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Dietary transitions to plant-based meat and milk alternatives and their impact on mycotoxin exposure: A risk profiling study in Italy and the UK

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ABSTRACT

The aim of this study was to evaluate how transitioning to plant-based meat alternatives (PBMAs) and plant-based beverages (PBBs) contributes to mycotoxin exposure among Italian and British consumers. We retrieved mycotoxin occurrence data from 413 PBMAs and PBBs from the Italian and British markets and combined them with age-specific modelling. The targeted mycotoxins were aflatoxin B_1, aflatoxin B_2, aflatoxin G_1, aflatoxin G_2, fumonisin B_1, fumonisin B_2, HT-2, T-2, ochratoxin A, zearalenone, deoxynivalenol, alternariol, alternariol monomethyl ether, tentoxin, enniatin B, enniatin B1, enniatin A, enniatin A1, and beauvericin. Dietary exposure was assessed under 25%, 50%, and 100% substitution scenarios of meat and milk with PBMAs and PBBs. Italian consumers were more exposed than British consumers to AFs, DON, and FUMs, while British consumers were more exposed to OTA, HT2 + T2, BEA, and ENNs. Infants and children were the most vulnerable groups due to higher intake per body weight. Across both countries, AFs resulted in total margins of exposure <10,000, indicating a potential health concern in nearly all scenarios. OTA risk increased with higher substitution levels, particularly in younger populations. The highest cumulative non-tolerable exposures were observed for *Alternaria* toxins (AOH + AME) and HT-2 + T-2, especially under combined PBMA and PBB scenarios. The level of substitution was associated with safety threshold exceedance, with increases from 25% to 100% substitutions indicating substantial amplification of cumulative risk indicators. These findings indicate that large-scale dietary transitions toward plant-based alternatives may modify mycotoxin exposure patterns, highlighting the need for targeted surveillance and alignment of sustainability and food safety objectives.

1. Introduction

Worldwide dietary guidelines are promoting a transition toward healthier and more sustainable diets centered around plant-based foods. The Lancet's EAT-Lancet Commission report from 2025 recommends the "Planetary Health Diet," a plant-based diet consisting of whole grains, fruits, vegetables, nuts, and legumes, while significantly reducing red meat, sugar, and processed foods, to improve both human health and sustainability (Rockström et al., 2025). This dietary framework calls for a global transformation of food systems by increasing plant-based production and decreasing animal product consumption to meet the needs of 10 billion people within planetary boundaries (Rockström et al., 2025).

The shift toward plant-based diets has led to an increased consumption of meat and dairy alternatives with the plant protein market projected to reach 10% of the global protein market by 2030 (Bartashus & Srinivasan, 2021). Plant-based meat alternatives (PBMAs) and plant-based beverages (PBBs) aim to mimic the texture, structure, and sensorial characteristics of the animal-based foods they aim to substitute (McClements & Grossmann, 2021).

While the benefits of the transition to plant-rich diets are well recognized (Bunge et al., 2024; Rockström et al., 2025; Willett et al., 2019), recent studies have indicated associations between adherence to healthy diets adherence and exposure to environmental contaminants (Yu et al., 2024). In Italy, Germany, and the UK, recent evidence showed an increased exposure to mycotoxins based on the consumption of PBMAs and PBBs and adherence to a vegan diet (Mihalache et al., 2024; Penczynski et al., 2022; Torrijos et al., 2026). Mycotoxins are toxic

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secondary metabolites produced by fungi like *Aspergillus, Penicillium, Fusarium, Alternaria,* and have a wide range of toxic effects, including cytotoxicity, genotoxicity, and carcinogenicity, posing a significant health risk for human health (WHO, 2023).

Food safety concerns related to dietary shifts have been raised also by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) (FAO and WHO, 2019). Consumption of PBMAs and PBBs is likely to alter exposure patterns to mycotoxins, therefore the overall food safety considerations for plant-based alternatives and any dietary transition require careful reshaping for food safety management (FAO, 2022).

While global and European regulatory frameworks cover maximum levels for mycotoxins in certain foods, there are currently no specific legislations for some of the ingredients used in the production of plant-based meat alternatives (PBMAs) and plant-based beverages (PBBs) like soya, beans, chickpeas, lentils, and vegetable-based matrices such as carrots, eggplants, and others (Codex Alimentarius Commission, 1995; Commission Regulation (EC), 2023)(.

Italy and the United Kingdom represent two dynamic yet distinct contexts for studying dietary transitions toward plant-based foods in Europe. In Italy, plant-based products have seen rapid market growth, with more than 15 million households purchasing plant-based foods in 2024 (Good Food Institute, 2025a) and a substantial share of Italian consumers reporting reduced meat consumption and increasing interest in plant-based alternatives, including beverages and meat substitutes (59% of meat eaters reducing intake) (ProVeg, 2024). In the UK, a growing proportion of the population is adopting plant-based or reduced-animal-product diets, with millions identifying as vegan or reducing animal foods for ethical, health, and environmental reasons, and a notable share of adults planning to increase plant-based food consumption (Good Food Institute, 2025b).

To our knowledge, this study represents the first large-scale, cross-country quantitative risk assessment integrating multi-mycotoxin occurrence data from over 400 plant-based meat alternatives (PBMAs) and plant-based beverages (PBBs) across two European markets within substitution-based dietary modelling scenarios. In this context, the present study aimed to evaluate dietary exposure to mycotoxins among Italian and British consumers under different substitution scenarios in which meat and milk are partially or fully replaced with PBMAs and PBBs. Italy and the UK represent distinct European dietary patterns and market penetration of plant-based alternatives. This comparison highlights how geographical dietary habits influence the risk-benefit trade-off when substituting traditional meat and milk with plant-based alternatives. By applying a risk profiling approach, this work seeks to contribute to the understanding of the food safety implications associated with large-scale dietary transitions toward plant-based alternatives.

2. Materials and methods

2.1. Occurrence data for mycotoxins in PBMAs and PBBs

Occurrence data for mycotoxins have been retrieved from our previously published studies which analyzed 105 PBMAs (Mihalache et al., 2024) and 96 PBBs (Torrijos et al., 2025) from Italy as well as 92 PBMAs and 120 PBBs from the UK (Torrijos et al., 2026). These three studies were selected because they were conducted using the same harmonized, validated UHPLC-MS/MS multi-mycotoxin method and a common sample preparation protocol, eliminating inter-laboratory variability that would arise from pooling data across independent published surveys. Italy and the UK were selected as representative of contrasting European dietary patterns (Mediterranean vs. Northern-European) with distinct food consumption survey frameworks.

The targeted mycotoxins were both regulated and emerging. Regulated mycotoxins included aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), aflatoxin G_2 (AFG_2), fumonisin B_1 (FB_1), fumonisin B_2 (FB_2), HT-2, T-2, ochratoxin A (OTA), zearalenone (ZEN), and deoxynivalenol (DON). Non-regulated mycotoxins were mainly alternariol (AOH), alternariol monomethyl ether (AME), tentoxin (TEN), enniatin B (ENNB), enniatin B1 (ENNB1), enniatin A (ENNA), enniatin A1 (ENNA1), and beauvericin (BEA).

The product categories were not pooled because they represent compositionally distinct matrices such as mixed cereal-legume versus mixed vegetable-legume formulations and pooling would risk masking ingredient-specific contamination patterns. The PBMAs and PBBs were therefore classified according to their primary ingredients as well as combinations of ingredients considering the complexity of some PBMAs formulation:

a) For Italy, PBMAs were classified into cereal-based meat alternatives (CBMA; $N = 33$), legume-based meat alternatives (LBMA; $N = 51$), and vegetable-based meat alternatives (VBMA; $N = 21$). PBBs were divided into oat-based beverages (OBBs) ($n = 28$), almond/nuts-based beverages (ABBs) ($n = 25$), soy-based beverages (SBBs) ($n = 27$), and rice-based beverages (RBBs) ($n = 16$).
b) For the, UK PBMAs consisted of cereal-based meat alternatives (CBMA) ($n = 10$), legume-based meat alternatives (LBMA) ($n = 30$), legumes and cereals-based meat alternatives (LCBMA) (n = 27) and legumes and vegetables-based meat alternatives (LVBMA) (n = 25). PBBs were divided into oat-based (OBB, $n = 57$), almond/nuts-based (ABB, $n = 32$), and soy-based (SBB, $n = 31$).

The number of samples censored and the percentage of left-censored data for each mycotoxin, product category, and country are presented in Supplementary Material 1.

2.2. Occurrence datasets and analytical methodology

Mycotoxin occurrence data used in the present exposure assessment were retrieved from our previously published studies on plant-based meat alternatives (PBMAs) and plant-based beverages (PBBs) from Italy and the United Kingdom (Mihalache et al., 2024; Torrijos et al., 2025, 2026). These studies generated the analytical occurrence data used as input for the present risk assessment.

Briefly, samples were extracted using an optimized salting-out assisted liquid-liquid extraction (SALLE) procedure and analyzed by a validated UHPLC-MS/MS multi-mycotoxin method developed for PBMAs and PBBs in accordance with the applicable SANTE guidance documents (SANTE/12089/2016, 2016; SANTE/11312/2021, 2021). Full details regarding sample collection, preparation, method validation, and analytical performance are available in the original publications and are not repeated here. The limits of detection (LODs) and limits of quantification (LOQs) are provided in Supplementary Material 2.

2.3. Food consumption data

Food consumption data for Italy and the UK were retrieved from the latest Italian Food Consumption Surveys (IV SCAI CHILD and IV SCAI ADULT 2017–2020) (Aida et al., 2021; Aida et al., 2022) and the National Diet and Nutrition Survey 2019 to 2023 (2026) (National Diet and Nutrition Survey 2019 to 2023, 2026).

