac{Exposure\ \ (\mu g/kg\ \ bw/day)}{HBGV\ \ or\ \ RPHC\ \ per\ \ contaminant\ \ (\mu g/kg\ \ bw/day)} \quad (2)$$

A hazard quotient exceeding 1 points towards a potential food safety risk. HBGVs and other reference values were obtained from the respective EFSA reports. When metabolites or modified forms of mycotoxins were analyzed, they were considered in the HQ calculation, if recommended in the EFSA report. For instance, EFSA (2017a) concluded that the phase I metabolites of HT-2 and T-2 toxin should be included in a group TDI. Neosolaniol (NEO) and T2-triol were included in the sum of HT-2 and T-2 after applying the relative potency factors of 0.3 for NEO and 0.1 for T2-triol. In addition, a relative potency factor of 1.5 was assigned to zearalanone (ZAN), and as such included in the exposure estimate and HQ calculation of ZEN and modified forms (EFSA, 2016a). When a TDI was unavailable, a RPHC was calculated based on the BMDL10 and a margin of exposure (MOE) (Hobé et al., 2023). An overview of the toxicological reference values used in the risk characterization is given in supplementary materials (Table S1). At this point, mycotoxins that lack toxicological reference values, i.e. roquefortine C, fusarenon X, mycophenolic acid, gliotoxin, circumdatin A, hydrolysed-FB1 and phomopsin A, were excluded for further analyses.

2.7. Level of substitution for safe consumption

The level of substitution of the current 'Meat and Meat Products' or

Table 1
Overview of publications and projects reporting mycotoxin levels in plant-based meat and dairy alternatives identified during the systematic review process from which data were extracted.

Mycotoxin	Samples (n)	Product category	Analytical method	Sensitivity of method	Publication
Scientific literature					
PHO A	Lupin-based vegetarian snack (1)	PBMA	LC-MS/MS	LOD: 1 μg/kg LOQ: 5 μg/kg	de Nijs et al. (2013)
AFB1, AFB2, AFG1, AFG2, FB1, FB2, DON, OTA, HT-2, T-2 and ZEN	Rice drink (3), soy drink (3), oat drink (3)	PBDA	LC-MS/MS	LOD: 0.001–0.25 μg/L LOQ: 0.003–0.9 μg/L	Miró-Abella et al. (2017)
FB1, FB2, DON, HT-2, T-2, ZEN and F-X	Rice drink (5), soy drink (8), oat drink (8)	PBDA	LC-MS/MS	LOD: 1.0–17.3 μg/L LOQ: 3.2–57.7 μg/L	Hamed et al. (2017)
CIT and OTA	Soybean drink (1), soy-based burger (2), falafel (1), tofu (1), Quorn (1), tempeh (1), vegetarian burger (11)	PBDA and PBMA	LC-MS/MS	LOD: 0.05 μg/kg LOQ: 0.1 μg/kg	Meerpoel et al. (2018)
AFB1, AFB2, AFG1 and AFG2	Oat drink (4), rice drink (5), coconut drink (3), almond drink (2)	PBDA	HPLC with photochemical derivatization and fluorescence detection	LOD: NA LOQ: 0.5 μg/L	Hamed et al. (2019)
AFB1, AFB2, AFG1, AFG2, AOH, AME, ENNA1, ENNA, ENNB, ENNB1, DAS, FB1, FB2, DON, 3-AcDON, 15-AcDON, OTA, HT-2, T-2 and ZEN	Soy-based burgers (19)	PBMA	LC-Q-Orbitrap HRMS	LOD: 0.01–0.1 μg/kg LOQ: 0.2–5.0 μg/kg	Rodriguez-Carrasco et al. (2019)
BEA, ENNA1, ENNA, ENNB and ENNB1	Rice drink (8), oat drink (8), soy drink (16)	PBDA	LC-MS/MS	LOD: 0.1–0.3 μg/L LOQ: 0.3–0.8 μg/L	Arroyo-Manzanares et al. (2019)
AFB1, AFB2, AFG1, AFG2, AOH, AME, TEN, BEA, ENNA, ENNA1, ENNB, ENNB1, OTA, HT-2, T-2 and ZEN	Oat drink (17), almond drink (11), rice drink (10), soy drink (18)	PBDA	LC-MS/MS	LOD: 0.1–3.6 μg/L LOQ: 0.3–18.0 μg/L	Juan et al. (2022)
CIT, OTA and ZEN	Rice drink (5) and oat drink (5)	PBDA	HPLC separation and fluorescence detection	LOD: 0.09–1.5 μg/L LOQ: 2.4–4.1 μg/L	Kholova et al. (2023)
AFB1, AFB2, AFG1, AFG2, AOH, AME, TEN, FB1, FB2, OTA and ZEN	Soy-based (6), pea-based (2), chickpea/wheat-based (1), lupin/wheat-based (2), soy/wheat-based (1) meat alternatives and seitan (1)	PBMA	LC-MS/MS	LOD: 0.01–0.75 μg/kg LOQ: 0.02–2.5 μg/kg	Mihalache et al. (2023)
AFB1, AFB2, AFG1, AFG2, STC, AOH, AME, BEA, ENNA, ENNA1, ENNB, ENNB1, CIT, DAS, FB1, FB2, DON, 15-AcDON, 3-AcDON, OTA, HT-2, T-2, NEO, T2 TRIOL, ZEN, ZAN, h-FB1, CTN, MPA and GLIO	Oat drink (16), almond drink (7), soy drink (9), rice drink (6)	PBDA	LC-MS/MS	LOD: 0.001–8.67 μg/L LOQ: 0.003–28.62 μg/L	Rodriguez-Canas et al. (2024)
Projects					
AFB1, AFB2, AFG1, AFG2, STC, AOH, AME, CIT, DAS, FB1, FB2, FB3, NIV, DON, 3-AcDON, 15-AcDON, DON-3G, OTA, sum EA, HT-2, T-2, NEO, ZEN, F-X and ROQ-C	Soy-based (30), rice-based (2), mycoprotein (2), pea-based (3), milk-based (1), lentil-based (2), chickpea/soy-based (1), chickpea-based (2) meat alternatives	PBMA	LC-MS/MS	LOD: 0.10–81.39 μg/kg LOQ: 0.33–163.38 μg/kg	Pavicich et al. (2024a)
AFB1, AFB2, AFG1, AFG2 and OTA	Vegetarian burgers (8), chickpea-based meat alternatives (9)	PBMA	LC-MS/MS	LOD: NA LOQ: 0.1–0.2 μg/kg	Belgian consumer organization

