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Exposure assessment of the European adult population to deoxynivalenol – Results from the HBM4EU Aligned Studies

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ABSTRACT

Mycotoxins are natural toxins produced by fungi that may cause adverse health effects thus constituting a public health concern. Deoxynivalenol (DON), a mycotoxin affecting the immune system and causing intestinal disorders, was selected as a priority under the European Human Biomonitoring Initiative (HBM4EU). Urinary total DON levels (tDON) of 1270 participants from six countries were used to characterize the internal exposure of the adult European population and identify the most relevant determinants of exposure. tDON concentrations' P50 and P95 were in the range of 0.41–10.16 μg/L (0.39–9.05 μg/g crt) and 3.25–46.58 μg/L (2.12–33.50 μg/g crt) respectively. Higher tDON levels were observed for (i) male participants from France and Germany, (ii) samples collected in spring and summer, (iii) participants with a lower educational level, (iv) participants living in rural areas, (v) individuals without a job in France and Luxembourg, while in Portugal higher exposure was observed in working individuals, (vi) individuals with higher consumption of cereals and bread. The proportion of individuals with exposure levels exceeding the HBM-GV of 23 μg/L was 12.3 %, ranging from 0.8 % to 20.7 % in the individual countries. This study on mycotoxins exposure has used post harmonized questionnaire data and validated analytical methodologies for analysis and covered countries representing the four geographical regions of Europe, having produced much needed knowledge on the exposure of the European adult population to deoxynivalenol.

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1. Introduction

Mycotoxins are secondary metabolites naturally produced by fungi that occur worldwide and can contaminate numerous raw materials, feed and food commodities. Contamination can occur at all stages of plant growth, processing and storage and thus ingestion of contaminated food is the main route of exposure to mycotoxins (Bennett & Klich, 2003; Koppen et al., 2010). Since mycotoxins are very stable compounds, they can resist storage and most standard food processing and cooking practices (Marin et al., 2013), which contributes to high levels of exposure. Exposure to these toxins constitutes a public health concern, as they can cause adverse health outcomes, namely carcinogenic, teratogenic, immunotoxic, nephrotoxic and endocrine disrupting effects (Fung & Clark, 2004; Omotayo et al., 2019; Silva et al., 2022). In the upcoming decades climate change is expected to impact fungal growth and geographical distribution leading to an increase in levels of mycotoxins in food commodities and, consequently, in human exposure (Assuncao et al., 2018; Battilani et al., 2016).

Traditionally, exposure to mycotoxins is assessed by combining data on mycotoxin levels in food commodities with food consumption data (collected through e.g. questionnaires, interviews, 24 h recall data), but this approach has some disadvantages since the levels in different foodstuffs may differ significantly and it may be difficult to obtain accurate consumption data. An alternative approach is the use of human biomonitoring (HBM), since this is a method that provides information on the total internal dose of exposure from all sources and via all routes and sources (Arce-López et al., 2020; Escrivá et al., 2017; Habschied et al., 2021).

HBM studies conducted in Europe have shown that the population is exposed to mycotoxins and knowledge on the determinants of this exposure is essential to reduce exposure and prevent possible adverse health effects. In recognition of this issue, under the European Human Biomonitoring Initiative (project HBM4EU co-funded by the participating countries and the Horizon 2020 research and innovation program) and following a prioritization strategy (Ougier et al., 2021) mycotoxins were selected as a prioritized substance group. Moreover, considering that the mycotoxins group comprises a wide variety of compounds, deoxynivalenol (DON) and fumonisin B_1 (FB_1) were selected as priority substances of concern to be investigated under HBM4EU. However, it was decided to analyse only total DON in urine samples of the adult population from the HBM4EU Aligned Studies (more information on the HBM4EU Aligned Studies can be found in (Gilles et al., 2021, 2022; Govarts et al., 2023)).

DON is one of the most common *Fusarium* mycotoxins, commonly found in oats, barley, rye, safflower seeds, wheat, maize and mixed feeds (Bennett & Klich, 2003; EFSA Panel on Contaminants in the Food Chain (CONTAM) et al., 2017), and in cereal-based products such as pasta, bread, pastries, biscuits, cereal snacks, breakfast cereals and cereal-based baby food (Assunção et al., 2018). As such, depending on the dietary habits, human exposure to DON can happen on a daily basis and several times a day. DON is also known as vomitoxin due to the strong emetic effects that can be felt after acute exposure. Results from the BIOMIN Mycotoxin Survey have identified DON as one of the most prevalent mycotoxins found in food commodities and feedstuffs in 2020 (DSM, n.d.), being one of the major foodborne mycotoxins of public health concern. Within HBM4EU, total DON (tDON, sum of free DON and its glucuronides after deconjugation) was selected as DON biomarker of exposure (Alvito et al., 2022).

Although DON is classified by the International Agency for Research on Cancer (IARC) as a group 3 compound (not classifiable as to its carcinogenicity to humans) (IARC, n.d.), a recent study (Claeys et al., 2020) has shown that more in-depth research on the carcinogenicity of mycotoxins, such as DON is needed. Moreover, existing data have demonstrated that DON can affect the immune system, can cause intestinal disorders and can exhibit developmental and reproductive toxicity (Pestka, 2010). In order to protect human health, the European Commission has introduced regulations to minimize exposure to mycotoxins, including DON. The Regulation (EC) N° 915/2023, replacing Regulation (EC) N°1881/2006 (European Commission, 2006), established maximum levels for several mycotoxins (including DON) in several foodstuffs. In addition to DON, the structurally related acetylated DON and modified forms of DON (e.g., plant-conjugates) have been found in the same type of matrices, of which 3-acetyl-DON, 15-acetyl-DON, and DON-3-glucoside are the most relevant ones. In the EFSA opinion, a group-TDI of 1 μg/kg bw per day for the sum of the four DON forms has been set (EFSA Panel on Contaminants in the Food Chain (CONTAM) et al., 2017).

In Europe, DON had been previously detected in HBM studies from Austria (Warth et al., 2012), Belgium (Huybrechts et al., 2015), Croatia (Sarkanj et al., 2013), France (Turner, Hopton, et al., 2010), Germany (Gerding et al., 2014), Hungary (Szabo-Fodor et al., 2021), Italy (Brera et al., 2015; Solfrizzo et al., 2014), Norway (Brera et al., 2015), Portugal (Martins et al., 2019), Spain (Vidal et al., 2016), Sweden (Turner et al., 2017; Wallin et al., 2015) and the UK (Brera et al., 2015; Turner, White, et al., 2010; Wells et al., 2017).

To assess exposure to DON (including 3-acetyl-DON, 15-acetyl-DON, and DON-3-glucoside), urine is the most suited matrix and either DON-15-glucuronide or tDON are suitable biomarkers (Thomsen, Cathrine, 2019; Vidal, Mengelers, et al., 2018). According to a recent EFSA report (EFSA Panel on Contaminants in the Food Chain (CONTAM) et al., 2017), the estimated mean chronic dietary exposure was above the group-TDI in infants, toddlers and other children, and at high levels in adolescents and adults, indicating a potential health concern. Thus, it urges to clarify this issue by characterizing the exposure of European citizens to DON using a harmonised study design.

The European Human Biomonitoring Initiative aimed to collect harmonized and quality controlled interal exposure data on the European population by building on existing HBM capacity in Europe and aligning national or regional HBM studies. The HBM4EU Aligned Studies were developed in three agre groups: children (6–11 years), teenagers (12–19 years) and adults (20–39 years), and biological samples were collected between 2014 and 2021 in 11–12 primary sampling units geographically distributed across Europe (Gilles et al., 2021, 2022; Govarts et al., 2023).

The present study aimed to characterize the current exposure of the European adult population to tDON using HBM data produced under the HBM4EU Aligned Studies, identify possible determinants of exposure and assess the associated risk from exposure.

2. Materials and methods

2.1. Data sources

This study uses data generated by six of the HBM4EU Aligned Studies that recruited adult participants (details on the HBM4EU Aligned Studies can be found elsewhere (Gilles et al., 2022)). HBM4EU Aligned Studies targeted the general population and were aligned concerning the sampling period (2014–2021), age group (20–39 years for the studies in adults), sampling size ($\leq$ 300 participants per study area), biomarkers analysed and basic data collected. Studies performed in hotspots, in patients or in occupational groups were excluded. The HBM4EU Aligned Studies were conducted to characterize exposure at European level, including countries from the four geographical regions of Europe according to the United Nations geo-scheme (North, East, South, West). Details on the alignment of studies can be found elsewhere (Gilles et al., 2021).

European countries (studies; organizations) providing data for this study included France (Etude de Santé sur l'Environnement, la Biosurveillance, l'Activité Physique et la Nutrition, ESTEBAN; Santé Publique France), Germany (Environmental Specimen Bank, ESB; German Environment Agency), Iceland (Icelandic National Dietary Survey, Diet-HBM; University of Iceland), Luxembourg (Observation of

Cardiovascular Risk Factors in Luxembourg, Oriscav-Lux2; Luxembourg Institute of Health and Laboratoire National de Santé), Poland (Polish Aligned Environmental Study, POLAES; Nofer Institute of Occupational Medicine) and Portugal (Exposure of the Portuguese Population to Environmental Chemicals: a study nested in INSEF, INSEF-ExpoQuim; National Institute of Health Doutor Ricardo Jorge).

Basic information on the HBM4EU Aligned Studies providing data for our analysis is presented in Table 1.