Consumers were divided into six age groups according to EFSA (EFSA, 2022): infants (up to and including 11 months old), toddlers (12–35 months old), other children (3–9 years old), adolescents ($N =$ 10–17 years old), adults (18–64 years old), and the elderly (65–74 years old). Since the surveys did not cover PBMAs and PBBs consumption, we simulated the replacement of meat consumption with PBMAs, and the consumption of cow milk with PBBs consumption. The mean consumption values are shown in Supplementary Material 3.

To cover different substitution scenarios and align with current dietary trends we simulated three consumption scenarios considering 25%, 50%, and 100% replacement of meat and milk products with PBMAs and PBBs. The weight-based substitution approach has been

adopted for consistency with published methodologies in dietary transitions mycotoxin risk assessment from Belgium (Schryvers et al., 2025) and Germany (BfR (Bundesinstitut für Risikobewertung), 2024). This approach allowed us to assess the range of exposure to mycotoxins and whether potential health risk would arise even at low substitution levels (25%) such as is the case for a flexitarian diet and/or increase in a full substitution scenario (100%) as is the case for a vegan diet.

2.4. Dietary exposure, risk characterization, and risk profiling

Before performing the exposure assessment, we applied the substitution method to treat left-censored data (LCD) (data below the LOD or LOQ). This method is currently recommended by WHO in "Principles and Methods for the Risk Assessment of Chemicals in Food" (WHO/IPCS (World Health Organization/International Programme on Chemical Safety), 2009) and is also used by the European Food Safety Authority (EFSA) (EFSA, 2010). Under this approach we created two scenarios: a) the lower-bound (LB) scenario which involves the replacement of any LCD with 0 and, b) the upper-bound (UB) scenario which involves the replacement of LCD with the corresponding LOD or LOQ. Using both LB and UB provides a more comprehensive perspective when considering optimistic and pessimistic exposure scenarios in risk assessment.

The estimated daily intake (EDI) of each mycotoxin was calculated using Eq. (1):

$$\text{EDI}\ (\mu g/\text{kg bw/day}) = \left(\frac{C \times CON}{bw \times 1000}\right) \quad (1)$$

where C = the average concentration of the mycotoxins found in the food items (μg/kg), CON = food consumption in g/day, and bw = bodyweight.

For the risk characterization we compared the exposure estimates with the health-based guidance values (HBGV) corresponding to each mycotoxin.

Considering the oestrogenic effects observed in pigs, EFSA established a TDI of 0.25 μg/kg bw/day for zearalenone (ZEN) (EFSA, 2011).

EFSA determined a tolerable daily intake (TDI) of 1 μg/kg bw/day for fumonisins (FUMs), based on the observed increase in megalocytic hepatocytes in mice (EFSA, 2018), and established the same TDI for deoxynivalenol (DON), derived from findings of reduced body weight gain in mice (EFSA, 2017a).

An in vivo toxicity study in rats demonstrated that exposure to T-2 and HT-2 toxins caused a decrease in total leukocyte count, which was identified as the critical effect for determining the group tolerable daily intake (TDI) (EFSA, 2017b). Based on these findings, EFSA set a group TDI of 0.02 μg/kg bw/day.

For *Alternaria* mycotoxins, EFSA applied the threshold of toxicological concern (TTC) approach, setting a TTC of 0.025 μg/kg bw/day for genotoxic compounds (AOH and AME), and 1.5 μg/kg bw/day for non-genotoxic compounds (TeA and TEN) (EFSA, 2016). We applied a similar approach also for BEA and ENNs considering that they are non-genotoxic (TTC = 1.5 μg/kg bw/day) (EFSA 2014; EFSA 2024).

For the risk characterization of mycotoxins that are both carcinogenic and genotoxic such as AFs and OTA we used the margin of exposure (MOE) approach because a HBGV cannot be established (EFSA, 2023). The MOE is presented by the ratio of the benchmark dose lower limit (BMDL) of the mycotoxin and the exposure of the consumer to the mycotoxin as shown in Eq. (2):

$$\text{MOE} = \frac{\text{BMDL10}}{EDI} \quad (2)$$

The $BMDL_{10}$ values were 0.4 μg/kg bw/day for AFs for the incidence of hepatocellular carcinoma in rats (EFSA, 2020a) and 14.5 μg/kg bw/day for OTA for the increased risk of neoplastic effects (EFSA, 2020b). MOEs equal or higher than 10,000 suggest low health risks while MOEs <10,000 indicate potential health concerns and further investigations for risk management measures (EFSA, 2023).

For the cumulative risk assessment of AFs, we applied the total margin of exposure (MOE_T). The MOET was calculated as the reciprocal of the sum of the reciprocals of the individual MOEs and indicated a potential health concern when below 10,000 (Eq. (3)).

$$\text{MOE}_T = 1/(1/\text{MOE}_{AFB1} + 1/\text{MOE}_{AFB2} + 1/\text{MOE}_{AFG1} + 1/\text{MOE}_{AFG2}) \quad (3)$$

For the individual and cumulative risk characterization of the other mycotoxins we used the hazard quotient (HQ), respectively the hazard index (HI). The HQ represents the ratio between the EDI and the HBGV of the mycotoxins (Eq. 4):

$$\text{HQ} = \frac{\text{EDI}}{HBGV} \quad (4)$$

The HI is the sum of the HQs of each mycotoxin used for an assessment group (EFSA, 2019). HQ or HI > 1, indicate that consumers are exposed to non-tolerable levels and potential health effects might occur (EFSA, 2019).

$$\text{HI} = \Sigma\ \text{HQs} \quad (5)$$

2.5. Statistical modelling

Data processing, statistical analysis, and visualizations were performed using R Statistical Software (version 4.5.3).

Age stratification was maintained throughout all analyses to identify vulnerable subpopulations, considering that children typically exhibit higher food consumption relative to body weight and may be more susceptible to certain toxicological effects. This stratification provides the foundation for age-specific dietary recommendations and targeted risk communication strategies.

Hierarchical cluster analysis was employed to identify similarities in risk profiles across different age groups and mycotoxin classes, allowing for the identification of specific consumer groups with unique exposure profiles.

To move beyond individual mycotoxin assessments and provide holistic risk evaluation, an integrated risk profiling approach was developed. This methodology combined all risk indicators (HQ, HI, MOE, and MOET) into unified analyses, recognizing that consumers are simultaneously exposed to multiple mycotoxins with varying toxicological profiles. For each combination of age group, country, food category, and substitution level, individual exposures were classified as either exceeding safety thresholds (HQ/HI ≥ 1 or MOE/MOET < 10,000) or remaining within acceptable limits.

Overall risk trends were visualized to show how the proportion of concerning exposures changes across age groups and substitution levels, with separate country comparisons revealing whether Italy or the UK exhibits systematically higher risk profiles.

3. Results

3.1. Dietary exposure

3.1.1. Exposure patterns in Italy

The EDIs for both Italy and the UK are presented in Figs. 1 and 2 while tabular data are presented in Supplementary Material 4.

Under the maximum exposure scenario (UB, 100% substitution), aflatoxin EDIs increased substantially, especially in the combined PBMAs + PBBs scenario. The highest aflatoxin exposure was observed for AFG_1, reaching 0.027 μg/kg bw/day in Italian toddlers based on the combined consumption of PBMAs + PBBs (SBB + VBMA).

OTA EDI was as low as 0.00005 μg/kg bw/day for adults in the (LB, 25% substitution) and as high as 0.011 μg/kg bw/day for infants (UB, 100% substitution of meat and milk with PBMAs + PBBs).

Under UB and full substitution, DON reached one of the highest EDIs in the dataset at 0.13 μg/kg bw/day in toddlers.