AFB1: aflatoxin B1; sum AF: sum AFB1, AFB2, AFG1 and AFG2; STC: sterigmatocystin; AME: alternariol monomethyl ether; AOH: alternariol; TEN: tentoxin; BEA: beauvericin; CIT: citrinin; DAS: 4,15-diacetoxyscirpenol; FB1: fumonisin B1; sum FUM: sum fumonisin B1, B2 & B3; NIV: nivalenol; sum DON: sum DON (deoxynivalenol), 3-acetyl-DON, 15-acetyl-DON and DON-3-glucoside; OTA: ochratoxin A; sum EA: sum ergocristinine, ergotcristine, ergokryptinine, ergokryptine, ergotaminine, ergotamine, ergocorninine, ergocornine, ergometrinine, ergotmetrine, ergosinine and ergosine; sum ENN: sum enniatin A, enniatin A1, enniatin B, enniatin B1; sum T-2 and HT-2: sum T-2 toxin and HT-2 toxin, NEO: neosolaniol; ZEN: zearalenone; ZAN: zearalanone, ROQ-C: roquefortine C; F-X: fusarenon X; MPA: mycophenolic acid; GLIO: gliotoxin; CTA: circumdatin A; h-FB1: hydrolysed-FB1; PHO A: phomopsin A; PBMA: plant-based meat alternative; PBDA: plant-based dairy alternative; LOD: limit of detection; LOQ: limit of quantification; NA: not available; LC-MS/MS: Liquid Chromatography Tandem Mass Spectrometry; HPLC: High Performance Liquid Chromatography; LC-HRMS: Liquid Chromatography coupled to High-Resolution Mass Spectrometry.

'Milk' consumption with their respective plant-based processed alternative at which a potential health risk could be expected was calculated as the consumption level at which the HQ was equal to 1, using Eq. (3).

$$Level\ of\ substitution\ (\%) = \frac{\left(\frac{HBGV\ or\ RPHC\ (\mu g/kg\ bw/day)}{mean\ mycotoxin\ concentration\ \left(\frac{\mu g}{kg}\right)}\right)}{P50\ consumption\ (kg/kg\ bw/day)} \times 100 \quad (3)$$

2.8. Scenario analysis

Some frequently used protein sources in both product categories were used in 100% replacement scenarios, to obtain more detailed information on the mycotoxin risks related to the different protein types generally used in such plant-based products. In this case, the mean mycotoxin concentration for each food category and a specific protein source was used in the exposure and HQ calculations. The specific scenarios include: soy-based PBMA, wheat-based PBMA, pulse-based PBMA (including lentil, pea, chickpea, chickpea/soy, chickpea/wheat, lupin/wheat), and oat-based PBDA, soy-based PBDA, almond-based PBDA and rice-based PBDA.

3. Results

3.1. Aggregated mycotoxin concentration database

In total 1,334 records were identified, from which 835 individual records remained after removal of duplicates and extracted from 11 eligible publications. More details on the review process can be found in the PRISMA flow diagram (supplementary materials Fig. S1). A summary of the selected publications and research projects is given in Table 1. The extracted data were used to develop an aggregated dataset containing mycotoxin levels in PBMA and PBDA. The final dataset contains mycotoxin levels in 313 samples, of which 182 correspond to PBDA and 131 to PBMA samples, collected and analyzed in Europe (Schryvers et al., 2024; Pavicich et al., 2024b).

3.2. Average mycotoxin concentrations in plant-based meat and drink alternatives

In Table 2 the calculated mean mycotoxin concentrations in PBMA and PBDA, with their 95% confidence intervals are given, for both the LB and UB scenario. In Fig. 1, the number of analyzed samples and number of detects (≥LOD) per mycotoxin are given for both PBMA and PBDA. In most cases, mycotoxins were detected in higher frequencies in milk alternatives, while the average concentration was generally higher in meat alternatives. For instance, AFB1 was more prevalent in PBDA (7.7%) than in PBMA (3.9%); however mean concentrations of AFB1 ranged between 0.25–0.65 μg/kg (LB – UB) in PBMA, and 0.01–0.33 μg/kg in PBDA. In PBMA, the most prevalent mycotoxins were sum of enniatins (89%), tentoxin (46%), alternariol monomethyl ether (35%) and sum of ergot alkaloids (27%). The most prevalent mycotoxins in PBDA were enniatins (56%), beauvericin (50%), sterigmatocystin (50%) and alternariol monomethyl ether (33%).

3.3. Exposure assessment: Substitution model (baseline scenario)

In Table 3 the mean chronic dietary exposure to mycotoxins based on the 100% substitution of meat and meat products, and milk with their processed plant-based alternatives is shown for children, adolescents and adults in the LB and UB scenarios. The highest estimated LB and UB exposure levels were 1273.65–1273.72 ng/kg bw/day for the sum of enniatins in children, based on the replacement of meat by PBMA; and 93.36–195.64 ng/kg bw/day for the sum of DON and metabolites in children, based on the replacement of milk by PBDA. In supplementary materials Table S2 the exposure estimates based on the P95 of the consumption are reported.