2.2. Total deoxynivalenol (tDON) determination

Total deoxynivalenol (tDON) was measured in urine samples from six HBM4EU Aligned Studies (n = 1296). Measurements were performed at laboratories that successfully passed the HBM4EU Quality Assurance/Quality Control (QA/QC) program (Esteban Lopez et al., 2021; Vorkamp et al., 2023), except for the data from Iceland. As such, analytical data from the DIET_HBM study is not quality assured by the HBM4EU quality assurance program.

tDON quantification was performed in six laboratories that measured tDON in urine using high-performance liquid chromatography (HPLC) coupled to mass spectrometry (MS) or to high-resolution MS (HRMS). The limit of detection was only available for Iceland, Luxembourg and Poland and ranged from 0.04 μg/L to 0.20 μg/L (Table 1). The limit of quantification (LOQ) was available for all the countries and ranged from 0.05 μg/L to 0.50 μg/L. The number of samples below the LOQ varied between countries, ranging from 1 to 39. Further information on the analytical methods and procedures used in each laboratory can be found in Table A.0 in the Supplementary Material.

For tDON data, measurements below the LOQ were imputed. A truncated lognormal distribution was fitted for the data of each study using the observed values above the limit (Feraldi et al., 2023; Lubin et al., 2004). Subsequently random values were imputed for the estimated distribution below the limits. For samples with values below the LOD a random value between 0 and the LOD was imputed; for samples with values between the LOD and the LOQ a random value between the limits was imputed; and for samples with values below the LOQ and no known LOD a random value between 0 and the LOQ was imputed.

Creatinine concentrations in urine were available for all data collections (n = 1269) and used for the standardisation of urinary tDON concentrations in the present study to take into account urinary dilution. The resulting concentrations in urine were expressed as μg/g creatinine.

2.3. Questionnaire data

HBM4EU Aligned Studies collected questionnaire data together with the biological samples. Selected questionnaire data for the present study included socio-demographic information (sex, age, education level, income, current occupation status, occupational sector and occupational activities potentially related to exposure to mycotoxins), information on residential environment (country of residence, degree of urbanization, and living in the vicinity of farmlands, orchards or vineyards), information on dietary habits (vegetarian diet, gluten free diet, and frequency of consumption during the last weeks of meat, poultry, vegetable and fruit, rice, cereals, hazelnut spread, butter, cheese, eggs, tea, coffee, dried fruits, bread, cookies, beer and milk), and health information (height, weight, body mass index, pregnancy status).

Educational level was used as a surrogate for socio-economic status. The classification used for the educational level was based on the International Standard Classification of Education (ISCED) (Eurostat, n.d.). Educational levels were categorized as low (individuals with no to lower secondary education: ISCED 0–2), medium (individuals with upper secondary to post-secondary non-tertiary education: ISCED 3–4) or high (individuals with tertiary education and higher: ISCED ≥ 5).

Studies were also classified into one of the four geographical regions defined by the United Nations geoscheme for Europe (North, West, South, East) (United Nations Statistics Division. Methodology - Standard Country or Area Codes for Statistical Use (M49), n.d.).

The individuals' living environment was classified according to the degree of urbanization using the Eurostat classification (Dijkstra,Lewis & Poelman, Hugo, 2014). Living areas were classified as densely populated areas (cities), intermediate populated areas (towns and suburbs) and thinly populated areas (rural areas).

Some of the HBM4EU Aligned Studies had already completed fieldwork and had available biological samples before the start of HBM4EU but collected within the defined timeframe (2014–2021); others were initiated before the start of HBM4EU but were still ongoing; and others were new studies using the HBM4EU protocols, guidelines and questionnaires (Iceland and Portugal). As such, the data from the different studies needed post-harmonisation (Gilles et al., 2021). The final codebooks with harmonized variables are available online at Zenodo: HBM4EU Harmonized Codebook Adults – Aligned studies (https://doi.org/10.5281/zenodo.6598404).

2.4. Statistical analysis

Descriptive statistics of tDON levels (volume based and standardised for creatinine) (N, arithmetic mean, geometric mean and 95 % CI, percentiles P5, P10, P25, P50, P75, P90, P95 and maximum) were calculated for each country and for the pooled European database. The normality of the distribution of tDON levels for the pooled European database and for each country separately was assessed using Kolmogorov-Smirnov (KS) normality tests. Differences between the countries were assessed using Kruskal-Wallis (KW) test and multiple pairwise-comparisons using Wilcoxon rank sum tests with Bonferroni continuity correction (WRS).

European exposure values for the pooled European database were calculated overall and stratified by geographical region, sampling season, sex, educational level, degree of urbanization and occupational status (geometric means, P95 and their 95 % confidence intervals were calculated). Data from Iceland was not considered for the calculation of the European exposure values as the analytical data from Iceland was not quality assured by HBM4EU (not subject to the HBM4EU Quality Assurance/Quality Control (QA/QC) program).

It was not possible to study the determinants of DON exposure for the pooled European database due to the discrepancies in the accessory data available from the different studies. As such, the study of the determinants of DON exposure was performed for each country separately with the variables that were available. Data from Iceland was considered

Table 1
Sample and analytical information from the HBM4EU Aligned Studies.

Study	Country	Representativeness	Sampling year	n	Sample type	LOD	LOQ	n < LOD	n < LOQ
ESTEBAN	France	National	2014–2016	300	First morning	–	0.05	–	20
ESB	Germany	Regional	2016–2021	120	24 h	–	0.30	–	2
Diet_HBM	Iceland	National	2019–2021	171	Spot sample	0.04	0.14	13	39
Oriscav-Lux2	Luxembourg	National	2016–2018	191	Spot sample	0.10	0.50	1	8
POLAES	Poland	Regional	2017	193	Spot sample	0.20	0.50	0	1
INSEF-ExpoQuim	Portugal	National	2019–2020	295	First morning	–	0.05	–	7

n: number of participants; LOD: Limit of Detection; LOQ: Limit of Quantification; n < LOD: number of samples below the limit of detection; n < LOQ: number of samples below the limit of quantification.

for these analyses as the analysis was performed independently for each country. Predictors of creatinine-standardised urinary concentrations of DON were determined by multiple linear regression analyses with log transformed imputed tDON levels as dependent variables and socio-demographic and food consumption frequency variables as independent variables. Sex, age and creatinine were included in all the multiple linear regression analysis to adjust for their potential effect. The validity of the models was assessed, namely in respect to linearity of the data, normality of residuals, homogeneity of residuals variance and independence of residuals error terms. The beta coefficients of the multiple linear regression models were exponentiated to be presented as ratios of levels between comparison groups.

A significance level of 0.05 was considered as statistically relevant for all the analysis. Statistical analysis was performed using the software R v4.1.1 and R Studio 2021.09.0.

2.5. Risk assessment

Exposure data obtained from the HBM4EU Aligned Studies was also considered for the characterization of the risk associated to exposure to DON through the determination of the hazard quotient (HQ), by comparing the levels of exposure with the correspondent reference values (e.g. HBM-GV and TDI). If the HQ presents a value above one, the exposure is considered as presenting concern (EFSA, 2013). Two approaches were considered:

Calculation of the external exposure (Probable Daily Intake, PDI) through reverse dosimetry and thereafter, determination of the HQ through comparison with the available external Health-Based Guidance Value (Tolerable Daily Intake (TDI) for DON: 1 μg / kg bw/day); and

Comparison of the HBM data (exposure) with the Human Biomonitoring Guidance Value (HBM-GV) for the general population determined for DON under HBM4EU (tDON: 0.69 μg DON/kg bw/ total 24 h ≈ 23 μg DON/L urine (Confidence Interval: 5–33 μg/L)) (Apel et al., 2023).

For the reverse dosimetry, the deterministic method of intake mass balance was applied, considering the concentration of tDON in urine (μg/L), the urinary volume produced in 24 h (L) (*Centers for Disease Control and Prevention.* Urine Output., 2020), the body weight (kg) and the excretion rate for DON (64.0 %) (Vidal, Claeys, et al., 2018). Body weights and urinary biomarker concentrations were considered at individual level, and the daily urinary volume was derived from the body weight (24 mL urine/kg body weight) (Castera & Borhade, 2023). For the cases where no body weight data was available (65 individuals) mean levels by country and gender were used (Average Height and Weight by Country, n.d.). In this study, risk assessment was performed for adults of the general population considering that human exposure to mycotoxins can be mostly attributed to consumption of contaminated food, and consequently generally can affect all population.

Calculations for risk assessment were performed using the software IBM SPSS v27.

3. Results

3.1. Characterization of the participants

Characteristics of the participants for each study and for the pooled European population are presented in Table 2. Considering the distribution by sex, the proportions of male and female participants were similar overall and in each country, except for Poland which had 67 % of female participants. As for the age of the participants, it ranged between 20 and 39 years with a mean of 32.2 years. France, Iceland and Poland covered the whole age group, while the other countries only covered part. The highest mean age was observed in the Portuguese study (34.6 years), which was the study with older participants (28–39 years), and the lowest in the German study (23.2 years), which was the study with younger participants (20–29 years).

Most samples were collected during the autumn–winter period (65.1 %). In France, Luxembourg and Portugal sampling was distributed over the year, while in Germany and in Poland the samples were all collected in one season (winter in Germany and autumn in Poland) and in Iceland samples were collected in summer, autumn and winter.

Most participants (63 %) had a high educational level (level 5 or higher according to ISCED) and only a small proportion (7 %) had a low educational level (level 2 or lower according to ISCED). Portugal was the country presenting a more evenly distributed sample concerning educational level, with 20 % of participants having a low educational level. All the other countries had less than 7 % of participants with a low educational level and more than 57 % with a high educational level. However, this skewed distribution towards a high educational level is usual in HBM studies, since individuals with a higher level of education may have a better understanding on the relevance of HBM studies and thus be more prone to participate.

Overall, a little over half of the participants lived in cities (55 %), while the rest were evenly distributed among towns and rural areas. The distribution presented differences between the countries. All the participants from Germany and Poland and the majority of participants from Iceland lived in cities. France, Luxembourg and Portugal presented more approximate distributions, although France had a higher proportion of participants living in cities while Luxembourg and Portugal had higher proportions of participants living in towns and in rural areas.