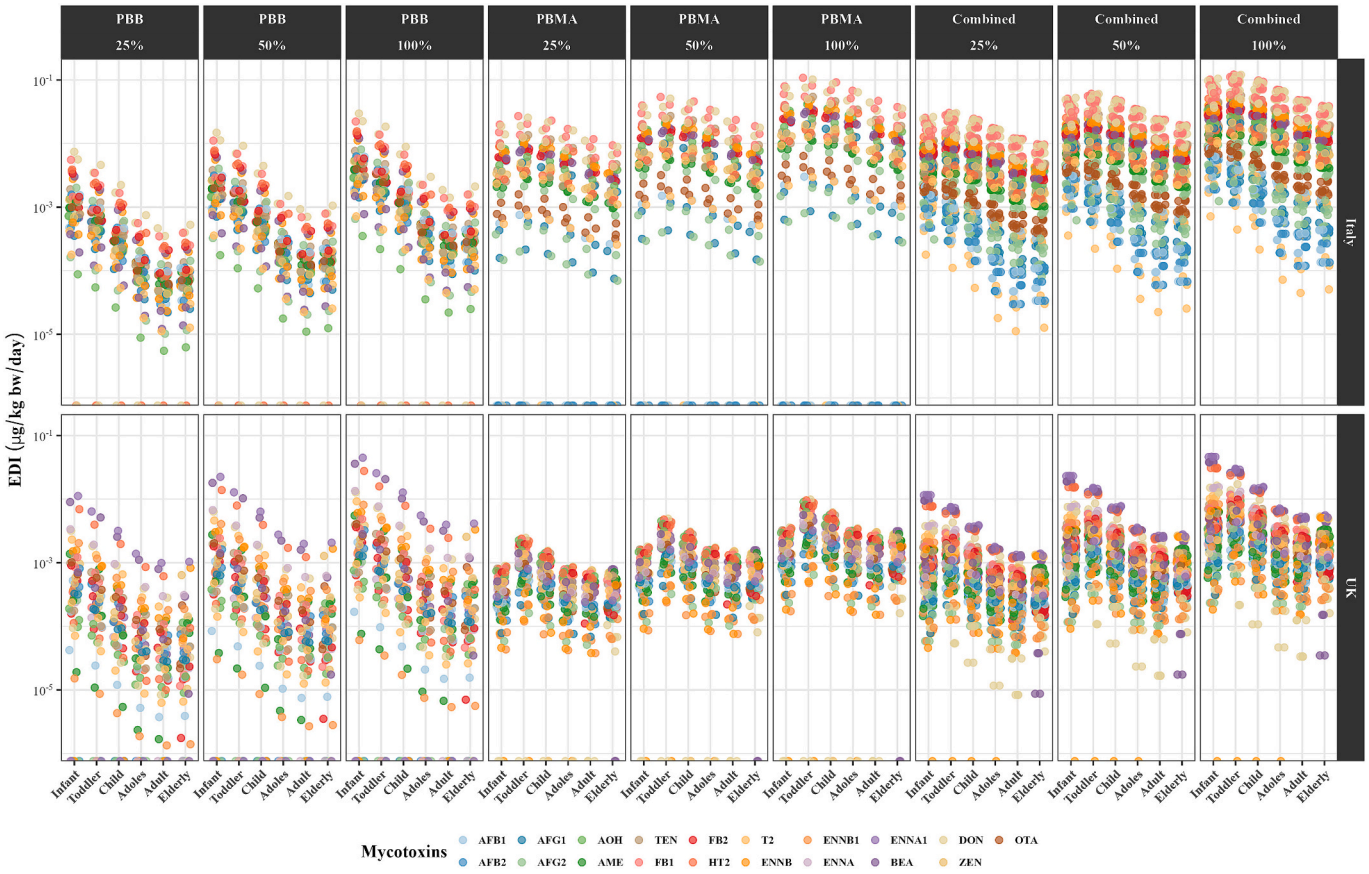

Fig. 1. Estimated daily intake (EDI) of mycotoxins due to consumption of PBBs, PBMAs, and PBMAs + PBBs for Italian and British consumers; 25%, 50%, 100% = substitution scenarios of meat and milk consumption with PBMAs and PBBs; each point represents one mycotoxin-age-group-food category combination; each colour represents a different mycotoxin.

FUMs showed a minimum EDI of 0.00006 µg/kg bw/day for adults (LB, 25% substitution), while the maximum EDI reached 0.13 µg/kg bw/day in toddlers (UB, 100% substitution).

For T-2 and HT-2 toxins, under UB conditions EDIs reached 0.07 µg/kg bw/day for toddlers.

ZEN showed minimal exposure under LB conditions at 0.00004 µg/kg bw/day but reached a maximum EDI of 0.023 µg/kg bw/day in Italian infants under full substitution and combined consumption of PBMAs + PBBs (SBB + VBMA).

Emerging mycotoxins like BEA and ENNB showed a minimum EDI of 0.00001 µg/kg bw/day under LB assumptions but reached 0.055 µg/kg bw/day in infants under UB and 100% substitution.

Alternaria toxins maximum EDIs reached 0.060 µg/kg bw/day in infants.

3.1.2. *Exposure patterns in the UK*

In the UK population, aflatoxin EDIs were minimal, with AFB1 showing the lowest EDI values (0.00001 µg/kg bw/day) across several age groups. For aflatoxins, the highest exposure was for AFG2 at 0.0050 µg/kg bw/day (UB, 100% substitution) for infants and toddlers when assuming consumption of PBMAs + PBBs (OBB + LVBMA).

Exposure to OTA varied between 0.00002 and 0.0062 µg/kg bw/day considering minimum and maximum substitution scenarios.

For DON, minimum EDIs were extremely low ~0.00008 µg/kg bw/day (LB, 25% substitution) while maximum EDIs reached 0.018 µg/kg bw/day for British toddlers (UB, 100% substitution).

FUMs EDI ranged from 0.0002 µg/kg bw/day for elderly based on SBB consumption and 0.012 µg/kg bw/day for toddlers due to combined consumption of PBMAs + PBBs (SBB + LCBMA).

EDIs for T-2 and HT-2 were between 0.00006 and 0.031 µg/kg bw/day, respectively, the higher exposure being noticed for infants and toddlers considering combined consumptions of PBB + PBMA.

ZEN reached a maximum EDI of 0.0074 µg/kg bw/day (ABB+LVBMA) for British children which is lower than the EDIs for Italian children.

BEA and ENNs EDIs were between 0.00001 and 0.052 µg/kg bw/day while exposure to *Alternaria* toxins was lower at 0.00001–0.011 µg/kg bw/day.

As observed for Italy, combined PBMAs + PBBs consumption resulted in the highest EDIs across all mycotoxins, particularly under upper-bound assumptions. Infants and toddlers were systematically the most exposed age groups.

Italian consumers generally exhibited higher EDIs than British consumers, particularly for AFs, DON, and FUMs, while British consumers were more exposed to OTA, HT2 + T2, BEA, and ENNs. These differences were most pronounced under upper-bound assumptions and full substitution scenarios, reflecting higher consumption levels of plant-based beverages and combined substitutes in younger age groups. Despite these differences, the relative contribution of individual mycotoxins and food categories to total exposure remained similar across both countries.

3.2. *Risk characterization for Italian consumers*

For the cumulative exposure to mycotoxins, we characterized the risk of genotoxic *Alternaria* toxins (AOH + AME), HT2 + T2, ENNs, and FUMs using the hazard index. For individual exposure to certain mycotoxins, we used the hazard quotient. For the total exposure to aflatoxins, we used the total margin of exposure, while for OTA we used the margin of exposure (Figs. 3 and 4).

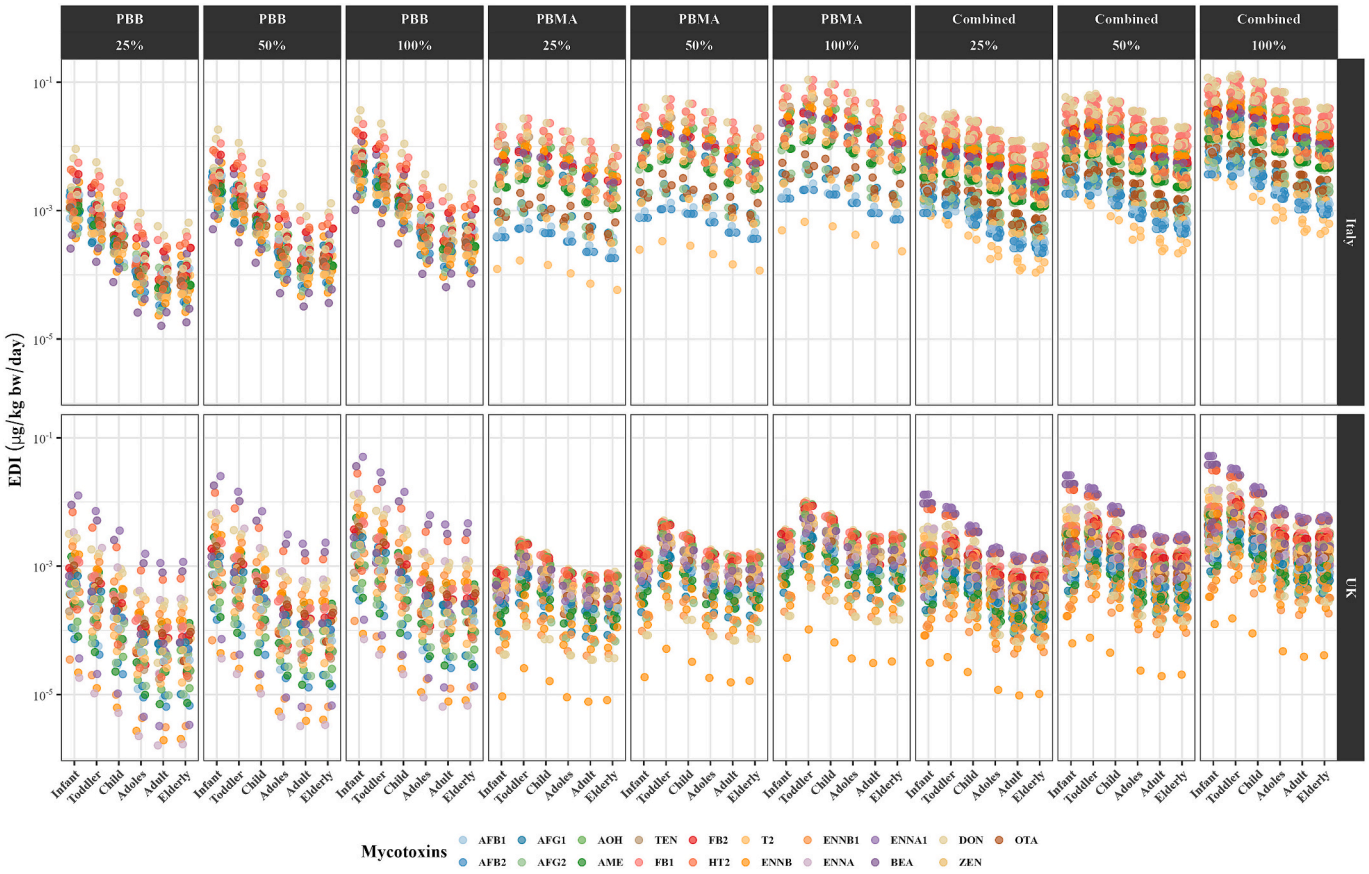

Fig. 2. Estimated daily intake (EDI) of mycotoxins due to consumption of PBBs, PBMAs, and PBMAs + PBBs for Italian and British consumers; 25%, 50%, 100% = substitution scenarios of meat and milk consumption with PBMAs and PBBs; each point represents one mycotoxin-age-group-food category combination; each colour represents a different mycotoxin.

Exposure to AFs indicated MOET <10,000 across all foods, age groups, and substitution scenarios, indicating that even at low exposure levels, it can lead to potential health risks. Although not an indicator of risk magnitude, some MOEs were 800 times lower than the MOE reference value (10,000), the lowest values being recorded for the younger consumer groups.