Table 2
Mean mycotoxin concentrations and 95% confidence intervals in the lower bound (LB) and upper bound (UB) scenario for both PBMA and PBDA. NA indicates that no contamination data were available, i.e. number of samples was zero.

Mycotoxins	Mean mycotoxin concentration (μg/kg) – PBMA			Mean mycotoxin concentration (μg/kg) – PBDA		
	LB	**UB**	**Number of samples**	**LB**	**UB**	**Number of samples**
AFB1	0.25 [0.02–0.56]	0.65 [0.42–0.94]	102	0.01 [0.0006–0.02]	0.33 [0.23–0.44]	117
Sum AF	1.28 [0.09–3.41]	3.71 [2.36–6.06]	102	0.02 [0.004–0.06]	1.29 [0.89–1.73]	117
STC	0.81 [0.36–1.25]	3.28 [2.69–3.86]	53	0.07 [0.02–0.13]	0.08 [0.04–0.13]	38
AME	44.12 [26.29–63.93]	52.16 [35.21–71.09]	85	0.09 [0.02–0.17]	2.25 [0.81–3.78]	94
AOH	2.83 [0.28–7.47]	4.58 [2.02–9.21]	85	0.03 [0.00–0.08]	2.21 [0.90–3.98]	94
TEN	1.94 [0.52–3.71]	1.95 [0.52–3.70]	13	3.86 [0.00–11.33]	6.02 [1.75–12.48]	56
BEA	NA	NA	0	0.39 [0.25–0.55]	0.89 [0.64–1.15]	126
CIT	0.08 [0.01–0.19]	1.33 [0.91–1.76]	32	0.07 [0.00–0.24]	6.25 [1.21–12.20]	49
DAS	0.00 [0.00–0.00]	0.71 [0.51–0.93]	72	0.00 [0.00–0.00]	0.04 [0.03–0.05]	38
FB1	7.07 [2.03–14.74]	43.71 [34.31–53.59]	85	0.12 [0.06–0.17]	5.35 [3.33–7.39]	69
Sum FUM	8.14 [2.49–15.83]	73.58 [58.27–89.03]	85	0.17 [0.09–0.26]	11.79 [7.82–15.75]	69
NIV	0.00 [0.00–0.00]	23.97 [23.79–23.97]	53	NA	NA	0
Sum DON	19.19 [0.40–55.75]	41.83 [21.31–78.79]	72	10.38 [0.00–28.6]	21.74 [11.11–39.30]	69
OTA	0.19 [0.01–0.42]	1.57 [1.22–1.94]	119	0.01 [0.004–0.03]	1.09 [0.70–1.48]	114
Sum EA	3.97 [0.61–8.18]	34.90 [31.79–38.77]	15	NA	NA	0
Sum ENN	336.27 [226.51–453.78]	336.29 [224.13–457.42]	19	4.59 [2.29–7.57]	6.83 [4.29–9.95]	126
Sum T-2 and HT-2	0.05 [0.00–0.14]	14.00 [11.21–16.75]	72	1.23 [0.71–1.78]	9.02 [6.42–11.87]	125
T-2 triol	NA	NA	0	0.00 [0.00–0.00]	0.13 [0.09–0.17]	38
NEO	0.00 [0.00–0.00]	4.27 [3.74–4.80]	53	0.00 [0.00–0.00]	0.40 [0.05–0.86]	38
ZEN	0.00 [0.00–0.00]	7.02 [5.64–8.44]	85	1.31 [0.61–2.15]	2.17 [1.45–3.02]	135
ZAN	NA	NA	0	0.00 [0.00–0.00]	0.17 [0.07–0.37]	38

AFB1: aflatoxin B1; sum AF: sum AFB1, AFB2, AFG1 and AFG2; STC: sterigmatocystin; AME: alternariol monomethyl ether; AOH: alternariol; TEN: tentoxin; BEA: beauvericin; CIT: citrinin; DAS: 4,15-diacetoxyscirpenol; FB1: fumonisin B1; sum FUM: sum fumonisin B1, B2 & B3; NIV: nivalenol; sum DON: sum DON (deoxynivalenol), 3-acetyl-DON, 15-acetyl-DON and DON-3-glucoside; OTA: ochratoxin A; sum EA: sum ergocristinine, ergotcristine, ergokryptinine, ergokryptine, ergotaminine, ergotamine, ergocorninine, ergocornine, ergometrinine, ergotmetrine, ergosinine and ergosine; sum ENN: sum enniatin A, enniatin A1, enniatin B, enniatin B1; sum T-2 and HT-2: sum T-2 toxin and HT-2 toxin; NEO: neosolaniol; ZEN: zearalenone; ZAN: zearalanone; PBMA: plant-based meat alternative; PBDA: plant-based dairy alternative.