The majority of the participants were employed at the time of sampling (77 %) overall and in France, Luxembourg and Portugal. Concerning the other countries, all the participants in the German study were students, while data was only available for 21.5 % of the Icelandic study participants and all the Polish study participants were employed.

Income data was only available for France, Luxembourg and Portugal and overall and for these countries the highest proportion of participants belonged to the medium income level category.

3.2. Urinary tDON levels

In the pooled European database, there were 77 participants (6.1 %) whose urine samples presented tDON levels below the limit of quantification (Table 3; LOQ provided in Table 1). This percentage was higher for Iceland (22.8 %) and lowest for Poland (0.5 %). LOQs varied between countries between 0.05 and 0.50 μg/L, but the countries with higher LOQs did not have higher percentages of participants with values below the LOQ and the countries with lower LOQs did not have lower percentages of participants with values below the limit. For the samples whose tDON values were below the LOQ random values were imputed (see details under section 2.2).

The concentrations of tDON in urine (volume based and standardised for creatinine) in the pooled European database and in each country are presented in Table 3. All the urine samples had creatinine levels above the limit of 5 mg/dL defined as the value for excluding samples that are too diluted in the screening of selected drugs of abuse in the workplace (Lauwerys & Hoet, 2001), and thereby no sample was excluded from the analysis based on these criteria.

The geometric mean tDON level in urine obtained for the pooled European database was 4.79 μg/L (4.38 μg/g crt), with a P95 of 33.70 μg/L (29.45 μg/g crt) and a maximum of 133.2 μg/L (189.8 μg/g crt). Not considering other factors, Iceland presented the lowest mean urinary tDON level (GM = 1.76 μg/L or 1.66 μg/g crt) and the differences between the tDON levels standardised by creatinine observed for Iceland and the other countries were statistically significant ($p < 0.001$; WRS). Poland presented the highest mean level (GM = 9.40 μg/L or 8.18 μg/g crt) and considering the tDON levels standardised by creatinine the difference between the values observed for Poland and the other countries were statistically significant except for France ($p = 0.935$, WRS)

Table 2
Characteristics of the study population for the pooled European database (ALL) and for each of the participating countries.

Variable	Category	France		Germany		Iceland		Luxembourg		Poland		Portugal		ALL	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%
Sex	Female	168	56.0 %	60	50.0 %	92	53.8 %	100	52.4 %	129	66.8 %	171	58.0 %	720	56.7 %
	Male	132	44.0 %	60	50.0 %	79	46.2 %	91	47.6 %	64	33.2 %	124	42.0 %	550	43.3 %
Age	Mean (Min-Max)	32.6 (20–39)		23.2 (20–29)		30.9 (20–39)		33.6 (25–39)		33.5 (20–39)		34.6 (28–39)		32.2 (20–39)	
Sampling season	Winter	79	26.3 %	120	100.0 %	23	13.5 %	41	21.5 %	–	–	53	18.0 %	316	24.9 %
	Spring	75	25.0 %	–	–	0	0.0 %	39	20.4 %	–	–	16	5.4 %	130	10.2 %
	Summer	59	19.7 %	–	–	52	30.4 %	50	26.2 %	–	–	152	51.5 %	313	24.6 %
	Autumn	87	29.0 %	–	–	96	56.1 %	61	31.9 %	193	100.0 %	74	25.1 %	511	40.2 %
Educational level	Low (ISCED 0–2)	7	2.3 %	–	–	8	4.8 %	9	4.7 %	0	0.0 %	59	20.0 %	83	6.6 %
	Medium (ISCED 3–4)	86	28.7 %	–	–	51	30.4 %	63	33.0 %	83	43.0 %	107	36.3 %	390	30.8 %
	High (ISCED ≥ 5)	207	69.0 %	120	100.0 %	109	64.9 %	119	62.3 %	110	57.0 %	129	43.7 %	794	62.7 %
Degree of urbanization*	Cities	136	45.3 %	120	100.0 %	129	76.8 %	39	20.4 %	193	100.0 %	80	27.1 %	697	55.0 %
	Towns/suburbs	82	27.3 %	–	–	18	10.7 %	81	42.4 %	–	–	109	36.9 %	290	22.9 %
	Rural areas	82	27.3 %	–	–	21	12.5 %	71	37.2 %	–	–	106	35.9 %	280	22.1 %
Occupational status	Working	250	83.3 %	–	–	36	100.0 %	169	88.5 %	–	–	257	92.8 %	712	77.1 %
	Not working	50	16.7 %	120	100.0 %	–	–	22	11.5 %	–	–	20	7.2 %	212	22.9 %
Income level	Low	40	13.4 %	–	–	–	–	18	9.4 %	–	–	71	26.3 %	129	17.0 %
	Medium	128	42.8 %	–	–	–	–	128	67.0 %	–	–	123	45.6 %	379	49.9 %
	High	123	41.1 %	–	–	–	–	12	6.3 %	–	–	57	21.1 %	192	25.3 %
	Doesn't know/ answer	8	2.7 %	–	–	–	–	33	17.3 %	–	–	19	7.0 %	60	7.9 %

*Classification according to the DEGURBA classification from EUROSTAT (2018).

Table 3
Tdon concentrations in urine in μg/l and μg/g creatinine in the pooled european database (all) and in each of the participating countries.

Country	n	% < LOD	% < LOQ	AM	GM	GM 95 % CI	P5	P10	P25	P50	P75	P90	P95	Max
tDON in urine (μg/L)														
ALL	1270	0.2 %	3.9 %	10.41	4.79	[4.38–5.23]	0.39	0.88	2.36	5.97	13.58	26.14	33.70	133.20
France	300	–	6.7 %	11.79	4.44	[3.49–5.66]	0.02	0.81	2.27	6.43	16.62	28.28	36.60	108.10
Germany	120	–	1.7 %	4.51	2.66	[2.14–3.32]	0.69	0.96	1.35	2.85	5.63	9.53	17.05	24.90
Iceland	171	1.2 %	6.4 %	4.00	1.76	[1.41–2.19]	0.12	0.29	0.82	2.03	4.44	9.00	16.23	33.45
Luxembourg	191	0.5 %	4.2 %	10.61	5.65	[4.74–6.75]	0.69	1.22	3.04	5.95	12.35	24.90	31.80	124.00
Poland	193	0.0 %	0.5 %	15.64	9.40	[8.02–11.00]	1.23	2.20	4.97	10.16	19.96	33.19	46.58	133.20
Portugal	295	–	2.4 %	11.58	6.75	[5.82–7.82]	0.84	1.70	4.08	7.99	14.82	26.57	36.06	77.87
tDON in urine (μg/g creatinine)														
ALL	1269	0.4 %	3.8 %	9.23	4.38	[4.02–4.77]	0.40	0.85	2.33	5.47	11.58	21.10	29.45	189.80
France	300	–	6.7 %	12.15	4.74	[3.72–6.03]	0.04	0.71	3.07	7.57	15.08	27.27	36.96	189.80
Germany	120	–	1.7 %	6.32	4.09	[3.31–5.05]	1.10	1.50	2.38	4.31	8.31	14.83	18.91	27.50
Iceland	170	0.6 %	1.8 %	3.08	1.66	[1.38–2.00]	0.22	0.40	0.80	1.97	3.73	6.89	10.71	21.58
Luxembourg	191	0.5 %	4.2 %	8.32	3.26	[2.66–3.99]	0.27	0.60	1.58	3.52	7.56	18.65	30.84	128.30
Poland	193	0.0 %	0.5 %	11.93	8.18	[7.16–9.35]	1.24	3.07	4.67	9.05	14.97	23.26	33.50	79.08
Portugal	295	–	2.4 %	9.83	5.86	[5.05–6.80]	0.73	1.69	3.73	7.21	12.44	20.94	28.20	75.65

LOD: Limit of Detection; LOQ: Limit of Quantification; AM: Arithmetic mean; GM: Geometric mean; 95 % CI: 95 % Confidence Interval; P5: percentile 5; P10: percentile 10; P25: percentile 25; P50: percentile 50; P75: percentile 75; P90: percentile 90; P95: percentile 95; Max – Maximum.

and Portugal ($p = 0.110$, WRS). The other differences between countries were statistically significant except the differences between Germany and Luxembourg ($p = 0.815$, WRS) and between France and Portugal ($p = 1$, WRS).

3.3. European DON exposure values

Table 4 presents the tDON exposure levels calculated for the European population using the data available from the HBM4EU Aligned Studies. Biomarker data from Iceland was not quality assured by the HBM4EU QA/QC program and was excluded from the calculations.

A geometric mean of 5.59 μg/L (5.09 μg/g crt) and a 95th percentile of 36.15 μg/L (31.21 μg/g crt) were obtained as European exposure values to DON (Table 4). Concerning the geographic region, the regions defined according to the United Nations geoscheme for Europe presented differences, with the lowest exposure value being observed in Western Europe, represented by France, Germany and Luxembourg (GM 4.34 μg/L or 4.09 μg/g crt) and the highest in Eastern Europe, represented by Poland (GM 9.40 μg/L or 8.18 μg/g crt). Differences were also found for the sampling season, with a lower exposure level observed in winter (GM 3.30 μg/L or 3.51 μg/g crt) and a higher exposure level in summer (7.83 μg/L or 6.83 μg/g crt). The difference observed for sex (GM 4.96 μg/L for women vs 6.57 μg/L for men) was not maintained after adjusting for creatinine (GM 5.05 μg/g crt for women vs 5.14 for men μg/g crt). The same happened for the occupational status, with no differences between the groups being observed after adjustment for creatinine. Exposure varied with the educational level, with higher exposure levels observed in individuals with a low educational level

Table 4
European tDON exposure levels (μg/L and μg/g creatinine) calculated with data from the HBM4EU Aligned Studies*.