In the 25% substitution scenario for PBBs and PBMAs consumption, exposure to OTA indicate a low risk except for infants (PBMAs consumption). Adolescents, adults, and the elderly were within the safe threshold even when considering higher substitution scenarios. However, when considering PBBs + PBMAs consumption coupled with the gradual increase of the substitution levels (50 and 100%), MOEs were <10,000 especially for children. This highlights that a partial transition to PBBs or PBMAs alone would not contribute to significant OTA exposure, especially for adults.

Exposure to DON, ZEN, ENNB, BEA, TEN, and FUMs was tolerable across all age groups and substitution scenarios, indicating that even at higher consumption assumptions, the low contamination levels would lead to a negligible exposure.

However, exposure to *Alternaria* toxins (AOH + AME) and HT2 + T2 indicated a non-tolerable exposure especially for younger consumer groups such as toddlers, infants, children, and adolescents (Fig. 5). Most exposures exceeded the safety threshold when considering both a partial substitution (25%, 50%) and a full substitution (100%) of meat and milk with PBMAs + PBBs or PBMAs.

Full substitution scenarios for adolescents, adults, and the elderly indicated exposures that may lead to potential health risks especially when considering the combined consumption of PBMAs + PBBs.

3.2.1. *Risk characterization for British consumers*

The cumulative exposure to AFs indicated a potential health risk regardless of exposure scenario, substitution level, and age group (MOET <10,000). This is consistent with the exposure trends for Italian consumers. The only exceptions are the lower bound scenarios considering only the substitution of milk with PBBs for adolescents, adults, and the elderly where exposure to AFs was unlikely to pose a concern (Fig. 3).

Exposure to OTA was tolerable only under 25% milk replacement with PBBs and in some lower-bound scenarios; MOEs fell below 10,000 mainly in younger age groups at higher substitution levels.

Comparable to the results for Italian consumers, even a full replacement of milk with PBBs would not lead to potential health risks concerning OTA exposure for adolescents, adults, and the elderly. Exposure from PBMAs and PBMAs + PBBs would however exceed the safety threshold. OTA exposure for adults and the elderly indicated potential health risks mainly when considering a 100% substitution scenario and consumption of PBMAs + PBBs (i.e., OBB + LVBMA, SBB + LVBMA, etc.).

Similar to the Italian exposure scenario, FUMs, DON, BEA, ENNs, ZEN, TEN were well below the health-based guidance values across all scenarios (HQ and HI < 1) (Fig. 6).

Partial substitution with PBBs was generally tolerable, whereas PBMAs and PBMAs + PBBs greatly increased *Alternaria* toxins and HT2 + T2 exposure, especially in infants and toddlers. For adolescents, adults, and the elderly exposure was non-tolerable only when considering a 100% substitution scenario of meat and milk with PBMAs + PBBs, indicating potential health risks of a full transition to plant-based alternatives.

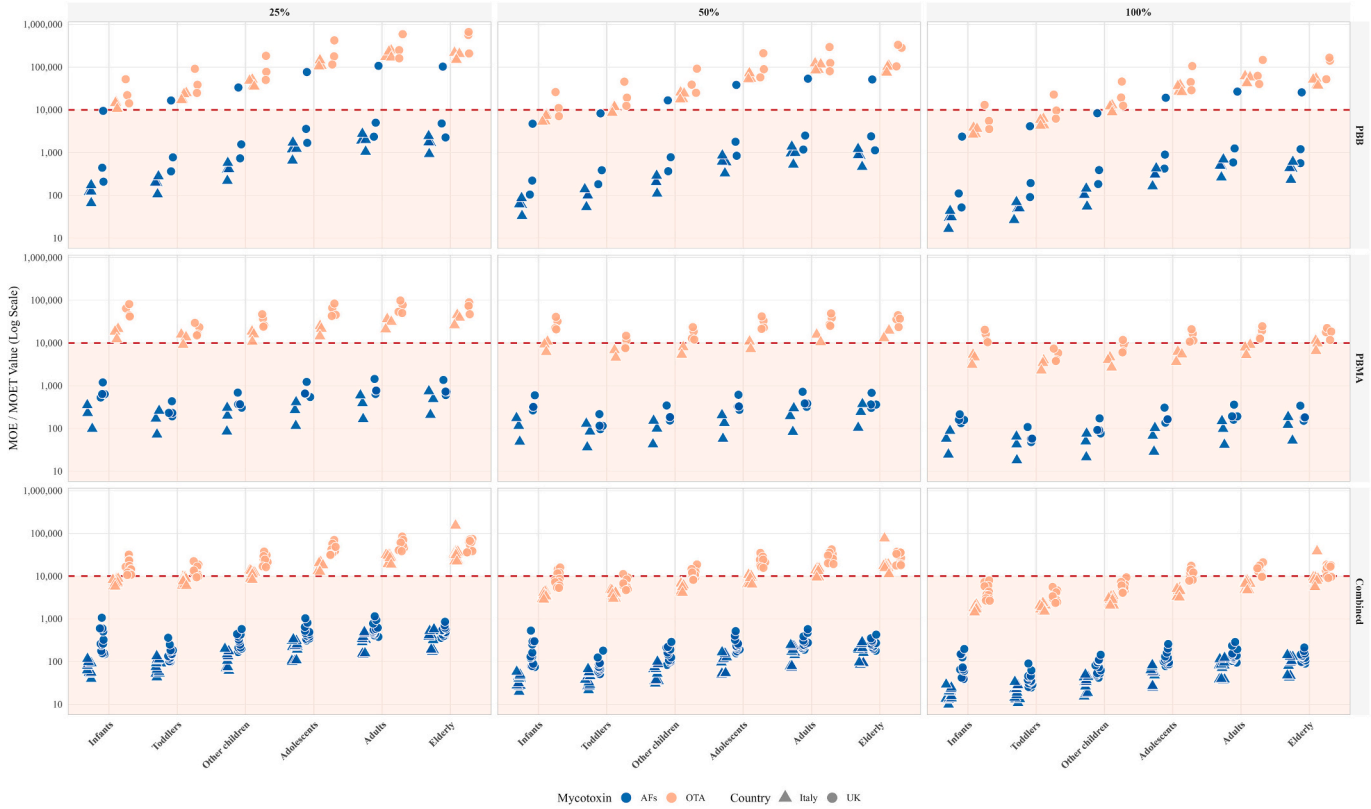

Fig. 3. Risk characterization in the lower bound scenario for the exposure to AFs and OTA considering 25%, 50%, and 100% replacement of meat and milk with PBMAs, PBBs, and PBMAs + PBBs; MOE/MOET = (total) margin of exposure expressed on log scale; the red dashed line indicates the safety threshold; each point represents one mycotoxin-age-group-food category combination; colors indicate the mycotoxins (AFs, OTA); the shape indicates the country. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3. Risk profiling for AFs and OTA in Italy and the UK

Hierarchical clustering was performed considering the lower-upper bound scenarios, 25%/50%/100% substitution levels, six age groups and the MOET and MOE values from AFs and OTA exposure (Supplementary Figures are in Supplementary Material 5). These mycotoxins provided the strongest contrast for clustering. For AFs, almost every population cluster in Italy and the UK were below safety thresholds. In contrast, OTA displayed a targeted risk, with the clustering partitioning the population into low-risk (adults, elderly) and high-risk (infants, children) categories.

Hierarchical clustering of MOET due to exposure to AFs revealed that risk stratification is driven by a synergy between age and substitution level. In both countries, infants, toddlers, and other children clustered at the lowest MOET values under 50% and 100% substitution. In the UK, the 25% substitution of milk with PBBs (LB) was the only low-risk cluster for adolescents, adults, and the elderly. Notably, all modeled AFs scenarios in Italy and nearly all in the UK remained below the 10,000 MOET threshold.

For OTA exposure in Italy, clustering isolated infants, toddlers, and children into a high-risk group, where MOE values were below the safety threshold. However, unlike aflatoxins, exposures for adults and the elderly were in the low-risk exposure range, particularly at 25% and 50% substitution levels and in some cases even at a 100% substitution scenario.

In the UK, the clustering is centered on toddlers at 100% substitution, together with infants and other children at 100%, and then extends into other 50% substitution scenarios. By contrast, adolescents, adults, and elderly at 25% substitution form the clearest low-risk cluster with the highest MOE values. The risk is concentrated in the youngest consumers under the highest replacement scenarios, while older groups remain comparatively protected, especially at 25% substitution.

Comparative analysis between Italy and the United Kingdom demonstrated consistent age-substitution-driven reductions in MOET values for aflatoxins across both countries. However, MOET levels were generally lower in Italy, suggesting higher baseline exposure likely driven by dietary structure and consumption patterns. These findings reflect increased use of cereal- and legume-based ingredients in plant-based alternatives, commodities which are susceptible to fungal contamination. Our assessments highlight the importance of mycotoxin surveillance during dietary transitions toward plant-based alternatives, especially for vulnerable populations.

3.4. Risk trends across age groups, substitution levels, and countries

The impact of dietary transition and substitution of meat and milk products with their plant-based counterparts is presented in Fig. 7 and Fig. 8. We chose to compare 25% and 100% substitution scenarios as these fall under undergoing dietary patterns such as flexitarianism and a more extreme plant-based focused diet like veganism. Key targets were cumulative exposure to *Alternaria* toxins (AOH + AME), HT2 + T2, and the carcinogenic mycotoxins AFs and OTA as these were the primary contributors to threshold exceedances in our study.

For *Alternaria* toxins, HI values under the PBB-only scenario remained below the safety threshold of 1 across most age groups under both LB and UB assumptions. However, substitution involving PBMAs exceeded the safety threshold for all age groups in Italy. The combined PBMAs + PBBs scenario revealed a notable change in risk when comparing 25% to 100% substitution especially for young British consumers and across all age groups for Italian consumers (HI > 1). This indicates that full substitution substantially increases mycotoxin exposure relative to partial substitution.