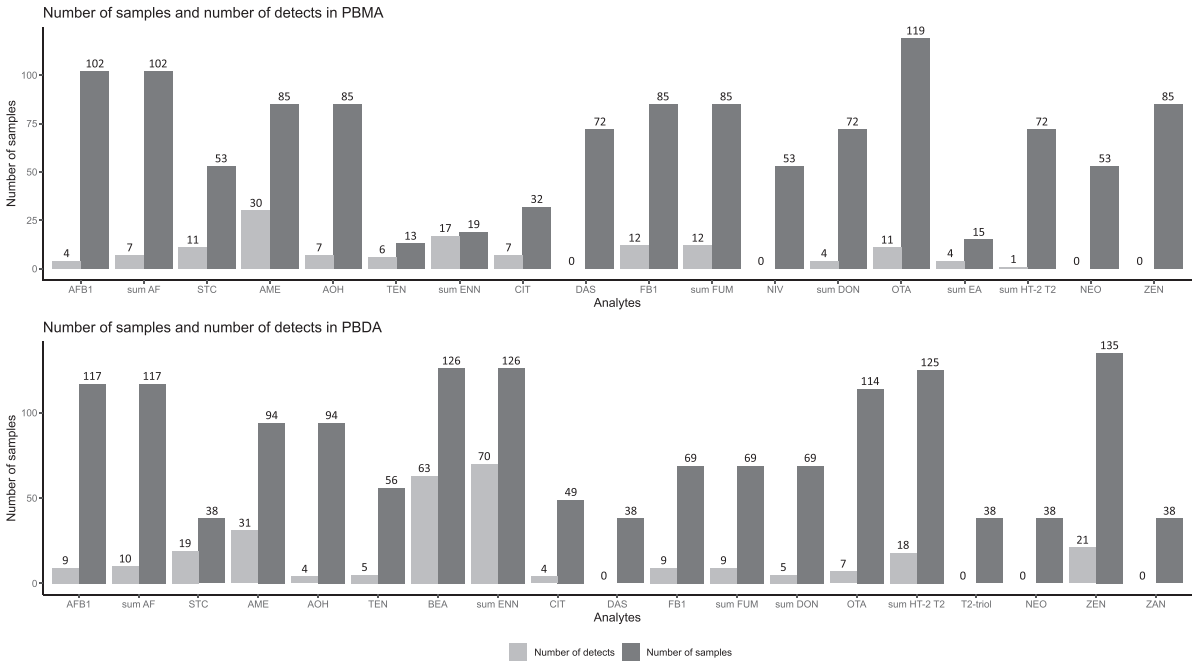

Fig. 1. Number of samples and detects (≥LOD) for mycotoxins in the plant-based meat alternatives (PBMA) and plant-based dairy alternatives (PBDA) product categories.

Table 3
Exposure (ng/kg bw/d) to mycotoxins based on the percentile of the consumption of 'Meat and meat products' and 'Milk' in Belgium for three age groups in both the LB and UB scenario. NA indicates that no contamination data were available, i.e. number of samples was zero.

Mycotoxins	PBMA						PBDA					
	Children		Adolescents		Adults		Children		Adolescents		Adults	
	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB
AFB1	0.96	2.44	0.64	1.63	0.50	1.26	0.05	2.95	0.02	1.05	0.01	0.41
sum AF	4.85	14.07	3.24	9.38	2.51	7.27	0.20	11.59	0.07	4.14	0.03	1.60
STC	3.08	12.41	2.06	8.28	1.59	6.41	0.67	0.76	0.24	0.27	0.09	0.10
AME	167.12	197.56	111.50	131.80	86.40	102.13	0.78	20.20	0.28	7.22	0.11	2.78
AOH	10.71	17.34	7.15	11.57	5.54	8.96	0.26	19.89	0.09	7.10	0.04	2.74
TEN	7.36	7.38	4.91	4.93	3.81	3.82	34.69	54.16	12.39	19.35	4.78	7.46
BEA	NA	NA	NA	NA	NA	NA	3.53	8.04	1.26	2.87	0.49	1.11
CIT	0.32	5.05	0.21	3.37	0.16	2.61	0.66	56.25	0.24	20.09	0.09	7.75
DAS	0.00	2.69	0.00	1.79	0.00	1.39	0.00	0.37	0.00	0.13	0.00	0.05
FB1	26.78	165.56	17.86	110.46	13.84	85.59	1.06	48.13	0.38	17.19	0.15	6.63
sum FUM	30.82	278.69	20.56	185.93	15.93	144.07	1.53	106.06	0.55	37.89	0.21	14.62
NIV	0.00	90.78	0.00	60.57	0.00	46.93	NA	NA	NA	NA	NA	NA
sum DON	72.70	158.43	48.50	105.70	37.58	81.90	93.36	195.64	33.35	69.89	12.87	26.96
OTA	0.73	5.95	0.49	3.97	0.38	3.08	0.11	9.82	0.04	3.51	0.02	1.35
sum EA	15.03	132.20	10.03	88.20	7.77	68.34	NA	NA	NA	NA	NA	NA
sum ENN	1273.65	1273.72	849.75	849.79	658.44	658.48	41.27	61.48	14.74	21.96	5.69	8.47
sum HT-2T2	0.18	56.59	0.12	37.75	0.09	29.25	11.04	81.55	3.94	29.13	1.52	11.24
sum ZEN	0.00	26.60	0.00	17.74	0.00	13.75	11.79	20.19	4.21	7.21	1.62	2.78

AFB1: aflatoxin B1; sum AF: sum AFB1, AFB2, AFG1 and AFG2; STC: sterigmatocystin; AME: alternariol monomethyl ether; AOH: alternariol; TEN: tentoxin; BEA: beauvericin; CIT: citrinin; DAS: 4,15-diacetoxyscirpenol; FB1: fumonisin B1; sum FUM: sum fumonisin B1, B2 & B3; NIV: nivalenol; sum DON: sum DON (deoxynivalenol), 3-acetyl-DON, 15-acetyl-DON and DON-3-glucoside; OTA: ochratoxin A; sum EA: sum ergocristinine, ergotcristine, ergokryptinine, ergokryptine, ergotaminine, ergotamine, ergocorninine, ergocornine, ergometrinine, ergotmetrine, ergosinine and ergosine; sum ENN: sum enniatin A, enniatin A1, enniatin B, enniatin B1; sum T-2 and HT-2: sum T-2 toxin and HT-2 toxin; NEO: neosolaniol; ZEN: zearalenone; ZAN: zearalanone; PBMA: plant-based meat alternative; PBDA: plant-based dairy alternative.