Variable	Category	tDON in urine (μg/L)			tDON in urine (μg/g creatinine)		
		n	GM [95 %CI]	P95 [95 %CI]	n	GM [95 %CI]	P95 [95 %CI]
Unstratified		1099	5.59 [5.10–6.14]	36.15 [33.0–41.2]	1099	5.09 [4.65–5.58]	31.21 [28.33–34.43]
Geographic region	East	193	9.40 [8.02–11.00]	46.14 [37.47–61.26]	193	8.18 [7.16–9.35]	33.01 [24.43–39.89]
	North*	–	–	–	–	–	–
	West	611	4.34 [3.78–4.98]	32.06 [28.71–37.68]	611	4.09 [3.56–4.71]	31.81 [27.50–39.31]
	South	295	6.75 [5.82–7.82]	36.01 [31.69–42.97]	295	5.86 [5.05–6.80]	28.08 [23.79–32.54]
Sampling season	Winter	293	3.30 [2.75–3.96]	21.13 [19.10–24.90]	293	3.51 [2.92–4.21]	21.14 [17.47–24.21]
	Spring	130	6.44 [4.85–8.56]	33.69 [28.71–46.06]	130	5.76 [4.34–7.63]	31.49 [23.53–62.00]
	Summer	261	7.83 [6.63–9.25]	41.30 [35.44–46.95]	261	6.83 [5.74–8.12]	37.01 [31.21–52.20]
	Autumn	415	6.28 [5.41–7.30]	41.18 [33.70–48.69]	415	5.30 [4.57–6.14]	33.01 [27.05–38.78]
Sex	Female	628	4.96 [4.36–5.63]	34.12 [31.27–38.10]	628	5.05 [4.46–5.72]	29.48 [26.54–34.43]
	Male	471	6.57 [5.76–7.50]	38.78 [33.49–45.02]	471	5.14 [4.49–5.89]	32.34 [28.51–37.01]
Educational level	Low (ISCED 0–2)	75	8.72 [6.50–11.69]	42.97 [36.01–48.80]	75	6.78 [4.97–9.26]	32.54 [27.05–39.31]
	Medium (ISCED 3–4)	339	6.78 [5.81–7.90]	37.47 [32.54–43.52]	339	5.70 [4.87–6.68]	31.49 [26.92–36.43]
	High (ISCED $\geq$ 5)	685	4.85 [4.29–5.48]	33.05 [29.95–39.74]	685	4.66 [4.14–5.26]	29.71 [25.25–36.00]
Degree of urbanization	Cities	568	5.24 [4.62–5.96]	36.48 [31.27–42.97]	568	5.16 [4.57–5.82]	29.09 [24.43–34.24]
	Towns/suburbs	272	5.13 [4.14–6.35]	32.54 [28.43–44.85]	272	4.27 [3.44–5.30]	31.45 [26.54–36.00]
	Rural areas	259	7.06 [6.00–8.30]	36.01 [32.84–42.53]	259	5.96 [5.02–7.08]	38.52 [28.88–52.48]
Occupational status†	Working	676	5.49 [4.83–6.23]	36.01 [32.66–41.46]	676	4.58 [4.02–5.22]	31.82 [28.88–38.52]
	Not working	212	3.68 [3.03–4.46]	27.76 [24.15–32.54]	212	4.69 [3.90–5.65]	26.75 [21.79–31.96]
Income levelΔ	Low	129	5.97 [4.65–7.66]	35.47 [27.76–48.80]	129	5.71 [4.40–7.41]	33.06 [25.30–52.20]
	Medium	379	5.24 [4.36–6.30]	35.91 [31.54–43.52]	379	4.24 [3.51–5.11]	31.96 [28.49–38.19]
	High	192	5.77 [4.57–7.28]	36.67 [31.23–48.69]	192	5.32 [4.23–6.71]	34.43 [26.70–53.20]
	Doesn't know/answer	60	5.21 [3.58–7.59]	30.11 [22.87–38.10]	60	3.62 [2.49–5.27]	23.36 [12.45–71.02]

*Data from Iceland was excluded as biomarker data for Iceland was not quality assured by HBM4EU. GM: Geometric mean; 95 %CI: 95 % Confidence interval; P95: Percentile 95; † Data not available for Poland (n = 888); $^{\Delta}$ Data not available for Germany and Poland (n = 760).

(GM 8.72 μg/L or 6.78 μg/g crt) and lower exposure levels observed in individuals with a high educational level (GM 4.85 μg/L or 4.66 μg/g crt). Individuals living in rural areas presented higher exposure levels (GM 7.06 μg/L or 5.96 μg/g crt) compared with individuals living in cities (GM 5.24 μg/L or 5.16 μg/g crt) and with individuals living in towns/suburbs (GM 5.13 μg/L or 4.27 μg/g crt).

3.4. *Determinants of exposure*

To study the factors contributing to exposure to DON in each of the participating countries, the exposure levels standardised for creatinine were stratified by socio-demographic characteristics and multiple linear regression models were fitted for the ln-transformed exposure levels standardized for creatinine. A model was fitted for each factor adjusting by sex, gender and creatinine values. Results are presented in Tables A.1 to A.6 (Supplementary material).

Male participants from France and Germany presented higher tDON levels than female participants. However, the differences between sex were very small for the other countries. Concerning the sampling season, higher tDON levels were observed in spring and summer for France, Luxembourg and Portugal, the three countries where samples were collected in all the four seasons. In general, a similar trend concerning exposure level and educational status of the study participants was observed, with participants with lower educational level having higher exposure level for all countries and participants with higher educational level being less exposed, except for Iceland where the values in the three categories were very similar. The only exception was Luxembourg where higher level educated participants had higher tDON concentrations than medium level educated participants. Concerning the degree of urbanization of the participants' area of residence, those living in rural areas had higher exposure levels than participants living in towns/suburbs or in cities. As for the participants' occupational status, individuals without a job had higher exposure values in France, Luxembourg and Portugal. Concerning income level and exposure, no clear pattern was observed between both variables.

Results from the multiple linear regression models confirmed the differences observed for gender, but showed that the differences were not statistically significant. Concerning the sampling season, statistically significant differences were observed for France, Luxembourg and Portugal. For France, samples collected in the summer presented a tDON level 2.00 [95 %CI: 0.97–4.11] times higher than the samples collected in winter, while in Luxembourg samples collected in the spring presented a tDON level 1.96 [95 %CI: 1.12–3.43] times higher than those collected in the winter. In Portugal, samples collected in the spring and in the summer presented tDON levels 2.43 [95 %CI: 1.21–4.89] and 2.48 [95 %CI: 1.68–3.65] times higher than samples collected in winter. The influence of educational level lost significance in the model, except for Portugal where individuals with a lower and a medium educational level had, respectively, tDON levels 1.72 [95 %CI: 1.15–2.57] and 1.49 [95 % CI: 1.08–2.07] times higher than individuals with a higher educational level. As for the degree of urbanization of the participants' area of residence, the differences observed lost significance in the models, except for France where individuals living in rural areas had tDON levels 1.97 [95 %CI: 1.10–3.52] times higher that individuals living in cities. No differences were observed concerning occupational status and income levels.

Concerning the consumption of foods from certain nutritional groups, statistically significant differences were observed in Iceland for the consumption of fruit and vegetables with individuals with a higher consumption pattern (≥ 7 times per week) presenting tDON levels 0.62 [95 %CI: 0.41–0.93] times lower than individuals with lower consumption (≤ 6 times per week). Cereal consumption has shown to be associated to higher tDON levels in Luxembourg and Poland with individuals with higher consumption presenting tDON levels 1.71 [95 % CI: 1.03–2.82] and 1.38 [95 %CI: 1.00–1.89] times higher than individuals with lower consumption. Bread consumption has also shown to be associated with higher tDON levels in France and Poland. Although without statistical significance, in France individuals eating bread more than 7 times per week presented tDON levels 1.98 [95 %CI: 0.91–4.30] times higher than individuals eating bread less than once per week. As for Poland, individuals eating bread more than 7 times and 2–6 times per week presented tDON levels, respectively, 1.57 [95 %CI: 0.92–2.65] and 1.59 [95 %CI: 1.02–2.47] times higher than individuals eating bread less than once per week, with the last difference being statistically significant. It is also worth mentioning that, although without statistical significance, the consumption of cookies in Luxembourg and the consumption of tea and coffee in Poland seemed to be associated to higher tDON levels, with individuals reporting the consumption of cookies more than once a week having tDON levels 1.42 [95 %CI: 0.94–2.15] times higher than those with lower consumption and individuals reporting the consumption of tea and coffee 2–6 times per week having tDON levels 1.40 [95 %CI: 0.97–2.03] times higher than those consuming tea and coffee less than once per week.

3.5. Risk assessment

Table 5 presents the results for HQ by country, considering the two approaches used (reverse dosimetry and probable daily intake estimation – PDI (RD), and comparison with the human biomonitoring guidance value – HBM-GV). Fig. 1 shows the HQ ordered from the lowest to the highest HQ, regarding all the participants from the six countries included in the present study. Results per country are presented in Table 5.

According to Table 5, median HQ were, in decreasing order, 0.59 (Poland), 0.44 (France), 0.43 (Portugal), 0.40 (Luxembourg), 0.17 (Germany), 0.15 (Iceland), considering the approach using PDI estimation through reverse dosimetry. All the participants from Germany and almost all from Iceland presented an exposure to DON below the TDI, thus not representing a potential health concern. For Luxembourg, Portugal, France and Poland, the percentage of individuals presenting a HQ above one was 8.4 %, 9.8 %, 13.3 % and 17.1 %, respectively. Considering a global perspective, for all the European countries considered, the percentage of individuals with a HQ above one was 9.4 %. This approach implied the assumption of urinary volume based on body weight and the use of the excretion rate determined by Vidal et al. (2018) of 64.0 %, which could lead to some uncertainty.