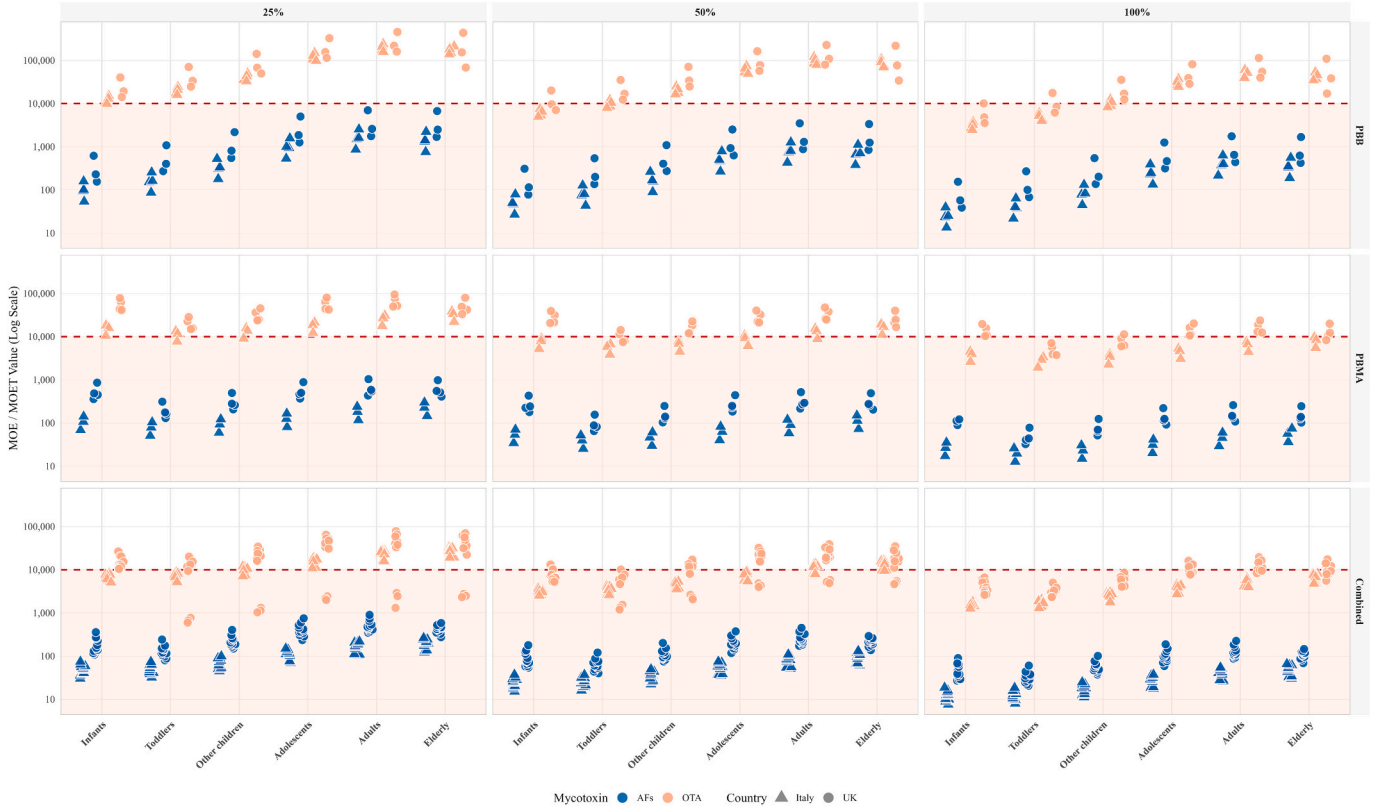

Fig. 4. Risk characterization in the upper bound scenario for the exposure to AFs and OTA considering 25%, 50%, and 100% replacement of meat and milk with PBMAs, PBBs, and PBMAs + PBBs; MOE/MOET = (total) margin of exposure expressed on log scale; the red dashed line indicates the safety threshold; each point represents one mycotoxin-age-group-food category combination; colors indicate the mycotoxins (AFs, OTA) the shape indicates the country. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

A similar pattern was observed for HT-2 + T-2. PBBs substitution was associated with the lowest HI values, generally remaining below or close to the threshold, whereas PBMAs and PBMAs + PBBs scenarios produced higher exposures and a clearer progression across age groups. In Italy, the youngest consumers showed the highest HI values, with risk decreasing progressively toward adults. In the UK, the same age gradient was present, although the magnitude of increase was less pronounced than in Italy.

Across both mycotoxin groups, the magnitude of risk amplification between 25% and 100% substitution was higher in PBMAs and PBMAs + PBBs scenarios, indicating that cereal- and legume-based matrices contribute disproportionately to cumulative exposure.

Fig. 8 presents the MOE/MOET values for Aflatoxins (AFs) and Ochratoxin A (OTA) across two substitution scenarios (25% and 100%) of meat and milk with PBBs, PBMAs, and PBMAs + PBBs under both LB and UB assumptions.

For AFs, MOET values in the PBBs dietary scenario remained below the safety threshold of 10,000 for all age groups in Italy. However, under LB conditions, adolescents, adults, and elderly British consumers were within safe exposures levels when considering 25% substitution scenarios. For the upper bound scenario and consumption of either PBMAs or PBMAs + PBBs MOETs were < 10,000 across the board.

For OTA, MOE values were generally higher than those observed for AFs. The lowest MOE values were observed for Italian consumers. While 25% substitution scenarios indicate low risks for British consumers, the picture is reversed when the transition increases to 100% and exposures lead to MOEs <10,000 for most consumers.

This indicates that plant-based meat alternatives, and diets combining both plant-based beverages and meat alternatives, drive the largest increases in our modeled mycotoxin-related risk. Older age groups generally tolerated partial substitution better than younger groups, whereas full replacement scenarios produced substantially less favorable risk profiles, particularly in Italy and in younger UK consumers.

To provide a comprehensive evaluation of consumer exposure to mycotoxins, we developed an integrated risk profiling approach that combines all risk characterization methods (Hazard Quotients, Hazard Indices, Margin of Exposure, and Total Margin of Exposure) into a unified analysis framework. Each individual exposure scenario (combination of country, age group, food category, mycotoxin, and substitution level) was classified according to whether it exceeded established safety thresholds or not (HI/HQ > 1; MOET/MOE ⟨10,000).

Fig. 9 presents the overall risk trends comparing Italy and the United Kingdom across the six consumer age groups and three substitution scenarios. The percentage of exposures exceeding safety thresholds demonstrated clear age-dependent patterns in both countries, with younger consumer groups consistently exhibiting higher proportions of exceedances compared to adults.

In Italy, the percentage of threshold exceedances ranged from 17.9% (adults at 25% substitution) to 38.4% (infants at 100% substitution). In the UK, the corresponding range was between 9.5% - 28.9% across the same age-substitution combinations. Across both countries the level of substitution was associated with safety threshold exceedance, with increases from 25% to 100% substitutions indicating substantial amplification of cumulative risk indicators.

4. Discussion

This study presents one of the first large-scale multi-mycotoxin risk assessments modelling dietary transitions toward plant-based meat alternatives (PBMAs) and plant-based beverages (PBBs) in Italy and the UK. By combining occurrence data, substitution modelling, age