3.4. Risk ranking: Substitution model (baseline scenario)

Fig. 2 shows the calculated hazard quotients for the PBMA and PBDA baseline LB scenario. The results obtained for the UB scenario are presented in supplementary materials Fig. S2. The sum of AFs (HQ = 121.3; LB children), AME (HQ = 66.8; LB children) and AFB1 (HQ = 24.0; LB children) resulted in the highest risks in processed PBMA. In addition, the HQs for AOH exceed 1, in both the LB and UB scenarios, for all age groups. The HQs based on the P95 of the exposure are given in Table S3. In this case, HQs exceeding 1 were also found for OTA (neoplastic effects) in PBMA, e.g. in children the LB – UB HQ estimates correspond to 1.2–9.9. The exposure to other mycotoxins through the consumption of PBMA is expected to be less of a concern. In PBDA, HQ values are considerably lower for all mycotoxins, and large differences between the LB and UB scenario can be observed (Fig. 2; Fig. S2). Here, the sum AF and AFB1 can be identified as most relevant. Additionally, based on the 95th percentile of the consumption, attention should go towards the sum of HT-2 and T-2 in plant-based milks, with HQs ranging from 1.7 to 12.3

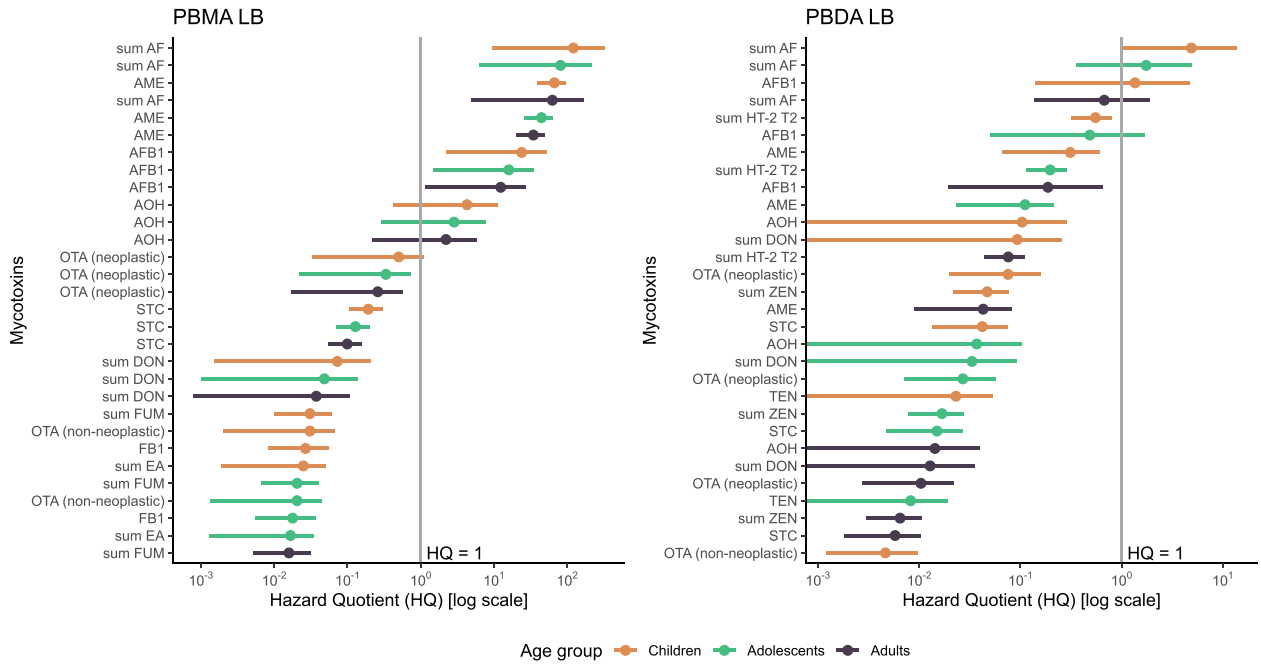

Fig. 2. Hazard quotients (HQs) and error bars indicating the 95% confidence interval (only representing sampling uncertainty in the occurrence data, not the consumption data) in both the plant-based meat alternatives (PBMA) and plant-based dairy alternatives (PBDA) product categories in the lower bound (LB) scenario for three age groups expressed on a log scale. The vertical line indicates an HQ equal to one.

(LB – UB) in children.

3.5. Level of substitution for safe consumption (%)

The lowest margins for safe consumption were found for the sum AF, AME, AFB1, AOH and OTA (neoplastic effects) in PBMA. For instance, a potential food safety risk can be predicted for children at 0.8–0.3% (LB – UB) replacement of meat consumption for the sum of AF, 1.5–1.3% replacement for AME, 4.2–1.6% replacement for AFB1, and 23.3–14.4% replacement for AOH. For OTA (neoplastic effects) the estimated level of substitution of meat amounts to 199.0–24.4% (LB – UB) for children. Here, 199% essentially means that the PBMA consumption should be double the current meat consumption for there to be a risk. Other mycotoxins in PBMA seem to be less of a concern, if only the consumption of PBMA is considered. In PBDA, attention should go towards the sum AF and AFB1. In this case, a potential food safety risk may be expected in children at 20.4–0.3% (LB – UB) and 73.5–1.4% milk replacement, for sum AF and AFB1, respectively. Similar outcomes (same order of magnitude) were obtained for the other age groups.

3.6. Risk ranking: Substitution model (100% replacement with specific protein source)

In Fig. 3, a heatmap of the HQ for the seven replacement scenarios are shown for Belgian children. The highest risk (LB HQ = 196) was found for the sum AF in soy-based PBMA, followed by AME in soy-based PBMA (LB HQ = 103) and sum AF in pulse-based PBMA (LB HQ = 73). Wheat-based products seem to be less of a concern regarding the studied mycotoxins. Again, much lower HQs were found for the PBDA scenarios. Here, oat- and almond-based drinks resulted in the highest HQ in the LB scenario for sum AF and AFB1. In soy-based drinks, the LB – UB HQs for HT-2 and T-2 toxin corresponded to 0.97 and 4.12. Again, similar outcomes (same order of magnitude) were obtained for the other age groups.