For the direct comparison with the HBM-GV approach (HBM-GV = 23 μg DON/L urine), the median HQ were, in decreasing order, 0.68 (Poland) > 0.51 (France) > 0.50 (Portugal) > 0.46 (Luxembourg) > 0.20 (Germany) > 0.17 (Iceland), with Germany being the only country where more than 99 % of participants presented a HQ below one (Table 5). The percentages of individuals presenting a HQ above one, i.e., above the HBM-GV, were 2.9 %, 11.5 %, 12.5 %, 17.0 % and 20.7 %, respectively for Iceland, Luxembourg, Portugal, France and Poland. Overall, the percentage of individuals with an HQ above one was 12.3 %.

Results obtained for HQ through the direct comparison with HBM-GV were generally higher than those obtained through reverse dosimetry and probable daily intake (Fig. 1).

Evaluating these results, it should be remarked that the HBM-GV was defined based on 24 h urine sampling, contrary to the aligned study

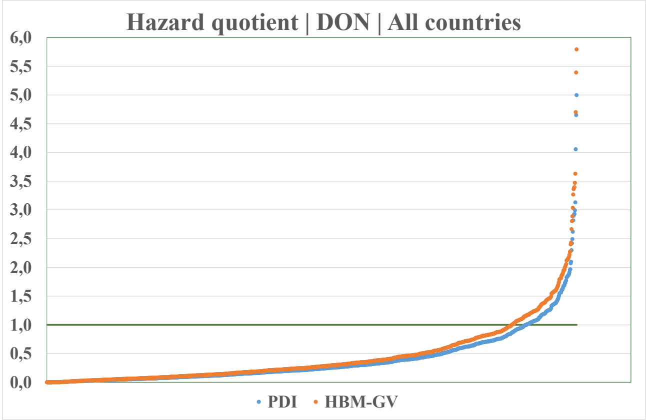

Fig. 1. Hazard quotient (HQ) in ascending order considering the participants from six countries (n = 1270). The green line corresponds to HQ = 1; HQ < 1 corresponds to exposure levels within safe limits. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 5
Hazard quotient determined through two different approaches: reverse dosimetry and probable daily intake estimation, and comparison with the human biomonitoring guidance value.

Country	n	HQ – PDI (RD)				HQ – HBM-GV			
		Mean	P90	P95	% participants HQ > 1	Mean	P90	P95	% participants HQ > 1
ALL	1270	0.39	0.98	1.27	9.4	0.45	1.14	1.47	12.3
France	300	0.44	1.07	1.41	13.3	0.51	1.24	1.64	17.0
Germany	120	0.17	0.38	0.67	0.0	0.20	0.44	0.78	0.8
Iceland	171	0.15	0.39	0.63	1.2	0.17	0.46	0.73	2.9
Luxembourg	191	0.40	0.94	1.22	8.4	0.46	1.09	1.42	11.5
Poland	193	0.59	1.27	1.79	17.1	0.68	1.47	2.08	20.7
Portugal	295	0.43	1.01	1.36	9.8	0.50	1.17	1.58	12.5

HQ: Hazard Quotient; PDI: Probable Daily Intake; RD: Reverse Dosimetry; HBM-GV: Human Biomonitoring Guidance Value; P90: Percentile 90; P95: Percentile 95.

developed under the scope of HBM4EU project where the majority of the samples considered were first-morning samples (only the German samples were 24 h urine samples), thus introducing uncertainty in the HQ determination.

4. Discussion

The concentrations of tDON in urine obtained for the countries participating in the HBM4EU Aligned Studies (Table 3) are in line with results obtained in other HBM studies conducted in Europe. A study developed in French male farmers in 1997–2000 has shown a 99 % detection rate and a tDON median level of 6.8 μg/L (Turner, Hopton, et al., 2010), similar to the level of 6.43 μg/L obtained in the French aligned study (ESTEBAN). A study conducted in Germany in 2012 has detected DON in 87 % of the samples and reported a mean value of 5.3 μg/g crt (Gerding et al., 2014), while another study conducted in 2013 has detected DON in all the samples and reported a tDON mean level of 10.5 μg/g crt (Ali et al., 2016). Concerning the data reported in this paper, DON was detected in 98 % of the German samples and a mean value of 6.32 μg/g crt was observed. Iceland was the only country from Northern Europe contributing with data on mycotoxins exposure to the HBM4EU Aligned Studies and was the country presenting the lowest mean urinary tDON level (Mean of 4.00 μg/L), with 93,6% of samples with detectable levels. No previous study with data on DON exposure for the Icelandic population was found, but previous studies developed in Norway and Sweden have reported similar values than the ones obtained for Iceland with high detection rates. Studies from Norway and Sweden have been able to detect DON in 99 % (Norway, n = 58, 2014, mean 6.1 μg/L for women and 6.3 for men) (Brera et al., 2015), 63 % (Sweden, n = 252, 2010–2011, mean 4.4 μg/g crt) (Wallin et al., 2015), 90 % (Sweden, n = 326, 2009–2011, mean 2.9 ng/mL) (Wallin et al., 2013) and 90 % (Sweden, n = 295, 2012, median 3.5 μg/g crt) of the samples (ref). No previous published studies were found for Luxembourg, but the values observed for Luxembourg (GM 10.61 μg/L; 8.32 μg/g crt) were of the same order of magnitude as the ones obtained for other countries from Western Europe, such as France, Belgium (2013–2014, n = 239, 37 % detection rate, mean 6.1 μg/g crt) (Heyndrickx et al., 2015) and Germany. As for Poland, data on mycotoxins exposure in countries from Eastern Europe is scarce and only a study from Hungary was found (n = 41, 2017, mean 3.63 μg/g crt) (Szabo-Fodor et al., 2021). The value obtained for Poland (GM 15.64 μg/L; 11.93 μg/g crt) was higher than the one reported for Hungary, which warrants further investigation to characterize the exposure in this region of Europe. Concerning Portugal, a previous study reported 30 % of samples containing measurable levels of tDON (n = 94, 2015–2016, 0.38 ug/L; 0.48 ug/g crt) (Martins et al., 2019), whereas in another study free DON and tDON were detected, respectively, in 15 % and 69 % of the samples (n = 13, mean 16.3 ng/mL) (Cunha & Fernandes, 2012).

The exposure values obtained for the European population under the HBM4EU Aligned Studies are also in line with the results obtained in studies developed in other European countries (Austria, n = 27, 59 % detected, mean 20.4 μg/L (Warth et al., 2012); Italy, n = 52, 2011, 96 % detected, mean 11.89 ng/mL (Solfrizzo et al., 2014); Italy, 2014, female n = 15, 80 % detected, 8.35 μg/g crt; male n = 16, 88 % detected, mean 7.60 μg/g crt (De Santis et al., 2019); Spain, 2013, female n = 10, 26.9 μg/g crt; male n = 12, 15.7 μg/g crt (Rodriguez-Carrasco et al., 2014); Scotland, n = 15, 2012, mean 7.2 μg/g crt, 2013, mean 19.7 μg/g crt (Gratz et al., 2014); UK, 2000–2001, n = 300, 99 % detected, GM 8.9 μg/g crt (Turner et al., 2008); UK, n = 35 samples, 94 % detected, mean 10.1 μg/g crt (Turner, White, et al., 2010), UK, 2014, n = 31, female, mean 12.7 μg/g crt, male, mean 24.3 μg/g crt (Wells et al., 2017)).

Regarding confounding variables, for sex, results from previous studies are not in agreement with our finding that it does not influence the tDON exposure, as some studies have shown differences between men and women (Heyndrickx et al., 2015; Turner et al., 2008) (with men having higher values as in the present study) while in others no differences were detected (Martins et al., 2021; Rodriguez-Carrasco et al., 2014; Solfrizzo et al., 2014; Wells et al., 2017). Concerning the sampling season, although differences were to be expected due to differences in mycotoxins' occurrence, previous studies have not found differences in exposure between sampling seasons (Turner, Hopton, et al., 2010; Wallin et al., 2015). However, differences were observed for the HBM4EU Aligned Studies, with higher exposure values observed in the summer and lower in the winter. These differences were observed at country level for France, Luxembourg and Portugal. On the other hand Iceland was the country with lowest tDON levels which may be due to the lowest temperatures in Iceland, although no differences were observed between seasons in this country.

To the best of the authors' knowledge, there are no previous studies examining the effect of occupational status or educational level on DON exposure levels, but differences between groups are commonly observed for other chemical compounds (Montazeri et al., 2019). The differences observed for the educational level can be due to different dietary habits and be explained by a higher consumption of foodstuffs with higher DON levels (e.g., cereals) by the individuals with higher educational level. Indeed, consumption of food with high fiber content, such as breakfast cereals or whole grain bread has been recommended due to their health benefits and these recommendations are usually better taken up by the most educated citizens. Concerning the degree of urbanization of the area of residence, a study from Bangladesh (Ali et al., 2016) including participants from urban and rural areas did not find differences in the DON exposure levels. In the HBM4EU Aligned Studies differences were found, but at country level only France has shown significant differences.

On the other hand, a higher consumption frequency either of bread or cereals was associated with higher tDON levels in some countries. These results were to be expected as cereal and cereal-based foods are well recognized as important determinants of exposure to mycotoxins (Wallin et al., 2013). However, it is worth mentioning that the increases observed in tDON levels were significant. Some previous studies have also found a direct association between the levels of urinary DON and consumption of cereals, namely one in Sweden (Wallin et al., 2013) and one in the UK (Turner et al., 2008). The study from Brera et al., showed that the consumption of pasta and pasta-like products (in Italy), the consumption of breakfast cereals, snacks, bread and bread-like foods (in Norway) and the consumption of biscuits (in the UK) were significantly associated with higher levels of total DON (Brera et al., 2015b). Another study done in Portugal has found associations between tDON levels and consumption of pasta and of cookies and biscuits (Martins et al., 2021), confirming the tendency observed in Luxembourg for higher tDON levels in individuals reporting most frequent consumption of cookies.