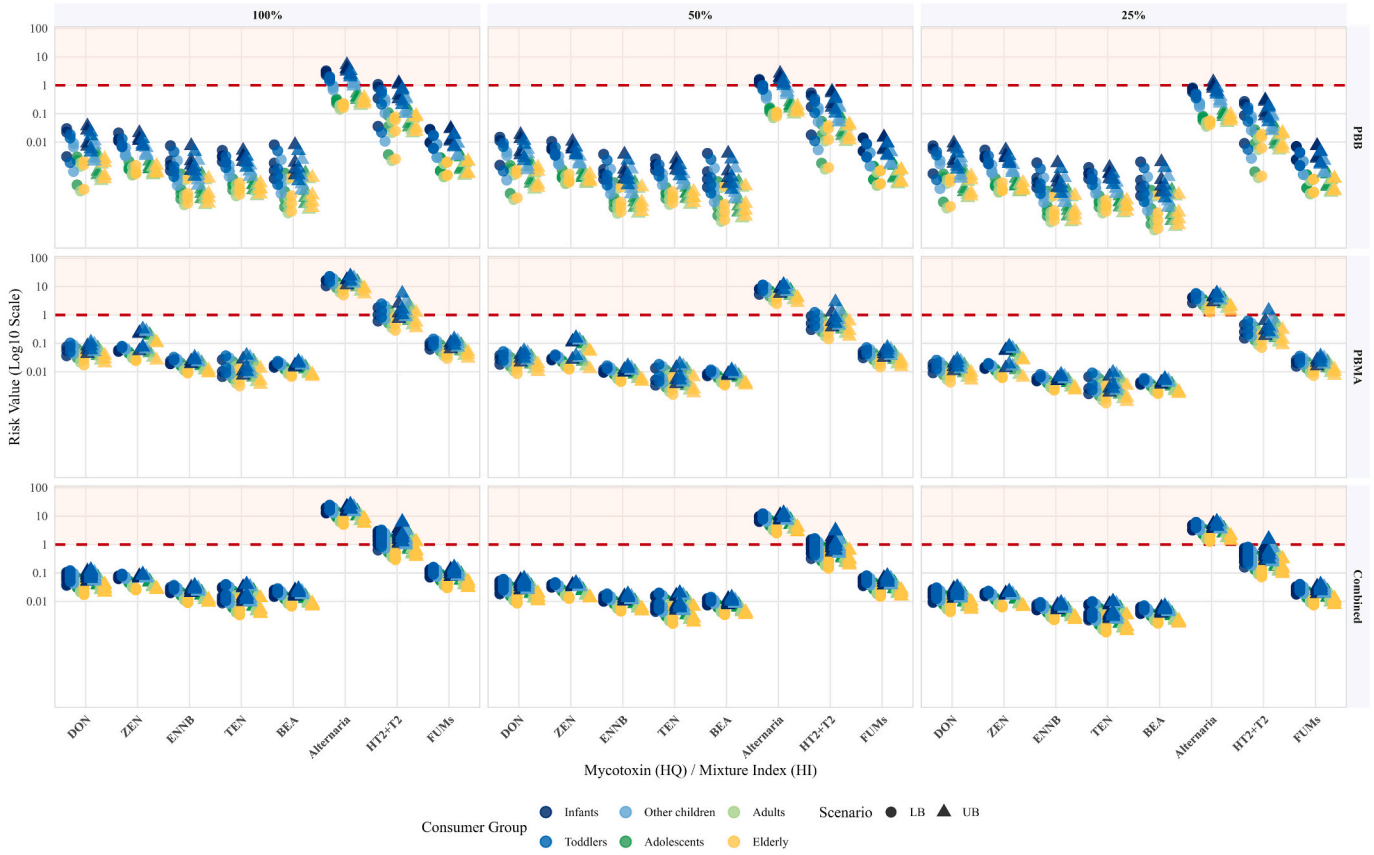

Fig. 5. Risk characterization for Italian consumers for the exposure to non-carcinogenic mycotoxins considering 25%, 50%, and 100% replacement of meat and milk with PBMAs, PBBs, and PBMAs + PBBs; HQ/HI = hazard quotient/hazard index; the red dashed line indicates the safety threshold; each point represents one mycotoxin-age-group-food category combination; colors indicate the different age groups; the shape indicates the scenario (LB = lower bound; UB = upper bound). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

stratification, and cumulative risk metrics, we show that increasing replacement of animal-source foods with plant-based alternatives may modify mycotoxin exposure patterns. Estimated intakes increased with substitution levels, and the highest exposures were from the combined PBMAs + PBBs scenarios consumption and upper-bound assumptions.

Aflatoxins resulted in MOET values below 10,000 across most scenarios, indicating continued regulatory concern, while ochratoxin A was mainly relevant for younger populations under high substitution levels. Among non-carcinogenic mycotoxins, *Alternaria* toxins (AOH + AME) and HT-2 + T-2 toxins were key drivers for most hazard index exceedances. In contrast, exposure to DON, TEN, ZEN, FUMs, ENNs, and BEA generally remained within health-based guidance values. Partial substitution (25%) was typically associated with tolerable exposure, whereas full substitution (100%) substantially increased exceedances, particularly in children.

4.1. Comparison with previous literature

A recent study evaluating mycotoxin exposure from seitan products and PBMAs and PBBs from the Belgian and German markets reported exposures higher than in our study, particularly for DON (HQ up to 51.4) and *Alternaria* toxins like AME (HQ > 900) in toddlers (Schneidemann-Bostelmann et al., 2026). These exposure levels are 30–50 times higher than in our study. Our findings indicate lower exposure levels both in Italy and the UK and HQ values below 1 for DON across all substitution scenarios, while exceedances were primarily associated with cumulative exposure to *Alternaria* toxins and HT-2/T-2.

The difference in risk may be related to contamination and consumption patterns as well as the product composition. The contamination levels from the Belgian and German markets were much higher than the data used in this study which includes Italian and British markets as well as a higher pool sample (Mihalache et al., 2024; Torrijos et al., 2025, 2026). Seitan-based products, predominantly derived from wheat gluten, may concentrate *Fusarium* and *Alternaria* contamination linked to cereal matrices. In contrast, the broader matrix diversity of PBMAs and PBBs evaluated in the present study may dilute single-ingredient contamination peaks. In Germany, seitan had the highest contamination levels (DON up to 333 μg/kg and AME up to 28 μg/kg) (Schneidemann-Bostelmann et al., 2026).Previous Italian studies indicated mycotoxin levels were below LOD for seitan (Mihalache et al., 2023) while in PBMAs from Italy DON values ranged between 22.4 and 105.80 μg/kg. Importantly, the analytical sensitivity of the German and Italian studies was comparable, with LOQs of 3.59 and 3.61 μg/kg for DON, respectively. Therefore, the lower contamination observed in the Italian products is unlikely to be explained by differences in analytical sensitivity alone, but rather reflects differences in the products analyzed, raw material composition, and/or contamination levels between the sampled markets.

Another study from Germany highlights the potential health risks from PBBs consumption by applying a similar approach to our study (Dick et al., 2026). Toddlers (1–3 years) were identified as the most vulnerable population group because of their substantially higher intake per body weight. Similar to our findings, in younger age groups aflatoxin B_1 and *Alternaria* toxins exceeded safety thresholds, while T-2 and HT-2 were highlighted as relevant contributors in oat-based beverages (Dick et al., 2026). Even when products complied with current EU maximum levels (AFs in almonds), margins of exposure for AFB1 in toddlers were well below the benchmark value of 10,000, indicating that

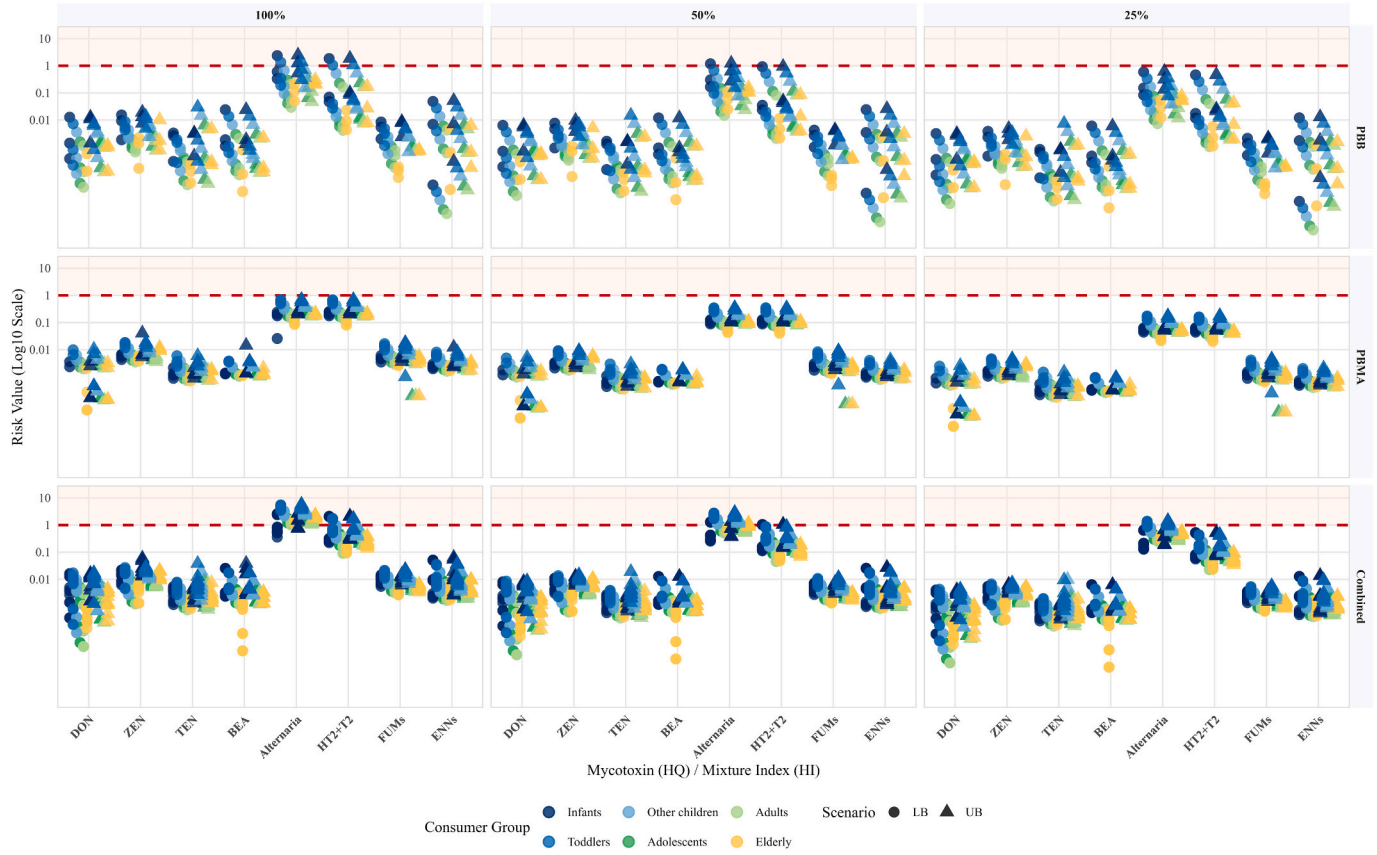

Fig. 6. Risk characterization for British consumers for the exposure to non-carcinogenic mycotoxins considering 25%, 50%, and 100% replacement of meat and milk with PBMAs, PBBs, and PBMAs + PBBs; HQ/HI = hazard quotient/hazard index; the red dashed line indicates the safety threshold; each point represents one mycotoxin-age-group-food category combination; colors indicate the different age groups; the shape indicates the scenario (LB = lower bound; UB = upper bound). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

regulatory compliance does not necessarily equate to negligible risk in high-consumption subgroups.

A parallel evaluation by the German Federal Institute for Risk Assessment (BfR (Bundesinstitut für Risikobewertung), 2024) concluded that children may approach health-based guidance values under certain contamination and intake conditions of PBBs, although uncertainties in representativeness prevented definitive conclusions on chronic risks. The BfR report concluded that consumption of almond drinks leads to exposure levels of AFs that may "cause health impairments in children in the age group of 0.5 to < 6 years old with a medium likelihood" (BfR (Bundesinstitut für Risikobewertung), 2024).