3.7. Risk ranking: Vegetarian and vegan consumers

In Fig. 4 the HQ's (LB scenario) obtained for the consumption of meat and milk alternatives, respectively, by vegans and vegetarians, are shown, based on data from the FFQ. HQ levels exceeding the threshold of 1 were found for the sum of aflatoxins, AME, AFB1 and AOH in meat alternatives in the LB scenario, indicating a potential food safety risk. The consumption of plant-based drinks indicates a food safety risk for the sum of aflatoxins, based on the LB scenario, while based on the UB scenario, a potential food safety risk for sum AF, AME, AFB1, AOH, OTA (neoplastic effects) sum HT-2 &T-2 cannot be excluded (Fig. S3). Overall, the absolute values of the calculated HQs appear to be lower than HQs based on the substitution models, indicating that they are probably overestimating the food safety risks. Exposure and risk estimates based on the 50th and 95th percentile of the consumption are given in supplementary materials Tables S4–S6.

4. Discussion

4.1. Composing an aggregated dataset for the occurrence of mycotoxins in PBMA and PBDA

In the assessment of emerging risks, here in the case of changing diet trends as potential driver, a lack of occurrence data is an important issue (EFSA, 2024b). Moreover, food safety data are scattered, often collected in varying contexts, using different sampling and analytical methods, with an overall lack of integrated databases (Yilmaz et al., 2023). To overcome this, the occurrence of mycotoxins in plant-based meat and milk alternatives in Europe was characterized by combining concentration data and relevant metadata from multiple sources, including scientific literature and project data, in an aggregated dataset.

Certain mycotoxins, e.g. enniatins, beauvericin and *Alternaria* toxins, were frequently detected in processed plant-based alternatives, which points to the contamination of the raw materials upon which these products are based, and to the chemical and thermal stability of mycotoxins during processing (Schaarschmidt & Fauhl-Hassek, 2021). This highlights the need for research on the fate of different mycotoxins

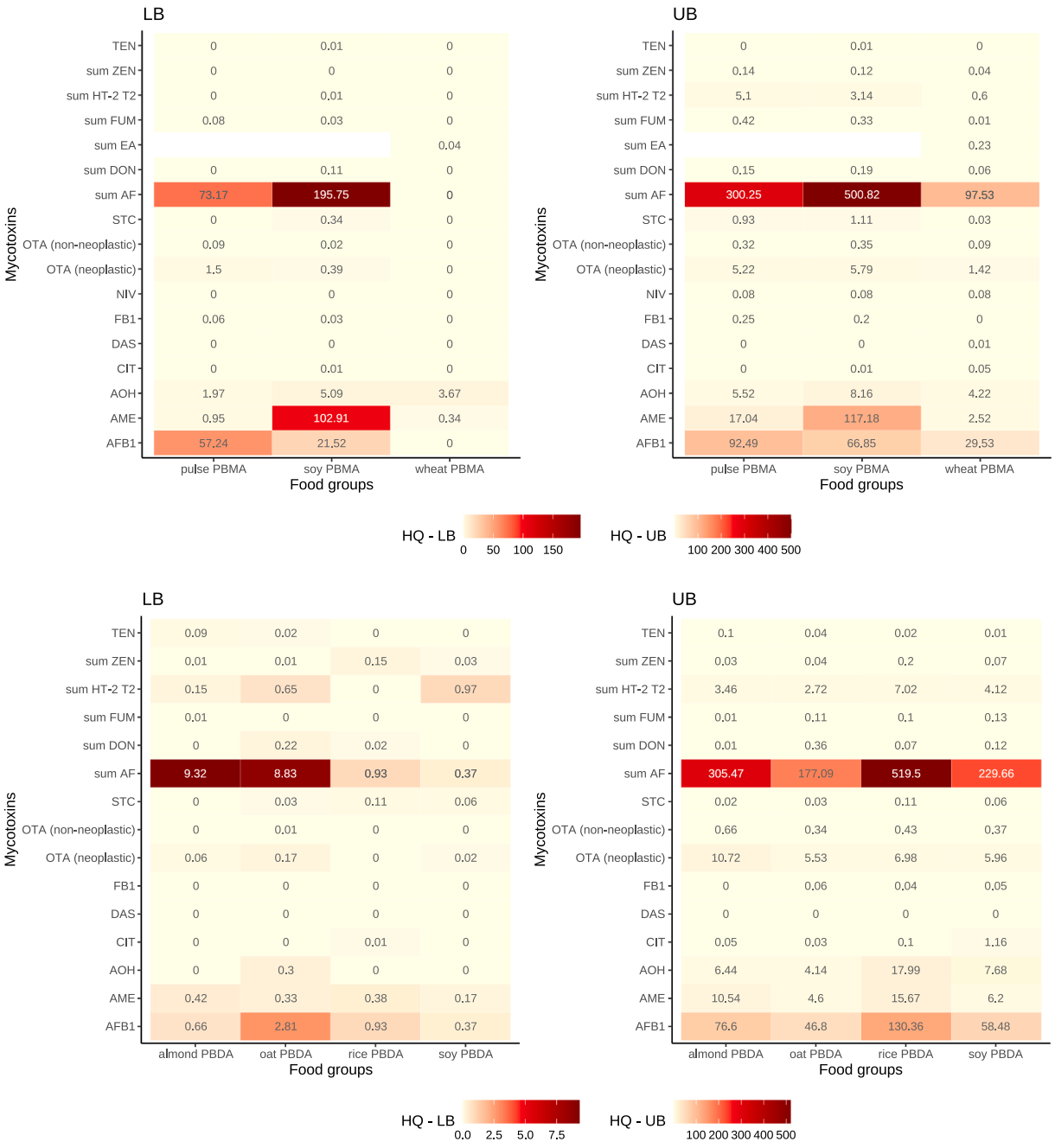

Fig. 3. Scenario analysis: heatmap of hazard quotient values calculated for specific protein sources for both the plant-based meat alternatives (PBMA) and plant-based dairy alternatives (PBDA) product categories and lower bound (LB) and upper bound (UB) scenario for the 'Children' age group.

during the processing of plant-based substitutes, e.g. during the extraction process of proteins from plant sources, and during the further processing, e.g. (high moisture) extrusion (Zhang et al., 2021). Vicente et al. (2019) studied the aflatoxin distribution in fractions during tofu production with soybeans contaminated with aflatoxins. They found that solid fractions (tofu and okara) were more contaminated than liquid fractions (soaking water and whey), and that of the initial AFB1 contamination between 0.5% and 13.2% remained in tofu, and between 6.2% and 67.7% in okara (insoluble fibrous fraction), demonstrating that even upon processing, AFB1 may end up in the final product. Furthermore, it has been shown that AME is persistent during the processing of legumes in the production of legume-derived protein-rich fractions (Pavicich et al., 2024a).