As for the consumption of fruit and vegetables, individuals with a higher consumption frequency presented tDON levels 0.66 times lower than individuals with a lower consumption frequency. To the best of the authors' knowledge, although some studies tried to evaluate tDON levels in vegetarians (Fleury et al., 2017; Wells et al., 2017), no study has reported associations between fruit and vegetables consumption and tDON levels. As such, the authors hypothesize that the lower tDON levels observed for individuals eating vegetables most frequently could be the result of a diet with lower consumption of cereal-based products.

Concerning risk assessment, previous studies available for European countries also presented a similar pattern of results for DON. These studies performed a risk assessment based on the approach of reverse dosimetry for PDI estimation and subsequent comparison with the already established TDI. Studies developed in Italy, Belgium, United Kingdom, Croatia, Austria, Sweden and Portugal, referred an exposure to DON above the TDI for 6 %-7% (De Santis et al., 2019; Solfrizzo et al., 2014), 29 % (Heyndrickx et al., 2015), 17 % (Turner, White, et al., 2010), 48 % (Sarkanj et al., 2013), 33 % (Warth, Sulyok, Fruhmann, Berthiller, et al., 2012), 1.3 %-1.6 % (Mitropoulou et al., 2018), and 10 % (Martins et al., 2019) of participants, respectively. For Spain (Valencia region), an exceedance of TDI was reported for 8 % of the participants in 2014 (Rodriguez-Carrasco et al., 2014), but more recent data referred no exceedance of TDI (Carballo et al., 2021). The results obtained in the HBM4EU Aligned Studies and in the studies mentioned above, confirm that, DON is indeed the most prevalent *Fusarium* toxin and the chronic dietary exposure represents a risk for about 12 % of the European population (EFSA Panel on Contaminants in the Food Chain (CONTAM) et al., 2017; Eskola et al., 2020). It is also worth mentioning that other toxins can co-occur with DON, justifying a higher concern over exposure. In addition, in the frame of climate changes, different occurrence patterns are expected across Europe and the globe, possibly resulting in higher dissemination and in higher amounts of mycotoxins contaminating food products and thereby in higher human exposure levels.

It should be noted that the approach of reverse dosimetry for PDI estimation and subsequent comparison with the already established TDI implied the assumption of urinary volume based on body weight and the use of the excretion rate determined by Vidal et al. (2018) of 64.0 %, while the HBM-GV used for the second approach was defined based on 24 h urine sampling and the majority of the samples considered were first-morning samples (only the German samples were 24 h urine samples), which could lead to uncertainty in the HQ determination in both approaches.

The inclusion of mycotoxins' HBM data remains an important aspect in the context of the assessment of risk from human exposure to chemicals and natural toxins, since it represents the internal exposure dose resulting from different exposure scenarios (e.g., food consumption and workplace environment) and exposure routes (mainly ingestion but also inhalation and dermal in occupational exposure scenarios) at individual level. Therefore, HBM data contributes to reduce the uncertainties associated with risk assessment, when performed at the population level and/or in indirect approaches (e.g., through combination of occurrence of chemical contaminants in food and food consumption data) (Choi et al., 2015). The use of HBM data for mycotoxins implies an extensive knowledge of the compounds' human metabolism and there are still some gaps regarding mycotoxins' toxicokinetic data that may hamper a proper risk assessment. If a risk assessment is developed for regulatory purposes, all these aspects should be properly considered. However, the establishment of an HBM-GV under HBM4EU project assumed a major relevance for performing a more accurate risk characterization, allowing a direct comparison of exposure obtained through HBM with a reference value, and reducing the uncertainty in estimates. It should be noted that the lack of knowledge on the toxicokinetics of a given compound impairs the establishment of a HBM-GV. In addition, limitations of the HBM-GV should be always described in detail.

5. Conclusion

The HBM4EU Aligned Studies were the first to obtain data on tDON exposure that covered countries representing the four regions of Europe, having produced new comparable data for tDON exposure in adults, with European coverage. Exposure levels obtained were similar to levels obtained in other studies, either done in the same or in other countries in Europe.

Higher exposure levels were observed for samples collected in spring and in summer, for individuals with a lower educational level and for individuals living in rural areas. Regarding occupation status, higher exposure levels were observed for individuals without a job in France and Luxembourg and for working individuals in Portugal. Concerning food consumption, individuals with higher consumption of cereals and bread had higher exposure levels.

Using two approaches to assess the risk from exposure to total DON (using PDI estimation through reverse dosimetry and comparing the tDON levels with the HBM-GV), similar trends were found: in around 10 % of the European population the chronic dietary exposure to DON may represent a health risk.

The results of this study might be used as background values for future studies and are expected to contribute to promote the development of national and European biomonitoring programmes on mycotoxins as a way to protect citizens' health from the expected increase in exposure as a consequence of climate change.

Ethical Statement

The HBM4EU Aligned Studies whose data was used in this study were conducted in accordance with the Declaration of Helsinki. Their study protocols have been approved by ethical review boards in each of the participating countries with the approvals being granted before recruitment of the study participants. ESTEBAN was approved by the Ile-de-France Protection to person committee on the 06.12.2012 (Internal number: CPP-IDF IX 12–012, EudraCT: 2012-A00459-34). The French Data Protection Agency gave its approval on the 14.02.2013. A Decree of the State Council establishing a processing of personal data relating to biomonitoring, health surveillance and nutrition (The Esteban study) was established after approval of the French Advisory Com-mittee on Information Processing for Research (CCTIRS). The French National Agency for Medi-cines and Health Products' Safety (ANSM) gave its approval for the use of biological samples and biobanking. ESB's study protocol has been reviewed by the ethics committee of the Medical Association Westfalen-Lippe, the Medical Faculty of the University of Münster and (since 2012) by the ethical committee of the Medical Association of the Saarland (Ha02/12). Diet_HBM was ap-proved by The National Bioethics Committee from Iceland (VSN-19–115; 31.10.2019). Oriscav-Lux2 was approved by the national ethical committee of Luxembourg (No. 201505/12). POLAES was approved by the ethical committee of the Nofer Institute of Occupational Medicine (10/2017). INSEF-ExpoQuim was approved by the ethical committees of the National Institute of Health Doutor Ricardo Jorge (12.02.2019), of the Regional Health Administrations of North (T1061; 02.04.2019), Center (63/2019; 24.06.2019), Lisbon and Tagus Valey (027/CES/INV/2019; 06.08.2019), Alentejo (14/2019/CE; 15.05.2019) and Algarve (07/2019; 11.04.2019), of the Health Service of the Autonomous Region of Madeira (19/2019; 17.06.2019) and of the Hospital of Horta (19.03.2019).

CRediT authorship contribution statement

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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.2024.115281.

Data availability

The authors do not have permission to share data. The data used in this manuscript is available through the Personal Exposure and Health Data Platform hosted at https://hbm.vito.be/peh-data-platform

References

Ali, N., Blaszkewicz, M., & Degen, G. H. (2016). Assessment of deoxynivalenol exposure among Bangladeshi and German adults by a biomarker-based approach. *Toxicology Letters, 258*, 20–28. https://doi.org/10.1016/j.toxlet.2016.06.006

Alvito, P., Assunção, R. M., Bajard, L., Martins, C., Mengelers, M. J. B., Mol, H., Namorado, S., van den Brand, A. D., Vasco, E., Viegas, S., & Silva, M. J. (2022). Current Advances, Research Needs and Gaps in Mycotoxins Biomonitoring under the HBM4EU—Lessons Learned and Future Trends. *Toxins, 14*(12). https://doi.org/10.3390/toxins14120826

Apel, P., Lamkarkach, F., Lange, R., Sissoko, F., David, M., Rousselle, C., Schoeters, G., & Kolossa-Gehring, M. (2023). Human biomonitoring guidance values (HBM-GVs) for priority substances under the HBM4EU initiative – New values derivation for deltamethrin and cyfluthrin and overall results. *International Journal of Hygiene and Environmental Health, 248*, Article 114097. https://doi.org/10.1016/j.ijheh.2022.114097

Arce-López, B., Lizarraga, E., Vettorazzi, A., & González-Peñas, E. (2020). Human Biomonitoring of Mycotoxins in Blood, Plasma and Serum in Recent Years: A Review. *Toxins, 12*(3), 147. https://doi.org/10.3390/toxins12030147

Assunção, R., Martins, C., Vasco, E., Jager, A., Oliveira, C., Cunha, S. C., Fernandes, J. O., Nunes, B., Loureiro, S., & Alvito, P. (2018). Portuguese children dietary exposure to multiple mycotoxins – An overview of risk assessment under MYCOMIX project. *Food and Chemical Toxicology, 118*, 399–408. https://doi.org/10.1016/j.fct.2018.05.040

Assuncao, R., Martins, C., Viegas, S., Viegas, C., Jakobsen, L. S., Pires, S., & Alvito, P. (2018). Climate change and the health impact of aflatoxins exposure in Portugal—An overview. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment, 35*(8), 1610–1621. https://doi.org/10.1080/19440049.2018.1447691

Average height and weight by country. (n.d.). Retrieved June 1, 2022, from https://www.worlddata.info/average-bodyheight.php.