A risk ranking of mycotoxins in PBMAs and PBBs from the Belgian market highlights that AFs and genotoxic *Alternaria* toxins (AOH + AME) should be prioritized by risk assessors and managers (Schryvers et al., 2025). The study revealed that potential risk thresholds could be exceeded with as little as 0.3–1.4% replacement of meat consumption for sum AFs and AME, respectively. While our UK and Italian data do not suggest such extreme margins, the pattern remains consistent: aflatoxins and *Alternaria* toxins drive risk prioritization under substitution scenarios.

All studies share similar findings that children represent the most vulnerable population group due to higher food intake per body weight. This age-dependent risk amplification was observed consistently across Italy, the UK, Germany, and Belgium.

4.2. Food safety and legislative implications for dietary transition and sustainability policies

Dietary guidelines promoting plant-forward diets, including those proposed by the EAT-Lancet Commission, emphasize environmental and health benefits (Rockström et al., 2025). However, as food systems transition, exposure patterns to naturally occurring contaminants may shift. Our findings highlight the need for adaptive food safety monitoring aligned with evolving dietary patterns.

Cereal- and legume-based ingredients are the main components of PBMAs and PBBs which are known to be susceptible to fungal contamination. As the market for alternative proteins increases, rigorous raw material screening, optimized storage, and targeted surveillance for vulnerable subpopulations (i.e., children) become increasingly important.

Maximum levels for certain mycotoxins are established at EU level under Commission Regulation (EC) 2023/915 (Commission Regulation (EC), 2023) and applied also in the UK. However, plant-based meat alternatives (PBMAs) and plant-based beverages (PBBs) are not regulated. While cereals for human consumption are generally monitored, among legumes only soybeans are monitored (for OTA), despite evidence that other pulses are also susceptible to toxigenic fungi (Acuña-Gutiérrez et al., 2022). Given the growing consumption of legumes and their widespread use in plant-based formulations, significant data gaps remain regarding mycotoxin occurrence in pulses and other vegetables used in the manufacturing of PBMAs (Mihalache, Dellafiora and Dall'Asta, 2022a).

Exposure to *Alternaria* toxins was already known to be higher in vegan populations at the European level in the past decade (EFSA (European Food Safety Authority), 2016), therefore our results showing non-tolerable levels for AOH + AME were expected. *Alternaria* toxins are currently not legislated in the EU, however the European Commission published indicative levels for certain foodstuff including

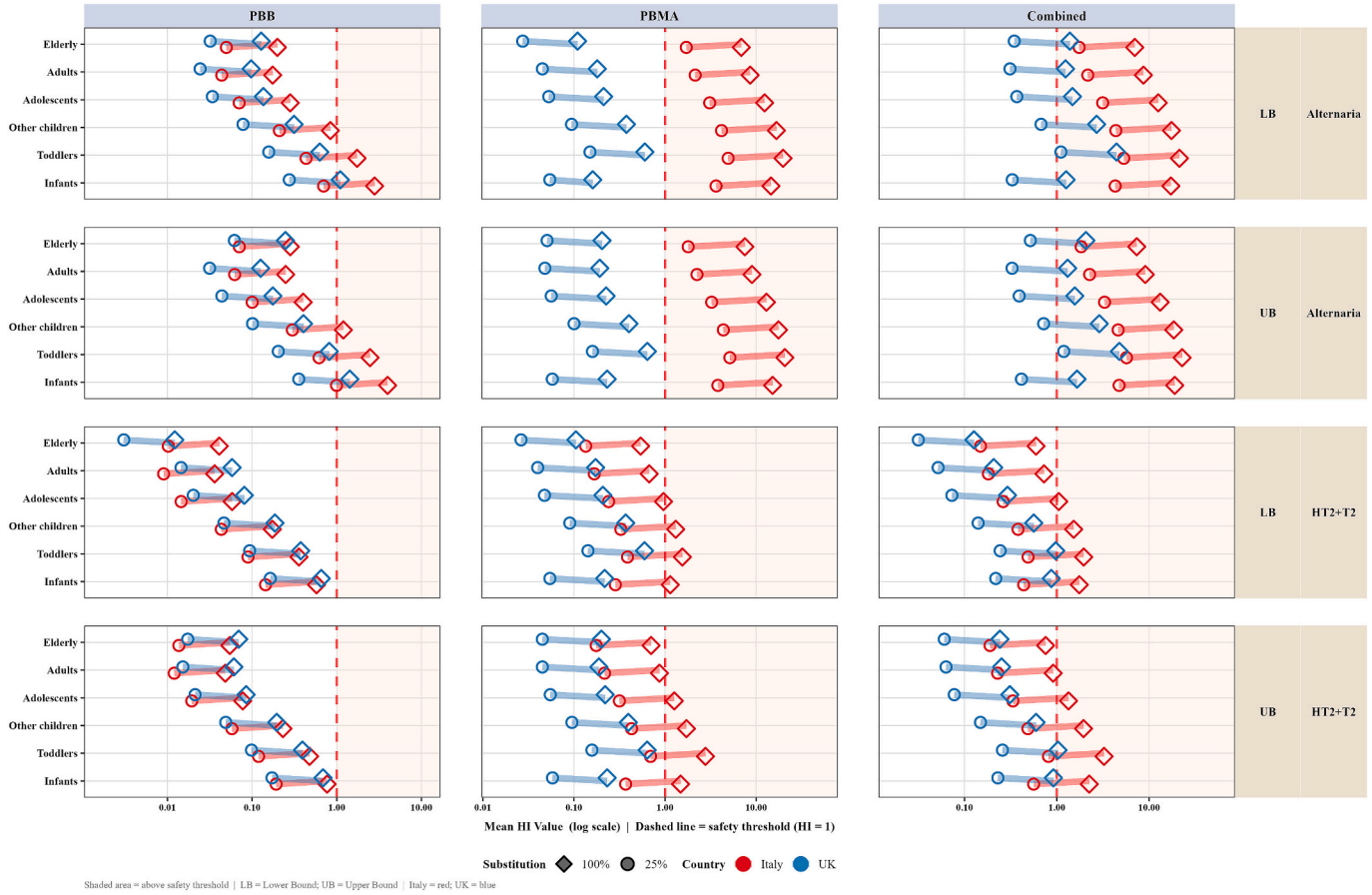

Fig. 7. Dumbbell plot showing the magnitude of risk from exposure to *Alternaria* (AOH + AME) and HT2 + T2 based on 25% and 100% transition to PBMAs and PBBs: LB = lower bound; UB = upper bound; the red dashed line indicates the safety threshold; each point represents one mycotoxin-age-group-food category combination; colors indicate the country; the shape indicates the substitution scenarios (25% and 100%). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

processed tomato products, tree nuts, paprika, sesame seeds, sunflower seeds and oil, and cereal-based foods for infants (European Commission, 2022).

In Belgium, soy-based PBMA resulted in the highest exposure for AFs and AME, while oat- and almond-based beverages were most relevant for aflatoxin exposure among plant-based dairy alternatives (PBDA) (Schryvers et al., 2025). Soybeans (*Glycine* max) are widely used in PBMA/PBDA due to their high protein content (35–40%) and functional properties (Day et al., 2022; van den Berg et al., 2022). EFSA (2020a) reported mean concentrations of 0.17–0.78 μg/kg for AFB1 in soybeans, demonstrating that raw material contamination remains a plausible contributor among other legumes. A modelling study also found that the replacement of soy-based meat alternatives increases the potential burden of disease due to increased exposure to AFs (Mihalache, Dellafiora and Dall'Asta, 2022b).

PBMAs and PBBs lead the market of plant-based foods in Europe (Smart Protein Project, 2021) and the exposure to mycotoxins is expected to grow as more consumers shift to more sustainable diets while the regulatory framework is not updated.

Conventional treatments (cooking, roasting, baking, pasteurization) have limited impact on mycotoxin reduction, and little is known about extrusion or hydrothermal processing used in alternative protein production. While some degradation may occur, the formation of bound (masked) mycotoxins cannot be excluded (Campagnollo et al., 2016; Liu et al., 2020). Environmental factors such as humidity and temperature further influence fungal growth and toxin production during cultivation and storage, yet data for legumes and pulses remain scarce (Ferrigo et al., 2016; Garcia-Cela et al., 2018; Medina et al., 2017; Nesic et al., 2015; Perincherry et al., 2019).

Current EU and UK regulations define maximum levels primarily for raw commodities rather than finished plant-based alternatives. As PBMAs frequently combine multiple cereal- and legume-derived ingredients, cumulative contamination trends may not be properly captured by current regulations. Consequently, the contribution of PBMAs and PBMAs + PBBs to dietary mycotoxin exposure in alternative diets is significant.

Our findings further highlight the need to update national food consumption surveys to explicitly capture plant-based alternatives within dietary records and food frequency questionnaires. Accurate consumption data would support future risk assessments and avoid under- or over-estimation of dietary exposure to chemical contaminants.

A safe-by-design approach that ranks hazard–ingredient combinations (van Asselt et al., 2024) during product formulation to support proactive mitigation strategies coupled with updated regulatory frameworks may be needed to reduce overall exposure levels and ensure a safe transition to plant-based diets.

We provide support to concerns previously highlighted by the Food and Agriculture Organization (FAO) which emphasized that large-scale dietary transitions toward plant-based foods may reshape mycotoxin exposure patterns and require adaptive food safety management frameworks (FAO and WHO, 2019; FAO, 2022). Our modelling study demonstrates that substitution-based shifts can increase exposure to specific mycotoxins, particularly in vulnerable age groups, highlighting the need to integrate chemical risk assessment into dietary transitions.