In particular the sum ENN and BEA were highly prevalent in both PBMA and PBDA. They are considered 'emerging' mycotoxins, that are frequently detected in food, feed and grains (Fraeyman et al., 2017; Gautier et al., 2020). For instance, Reinholds et al. (2021) detected enniatins in 94% of Latvian cereal samples (3.5–2073 μg/kg) (n = 110) and in 13% of the pulse samples (4.4–17 μg/kg) (n = 23). EFSA (2014) reported a mean BEA concentration in 'Legumes, nuts and oilseeds' of 2.24–2.46 μg/kg (LB – UB, n = 6), while for the sum of the four enniatins they found a mean concentration of 163–166 μg/kg (n = 67). In the EU, they are not (yet) regulated, as evidence on their toxicological relevance remains inconclusive, with an overall lack of *in vivo* toxicity data, precluding a human risk assessment (EFSA, 2014; Maranghi et al., 2018; Mihalache, De Boevre, et al., 2023).

In a screening of plant-based meat alternatives (published after the review process was conducted) Mihalache et al. (2024) analyzed sixteen mycotoxins in 105 meat alternatives available in Italy. Similarly to our findings, they obtained highest occurrences for ENNB (97.1%) and BEA

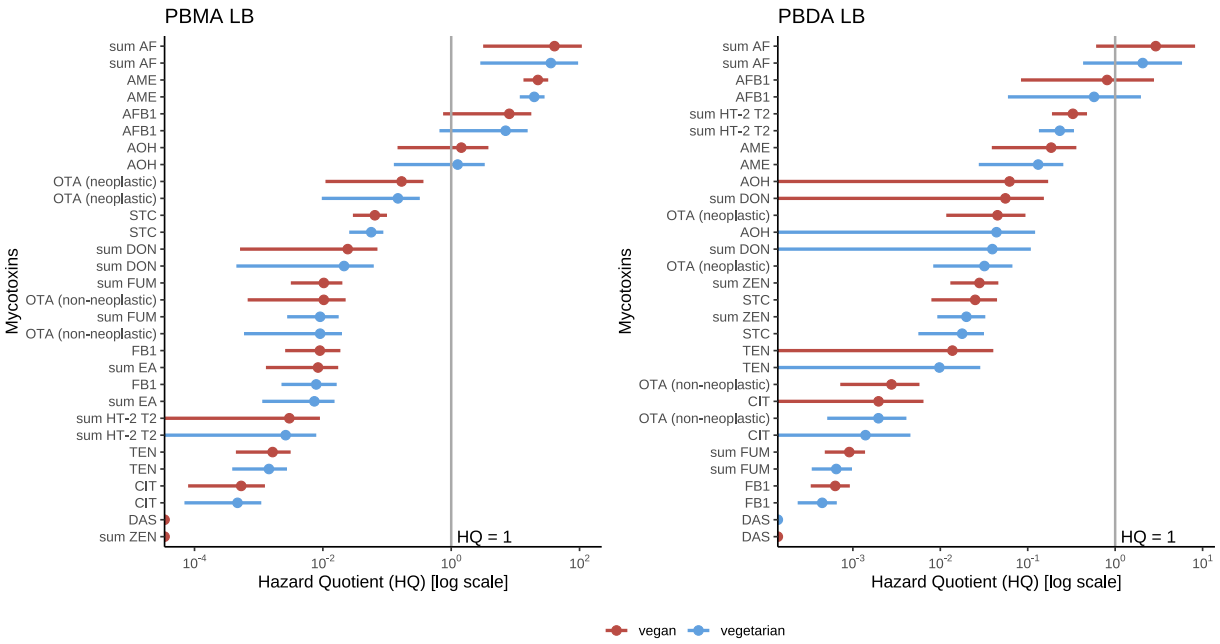

Fig. 4. Hazard quotients (HQs) and error bars indicating the 95% confidence interval (only representing sampling uncertainty in the occurrence data, not the consumption data) in both the plant-based meat alternatives (PBMA) and plant-based dairy alternatives (PBDA) product categories in the lower bound (LB) scenario for the vegetarian and vegan population subgroup expressed on a log scale. The vertical line indicates an HQ equal to one.

(99.1%). However, mean concentrations were clearly lower, with average concentrations of 10.9 μg/kg for ENNB and 6.5 μg/kg for BEA in legume-based meat alternatives. Certain other mycotoxins were clearly more prevalent in their PBMA sample set. For instance, they observed much higher occurrences for both the sum FUM (FB1: 83.8%) and ZEN (61%), with mean mycotoxin concentrations ranging from 6.7 to 24.0 μg/kg and 4.0 to 4.4 μg/kg, respectively, depending on the type of meat alternative.