Battilani, P., Toscano, P., Van der Fels-Klerx, H. J., Moretti, A., Camardo Leggieri, M., Brera, C., Rortais, A., Goumperis, T., & Robinson, T. (2016). Aflatoxin B1 contamination in maize in Europe increases due to climate change. *Scientific Reports, 6*(1), 24328. https://doi.org/10.1038/srep24328

Bennett, J. W., & Klich, M. (2003). Mycotoxins. *Clinical Microbiology Reviews, 16*(3), 497–516. https://doi.org/10.1128/CMR.16.3.497-516.2003

Brera, C., de Santis, B., Debegnach, F., Miano, B., Moretti, G., Lanzone, A., Del Sordo, G., Buonsenso, D., Chiaretti, A., Hardie, L., White, K., Brantsæter, A. L., Knutsen, H., Eriksen, G. S., Sandvik, M., Wells, L., Allenf, S., & Sathyapalanf, T. (2015). Experimental study of deoxynivalenol biomarkers in urine. *EFSA Supporting. Publication, 12*(6). https://doi.org/10.2903/sp.efsa.2015.EN-818

Carballo, D., Pallares, N., Ferrer, E., Barba, F. J., & Berrada, H. (2021). Assessment of Human Exposure to Deoxynivalenol, Ochratoxin A, Zearalenone and Their Metabolites Biomarker in Urine Samples Using LC-ESI-qTOF. *Toxins (Basel), 13*(8). https://doi.org/10.3390/toxins13080530

Castera, M. R., & Borhade, M. B. (2023). *Fluid Management*. In StatPearls: StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK532305/.

Urine Output., (2020) https://www.cdc.gov/dengue/training/cme/ccm/page57297.html.

Claeys, L., Romano, C., De Ruyck, K., Wilson, H., Fervers, B., Korenjak, M., Zavadil, J., Gunter, M. J., De Saeger, S., De Boevre, M., & Huybrechts, I. (2020). Mycotoxin exposure and human cancer risk: A systematic review of epidemiological studies. *Comprehensive Reviews in Food Science and Food Safety, 19*(4), 1449–1464. https://doi.org/10.1111/1541-4337.12567

Cunha, S. C., & Fernandes, J. O. (2012). Development and validation of a gas chromatography-mass spectrometry method for determination of deoxynivalenol and its metabolites in human urine. *Food and Chemical Toxicology : An International Journal Published for the British Industrial Biological Research Association, 50*(3–4), 1019–1026. https://doi.org/10.1016/j.fct.2011.12.028

De Santis, B., Debegnach, F., Miano, B., Moretti, G., Sonego, E., Chiaretti, A., Buonsenso, D., & Brera, C. (2019). Determination of Deoxynivalenol Biomarkers in Italian Urine Samples. *Toxins (Basel), 11*(8). https://doi.org/10.3390/toxins11080441

Dijkstra, L., & Poelman, H. (2014). *A harmonised definition of cities and rural areas: The new degree of urbanisation* (Working Papers). *European Commission Directorate-General for Regional and Urban Policy (DG REGIO)*. https://ec.europa.eu/regional_policy/sources/docgener/work/2014_01_new_urban.pdf.

DSM. (n.d.). *World Mycotoxin Survey: Impact 2021*. Retrieved June 1, 2022, from https://www.biomin.net/science-hub/world-mycotoxin-survey-impact-2021/.

Efsa. (2013). International Frameworks Dealing with Human Risk Assessment of Combined Exposure to Multiple Chemicals. *EFSA Journal, 11*(7), 3313. https://doi.org/10.2903/j.efsa.2013.3313

EFSA Panel on Contaminants in the Food Chain (CONTAM), Knutsen, H. K., Alexander, J., Barregård, L., Bignami, M., Brüschweiler, B., Ceccatelli, S., Cottrill, B., Dinovi, M., Grasl-Kraupp, B., Hogstrand, C., Hoogenboom, L. (Ron), Nebbia, C. S., Oswald, I. P., Petersen, A., Rose, M., Roudot, A., Schwerdtle, T., Vleminckx, C., … Edler, L. (2017). Risks to human and animal health related to the presence of deoxynivalenol and its acetylated and modified forms in food and feed. *EFSA Journal*, *15*(9). https://doi.org/10.2903/j.efsa.2017.4718.

Escrivá, L., Font, G., Manyes, L., & Berrada, H. (2017). Studies on the Presence of Mycotoxins in Biological Samples: An Overview. *Toxins, 9*(8), 251. https://doi.org/10.3390/toxins9080251

Eskola, M., Elliott, C. T., Hajšlová, J., Steiner, D., & Krska, R. (2020). Towards a dietary-exposome assessment of chemicals in food: An update on the chronic health risks for the European consumer. *Critical Reviews in Food Science and Nutrition, 60*(11), 1890–1911. https://doi.org/10.1080/10408398.2019.1612320

Esteban Lopez, M., Goen, T., Mol, H., Nubler, S., Haji-Abbas-Zarrabi, K., Koch, H. M., Kasper-Sonnenberg, M., Dvorakova, D., Hajslova, J., Antignac, J. P., Vaccher, V., Elbers, I., Thomsen, C., Vorkamp, K., Pedraza-Diaz, S., Kolossa-Gehring, M., & Castano, A. (2021). The European human biomonitoring platform—Design and implementation of a laboratory quality assurance/quality control (QA/QC) programme for selected priority chemicals. *International Journal of Hygiene and Environmental Health, 234*, Article 113740. https://doi.org/10.1016/j.ijheh.2021.113740

European Commission. (2006). Commission regulation EC 1881/2006, setting maximum levels for certain contaminants in foodstuffs. *Off. J. Eur. Communities*, *L364*, 5e24.

Eurostat. (n.d.). (2022). International Standard Classification of Education (ISCED). *Retrieved June 1, from.* https://ec.europa.eu/eurostat/statistics-explained/index.php?title=International_Standard_Classification_of_Education_(ISCED)#Implementation_of_ISCED_2011_.28levels_of_education.29.

Feraldi, A., De Santis, B., Finocchietti, M., Debegnach, F., Mandile, A., & Alfò, M. (2023). Evaluation of Statistical Treatment of Left-Censored Contamination Data: Example Involving Deoxynivalenol Occurrence in Pasta and Pasta Substitute Products. *Toxins, 15*(9), 521. https://doi.org/10.3390/toxins15090521

Fleury, S., Rivière, G., Allès, B., Kesse-Guyot, E., Méjean, C., Hercberg, S., Touvier, M., & Bemrah, N. (2017). Exposure to contaminants and nutritional intakes in a French vegetarian population | Elsevier Enhanced Reader. *Food and Chemical Toxicology, 109*, 218–229. https://doi.org/10.1016/j.fct.2017.07.048

Fung, F., & Clark, R. F. (2004). Health Effects of Mycotoxins: A Toxicological Overview. *Journal of Toxicology: Clinical Toxicology, 42*(2), 217–234. https://doi.org/10.1081/CLT-120030947

Gerding, J., Cramer, B., & Humpf, H. U. (2014). Determination of mycotoxin exposure in Germany using an LC-MS/MS multibiomarker approach. *Molecular Nutrition & Food Research, 58*(12), 2358–2368. https://doi.org/10.1002/mnfr.201400406

Gilles, L., Govarts, E., Rambaud, L., Vogel, N., Castano, A., Esteban Lopez, M., Rodriguez Martin, L., Koppen, G., Remy, S., Vrijheid, M., Montazeri, P., Birks, L., Sepai, O., Stewart, L., Fiddicke, U., Loots, I., Knudsen, L. E., Kolossa-Gehring, M., & Schoeters, G. (2021). HBM4EU combines and harmonises human biomonitoring data across the EU, building on existing capacity—The HBM4EU survey. *International Journal of Hygiene and Environmental Health, 237*, Article 113809. https://doi.org/10.1016/j.ijheh.2021.113809

Gilles, L., Govarts, E., Rodriguez Martin, L., Andersson, A.-M., Appenzeller, B. M. R., Barbone, F., Castaño, A., Coertjens, D., Den Hond, E., Dzhedzheia, V., Eržen, I., López, M. E., Fábelová, L., Fillol, C., Franken, C., Frederiksen, H., Gabriel, C., Haug, L. S., Horvat, M., & Schoeters, G. (2022). Harmonization of Human Biomonitoring Studies in Europe: Characteristics of the HBM4EU-Aligned Studies

Participants. *International Journal of Environmental Research and Public Health, 19*(11), 6787.

Govarts, E., Gilles, L., Rodriguez Martin, L., Santonen, T., Apel, P., Alvito, P., Anastasi, E., Andersen, H. R., Andersson, A.-M., Andryskova, L., Antignac, J.-P., Appenzeller, B., Barbone, F., Barnett-Itzhaki, Z., Barouki, R., Berman, T., Bil, W., Borges, T., Buekers, J., & Schoeters, G. (2023). Harmonized human biomonitoring in European children, teenagers and adults: EU-wide exposure data of 11 chemical substance groups from the HBM4EU Aligned Studies (2014–2021). *International Journal of Hygiene and Environmental Health, 249*, Article 114119. https://doi.org/10.1016/j.ijheh.2023.114119

Gratz, S. W., Richardson, A. J., Duncan, G., & Holtrop, G. (2014). Annual variation of dietary deoxynivalenol exposure during years of different Fusarium prevalence: A pilot biomonitoring study. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment, 31*(9), 1579–1585. https://doi.org/10.1080/19440049.2014.937772

Habschied, K., Kanižai Šarić, G., Krstanović, V., & Mastanjević, K. (2021). *Mycotoxins-Biomonitoring and Human Exposure. Toxins, 13*(2), 113. https://doi.org/10.3390/toxins13020113

Heyndrickx, E., Sioen, I., Huybrechts, B., Callebaut, A., De Henauw, S., & De Saeger, S. (2015). Human biomonitoring of multiple mycotoxins in the Belgian population: Results of the BIOMYCO study. *Environment International, 84*, 82–89. https://doi.org/10.1016/j.envint.2015.06.011

Huybrechts, B., Martins, J. C., Debongnie, P., Uhlig, S., & Callebaut, A. (2015). Fast and sensitive LC-MS/MS method measuring human mycotoxin exposure using biomarkers in urine. *Arch Toxicol, 89*(11), 1993–2005. https://doi.org/10.1007/s00204-014-1358-8

IARC. (n.d.). (2022). Agents Classified by the IARC Monographs, Volumes 1–131. from *Retrieved June 1* https://monographs.iarc.who.int/agents-classified-by-the-iarc/.