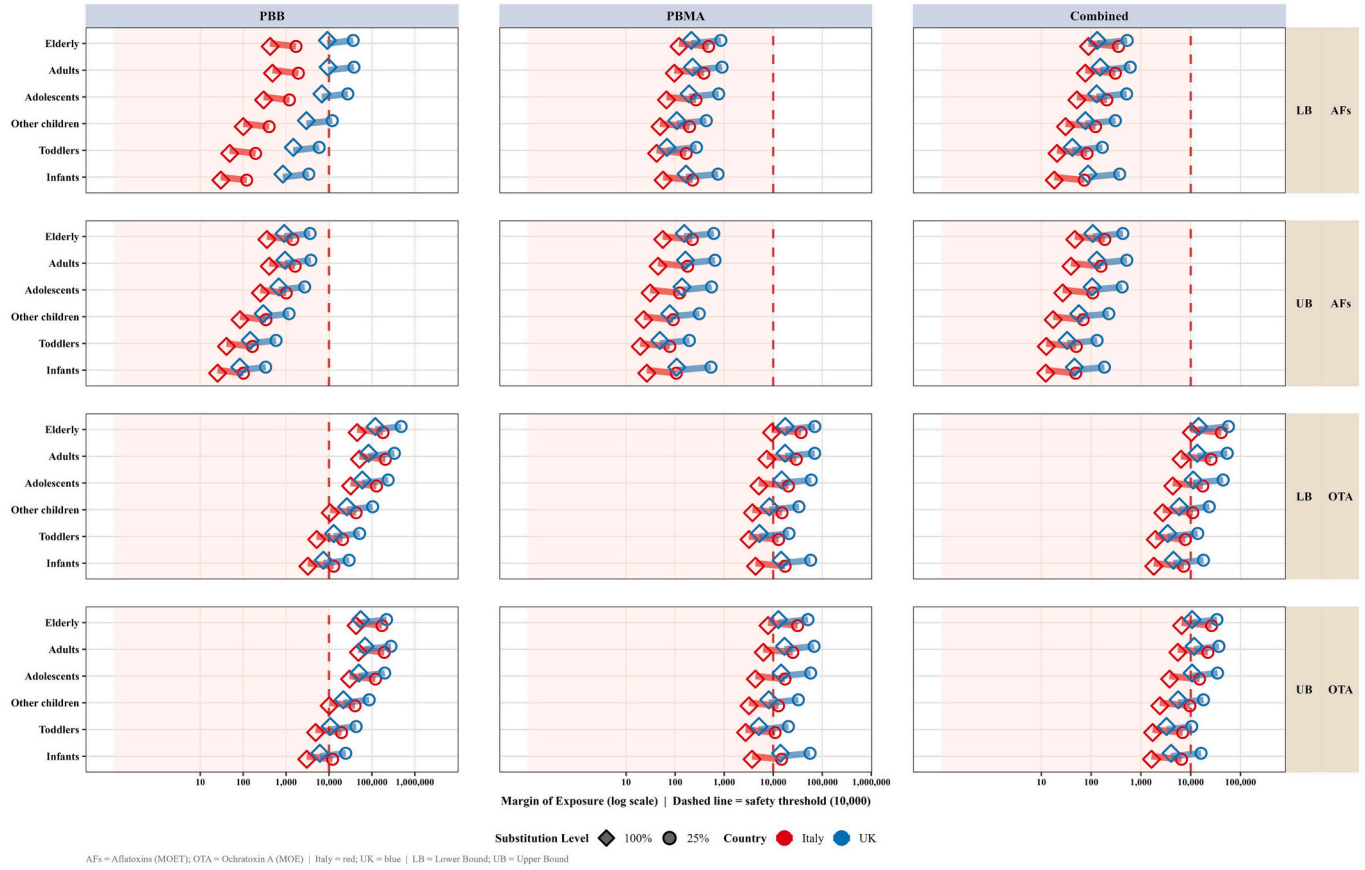

Fig. 8. Dumbbell plot showing the magnitude of risk from exposure to AFs and OTA based on 25% and 100% transition to PBMAs and PBBs: LB = lower bound; UB = upper bound; the red dashed line indicates the safety threshold; each point represents one mycotoxin-age-group-food category combination; colors indicate the country; the shape indicates the substitution scenarios (25% and 100%). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4.3. Study limitations

This study has several limitations that need to be acknowledged. First, the weight-based substitution approach in which the gram-weight of replaced meat or milk is assumed to be fully consumed as a PBMA or PBB may not reflect real-world serving size differences or nutritional equivalence considerations and is best interpreted as a conservative exposure scenario. Second, sample sizes for certain product categories are limited, and occurrence estimates for these matrices should be interpreted with appropriate caution. Third, the deterministic approach based on mean occurrence concentrations may not capture the full distribution of individual-level exposures.

5. Conclusions

This study provides the first cross-country quantitative risk assessment of multi-mycotoxin exposure associated with plant-based meat alternatives (PBMAs) and plant-based beverages (PBBs), based on occurrence data from 413 commercially available products across Italy and the United Kingdom.

In both countries, the estimated dietary exposure increased with substitution level, with combined PBMAs + PBBs consumption scenarios producing the highest risk estimates. Infants and toddlers represented the most vulnerable population groups due to higher intake per body weight. While partial substitution scenarios (25%) were generally in line with existing health-based guidance values for adolescents and adults, full substitution scenarios (100%) led to non-tolerable exposures for AFs, OTA, and *Alternaria* toxins (AOH and AME), and HT2 + T2 toxins.

Our findings indicate that large-scale dietary shifts may alter mycotoxin exposure patterns if contamination levels remain unchanged. These results highlight the need to integrate food safety considerations into the ongoing sustainability-focused dietary transitions.

Future work should incorporate processing fate studies and biomonitoring data to improve exposure assessments and reduce uncertainty. As plant-based alternatives continue to expand within European markets, ensuring that sustainability transitions remain aligned with food safety objectives will be essential.

CRediT authorship contribution statement

Octavian Augustin Mihalache: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Formal analysis, Conceptualization. **Raquel Torrijos:** Investigation, Formal analysis, Data curation. **Andrea Patriarca:** Writing – review & editing, Formal analysis, Data curation. **Angel Medina:** Writing – review & editing, Supervision, Funding acquisition. **Chiara Dall'Asta:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

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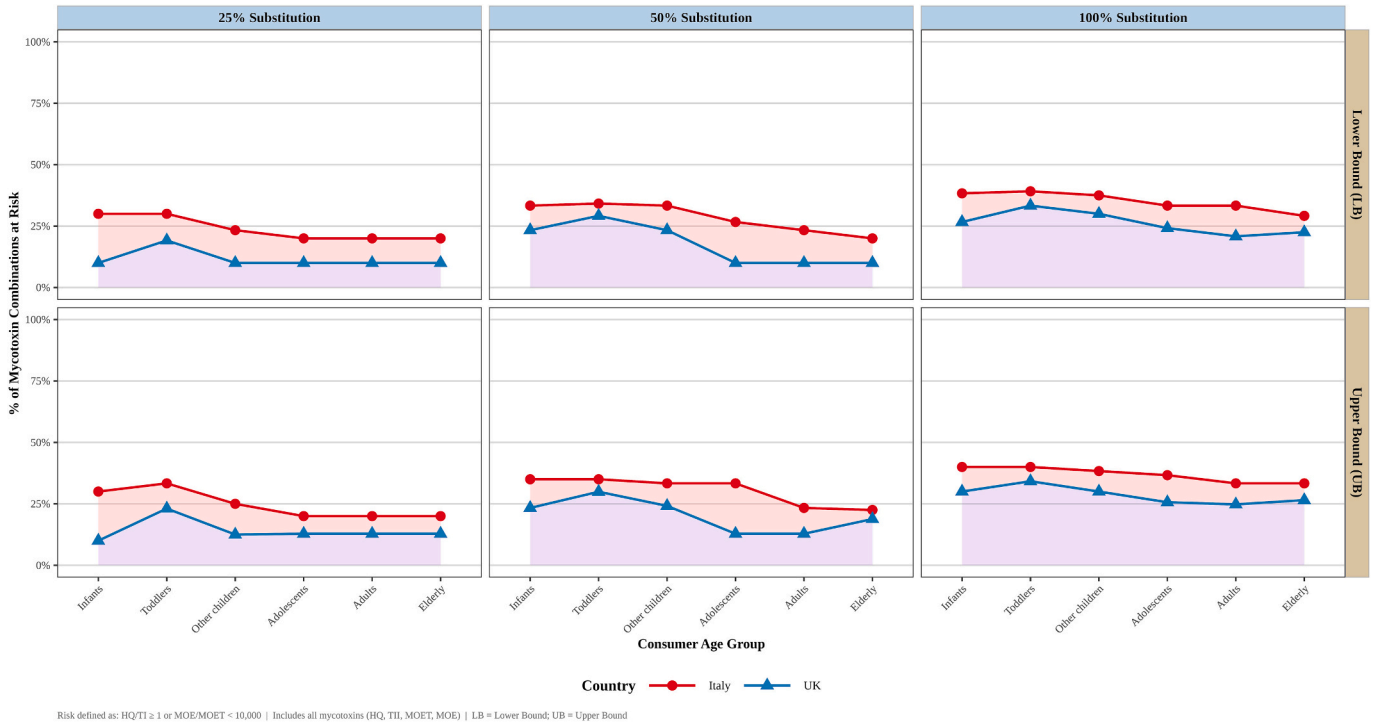

Fig. 9. Percentage of all mycotoxin–food combinations exceeding safety thresholds in Italy and the UK across all consumer age groups in the lower and upper bound scenarios considering all health risks indices such as MOE, MOET, HQ, HI; colors indicate the country - red = Italy; blue = UK; each point represents a mycotoxin-food combination exceeding safety threshold for each consumer group. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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.2026.120167.

Data availability

Data will be made available on request.

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