4.2. *Exposure assessment and risk ranking of mycotoxins*

The risk ranking of mycotoxins demonstrated that aflatoxins (sum AF and AFB1) and genotoxic *Alternaria* toxins (AOH and AME) in plant-based meat alternatives should be prioritized by risk assessors and managers. While results were less conclusive in PBDA, the exposure assessment and risk ranking pointed out that the exposure to aflatoxins could result in a potential health concern, in particular for children, in the case of 100% replacement of cow's milk. Almond drink and oat drink were identified as highest risk product categories, while in meat alternatives soy-based products resulted in highest HQs, especially for AME and AOH, and aflatoxins. Soybeans (*Glycine* max) are an often used protein source in PBMA/PBDA, because of their high protein content (35–40%) and good protein quality, e.g. good amino acid composition and digestibility, and functional properties, e.g. water and fat binding, emulsification and gelation (Day et al., 2022; Van Den Berg et al., 2022; Velasquez & Bhathena, 2007). With regard to the contamination of the raw material, EFSA (2020) reported a mean LB – UB concentration of 0.17–0.78 μg/kg (n = 146) and 0.24–2.02 μg/kg (n = 102) for AFB1 and the sum of AF, respectively, in soybeans. Concerning AOH and AME, EFSA (2016b) didn't report concentration levels at such a specific level, but in the 'Legumes, beans, dried' category, AOH and AME concentrations corresponded to 0.0–0.0087 μg/kg (LB – UB) and 0.0–0.0074 μg/kg, respectively, indicating 100% left-censored data. In contrary to Oviedo et al. (2012), who detected genotoxic *Alternaria* toxins in 60% of Argentinian soybean samples at concentrations ranging from 25 to 211 μg/kg for AOH and 62 to 1153 μg/kg for AME. In line with our outcomes, Mihalache et al. (2024) concluded that *Alternaria* toxin exposure from the consumption of PBMA may result in a public health risk. A preliminary analysis by EFSA (2016b) indicated that the exposure to *Alternaria* mycotoxins is expected to be higher in a vegetarian population than in the general (omnivore) population. Further research is warranted considering their toxicological relevance. *In vivo* data suggests genotoxic effects, including DNA damage, induction of gene mutations and micronuclei formation, for both AOH and AME (Louro et al., 2024; Miao et al., 2022; Tang et al., 2022). The European Commission recognized the potential public health risk related to *Alternaria* toxins and recommended the monitoring of AOH, AME and TeA in foods such as oilseeds, tomato-based products and tree nuts, and has set indicative maximum levels (Recommendation EC 2022/553).

4.3. *Plant-based meat and dairy alternatives as product category in the protein transition*

The role of processed plant-based meat and dairy substitutes in healthy diets is still a topic of debate, given the lack of understanding of their long-term health impacts, in particular because of their high level of processing, and NOVA categorization as ultra-processed food (UPF) (Gehring et al., 2020; MacDiarmid, 2021). Messina et al. (2023), on the other hand, argued that (legume-based) PBMA can be considered gateway foods to facilitate the transition to plant-based diets, and also highlighted the potential for fortification with micronutrients. Our results add another layer to this discussion, as both PBMA and PBDA have been identified as potentially significant sources of exposure to certain mycotoxins. In this regard, Van Asselt et al. (2024b) suggested a safe-by-design approach in the development of plant-based meat alternatives, by ranking chemical food safety hazards (hazard – ingredient combinations) and identifying the most optimal plant-based product recipe.

4.4. *Study limitations and further research needs*

In general, the uncertainty associated with the exposure assessment is considered high due to potential biases in product sampling and the possible overestimation of consumption, in particular for PBMA. The 'Meat and Meat Products' category represents the highest level of reporting in the FoodEx2 classification system for meat, including all types of meat consumption (e.g. meat preparations, meat products,

minced meat, etc.). This issue was addressed by calculating the percentage of replacement for safe consumption, with some of the obtained estimates already being quite low. Updated and more detailed consumption data in the frame of the ongoing shift to plant-based diets are needed to better understand the exposure to chemical contaminants and nutrient intakes, the associated health risks, and their distribution within a population. In addition, epidemiological and biomonitoring studies are needed to get more scientifically reliable data on the (long-term) health effects of different plant-based diets. Finally, it should be emphasized that the obtained set of samples should not be considered representative for the European PBMA/PBDA market, and should rather be seen as a convenience sample at best, given that the sampling of PBMA and PBDA products, may have been subject to selection bias. Moreover, publication bias, i.e. the more frequent publication of 'positive' outcomes over 'negative' outcomes, may have resulted in an overestimation of the mycotoxin levels (Rothstein et al., 2005).

5. Conclusion

To our knowledge, this is the first study to model the exposure to mycotoxins through the consumption of both plant-based meat and milk alternatives, by simulating the substitution of animal-source foods with their plant-based alternatives (PBMA and PBDA), and rank their associated health risks. In the frame of the transition to plant-based diets, more attention should go towards changing exposures to chemical hazards, in particular mycotoxins. It was demonstrated that a potential health risk due to the exposure to aflatoxins, in particular AFB1, and genotoxic *Alternaria* toxins (AOH and AME) cannot be excluded, especially in soy-based meat alternatives, and almond and oat drinks. Emerging mycotoxins, such as ENNs and BEA, were shown to be highly prevalent in both plant-based meat and milk substitutes, and while their toxicity remains unclear, their exposure will probably increase in this transition to plant-based diets.

Multiple uncertainties, particularly selection bias, affect the concentration data and should be considered when interpreting the results. Hence, we recommend a more systematic screening of the PBMA and PBDA market in Europe, to further refine risk assessments. As well as the collection of consumption data that more accurately capture the consumption of plant-derived proteins in a population undergoing such a protein transition.

CRediT authorship contribution statement

Sofie Schryvers: Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Christian Jung:** Writing – review & editing, Methodology. **Maria Agustina Pavicich:** Writing – review & editing. **Sarah De Saeger:** Writing – review & editing. **Carl Lachat:** Writing – review & editing. **Liesbeth Jacxsens:** Writing – review & editing, Supervision, Methodology, Conceptualization.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2024.115422.

Data availability

A link to the dataset is shared in the manuscript.

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