Koppen, R., Koch, M., Siegel, D., Merkel, S., Maul, R., & Nehls, I. (2010). Determination of mycotoxins in foods: Current state of analytical methods and limitations. *Applied Microbiology and Biotechnology, 86*(6), 1595–1612. https://doi.org/10.1007/s00253-010-2535-1

Lauwerys, R. R., & Hoet, P. (2001). *Industrial chemical exposure: Guidelines for biological monitoring (12269475* (3rd ed.). Publisher description: Lewis Publishers. http://www.loc.gov/catdir/enhancements/fy0646/2001016452-d.html.

Lubin, J. H., Colt, J. S., Camann, D., Davis, S., Cerhan, J. R., Severson, R. K., Bernstein, L., & Hartge, P. (2004). Epidemiologic Evaluation of Measurement Data in the Presence of Detection Limits. *Environmental Health Perspectives, 112*(17), 1691–1696. https://doi.org/10.1289/ehp.7199

Marin, S., Ramos, A. J., Cano-Sancho, G., & Sanchis, V. (2013). Mycotoxins: Occurrence, toxicology, and exposure assessment. *Food and Chemical Toxicology, 60*, 218–237. https://doi.org/10.1016/j.fct.2013.07.047

Martins, C., Torres, D., Lopes, C., Correia, D., Goios, A., Assuncao, R., Alvito, P., Vidal, A., De Boevre, M., De Saeger, S., & Nunes, C. (2021). Deoxynivalenol exposure assessment through a modelling approach of food intake and biomonitoring data—A contribution to the risk assessment of an enteropathogenic mycotoxin. *Food Research International, 140*, Article 109863. https://doi.org/10.1016/j.foodres.2020.109863

Martins, C., Vidal, A., De Boevre, M., De Saeger, S., Nunes, C., Torres, D., Goios, A., Lopes, C., Assuncao, R., & Alvito, P. (2019). Exposure assessment of Portuguese population to multiple mycotoxins: The human biomonitoring approach. *International Journal of Hygiene and Environmental Health, 222*(6), 913–925. https://doi.org/10.1016/j.ijheh.2019.06.010

Mitropoulou, A., Gambacorta, L., Lemming, E. W., Solfrizzo, M., & Olsen, M. (2018). Extended evaluation of urinary multi-biomarker analyses of mycotoxins in Swedish adults and children. *World Mycotoxin Journal, 11*(4), 647–659. https://doi.org/10.3920/WMJ2018.2313

Montazeri, P., Thomsen, C., Casas, M., de Bont, J., Haug, L. S., Maitre, L., Papadopoulou, E., Sakhi, A. K., Slama, R., Saulnier, P. J., Urquiza, J., Grazuleviciene, R., Andrusaityte, S., McEachan, R., Wright, J., Chatzi, L., Basagaña, X., & Vrijheid, M. (2019). Socioeconomic position and exposure to multiple environmental chemical contaminants in six European mother-child cohorts. *International Journal of Hygiene and Environmental Health, 222*(5), 864–872. https://doi.org/10.1016/j.ijheh.2019.04.002

Omotayo, O. P., Omotayo, A. O., Mwanza, M., & Babalola, O. O. (2019). Prevalence of Mycotoxins and Their Consequences on Human Health. *Toxicological Research, 35*(1), 1–7. https://doi.org/10.5487/TR.2019.35.1.001

Ougier, E., Ganzleben, C., Lecoq, P., Bessems, J., David, M., Schoeters, G., Lange, R., Meslin, M., Uhl, M., Kolossa-Gehring, M., Rousselle, C., & Vicente, J. L. (2021). Chemical prioritisation strategy in the European Human Biomonitoring Initiative (HBM4EU)—Development and results. *International Journal of Hygiene and Environmental Health, 236*, Article 113778. https://doi.org/10.1016/j.ijheh.2021.113778

Pestka, J. J. (2010). Deoxynivalenol: Mechanisms of action, human exposure, and toxicological relevance. *Archives of Toxicology, 84*(9), 663–679. https://doi.org/10.1007/s00204-010-0579-8

Rodriguez-Carrasco, Y., Molto, J. C., Manes, J., & Berrada, H. (2014). Exposure assessment approach through mycotoxin/creatinine ratio evaluation in urine by GC-MS/MS. *Food and Chemical Toxicology, 72*, 69–75. https://doi.org/10.1016/j.fct.2014.07.014

Sarkanj, B., Warth, B., Uhlig, S., Abia, W. A., Sulyok, M., Klapec, T., Krska, R., & Banjari, I. (2013). Urinary analysis reveals high deoxynivalenol exposure in pregnant women from Croatia. *Food and Chemical Toxicology, 62*, 231–237. https://doi.org/10.1016/j.fct.2013.08.043

Silva, J. V. B. D., Oliveira, C. A. F. D., & Ramalho, L. N. Z. (2022). An overview of mycotoxins, their pathogenic effects, foods where they are found and their diagnostic biomarkers. *Food Science and Technology, 42*, e48520.

Solfrizzo, M., Gambacorta, L., & Visconti, A. (2014). Assessment of multi-mycotoxin exposure in southern Italy by urinary multi-biomarker determination. *Toxins (Basel), 6*(2), 523–538. https://doi.org/10.3390/toxins6020523

Szabo-Fodor, J., Szeitzne-Szabo, M., Bota, B., Schieszl, T., Angeli, C., Gambacorta, L., Solfrizzo, M., Szabo, A., & Kovacs, M. (2021). Assessment of Human Mycotoxin Exposure in Hungary by Urinary Biomarker Determination and the Uncertainties of the Exposure Calculation: A Case Study. *Foods, 11*(1). https://doi.org/10.3390/foods11010015

Thomsen, Cathrine. (2019). *Deliverable Report D9.5—Prioritised list of biomarkers, matrices and analytical methods for the 2nd prioritisation round of substances*. HBM4EU. https://www.hbm4eu.eu/work-packages/deliverable-9-5-prioritised-list-of-biomarkers-matrices-and-analytical-methods-for-the-2nd-prioritisation-round-of-substances/.

Turner, P. C., Hopton, R. P., Lecluse, Y., White, K. L., Fisher, J., & Lebailly, P. (2010). Determinants of urinary deoxynivalenol and de-epoxy deoxynivalenol in male farmers from Normandy. *France. J Agric Food Chem, 58*(8), 5206–5212. https://doi.org/10.1021/jf100892v

Turner, P. C., Rothwell, J. A., White, K. L., Gong, Y., Cade, J. E., & Wild, C. P. (2008). Urinary deoxynivalenol is correlated with cereal intake in individuals from the United kingdom. *Environmental Health Perspectives (Research Triangle Park, NC), 116*(1), 21–25. https://doi.org/10.1289/ehp.10663

Turner, P. C., Solfrizzo, M., Gost, A., Gambacorta, L., Olsen, M., Wallin, S., & Kotova, N. (2017). Comparison of Data from a Single-Analyte and a Multianalyte Method for Determination of Urinary Total Deoxynivalenol in Human Samples. *Journal of Agricultural and Food Chemistry, 65*(33), 7115–7120. https://doi.org/10.1021/acs.jafc.6b04755

Turner, P. C., White, K. L., Burley, V. J., Hopton, R. P., Rajendram, A., Fisher, J., Cade, J. E., & Wild, C. P. (2010). A comparison of deoxynivalenol intake and urinary deoxynivalenol in UK adults. *Biomarkers, 15*(6), 553–562. https://doi.org/10.3109/1354750X.2010.495787

Retrieved June 1, from, (2022). https://unstats.un.org/unsd/methodology/m49/.

Vidal, A., Cano-Sancho, G., Marin, S., Ramos, A. J., & Sanchis, V. (2016). Multidetection of urinary ochratoxin A, deoxynivalenol and its metabolites: Pilot time-course study and risk assessment in Catalonia. *Spain. World Mycotoxin Journal, 9*(4), 597–612. https://doi.org/10.3920/Wmj2015.2006

Vidal, A., Claeys, L., Mengelers, M., Vanhoorne, V., Vervaet, C., Huybrechts, B., De Saeger, S., & De Boevre, M. (2018). Humans significantly metabolize and excrete the mycotoxin deoxynivalenol and its modified form deoxynivalenol-3-glucoside within 24 hours. *Scientific Reports, 8*(1), 5255. https://doi.org/10.1038/s41598-018-23526-9

Vidal, A., Mengelers, M., Yang, S., De Saeger, S., & De Boevre, M. (2018). Mycotoxin Biomarkers of Exposure: A Comprehensive Review: Mycotoxin biomarkers of exposure…. *Comprehensive Reviews in Food Science and Food Safety, 17*(5), 1127–1155. https://doi.org/10.1111/1541-4337.12367

Vorkamp, K., Esteban López, M., Gilles, L., Göen, T., Govarts, E., Hajeb, P., Katsonouri, A., Knudsen, L. E., Kolossa-Gehring, M., Lindh, C., Nübler, S., Pedraza-Díaz, S., Santonen, T., & Castaño, A. (2023). Coordination of chemical analyses under the European Human Biomonitoring Initiative (HBM4EU): Concepts, procedures and lessons learnt. *International Journal of Hygiene and Environmental Health, 251*, Article 114183. https://doi.org/10.1016/j.ijheh.2023.114183

Wallin, S., Gambacorta, L., Kotova, N., Lemming, E. W., Nalsen, C., Solfrizzo, M., & Olsen, M. (2015). Biomonitoring of concurrent mycotoxin exposure among adults in Sweden through urinary multi-biomarker analysis. *Food and Chemical Toxicology, 83*, 133–139. https://doi.org/10.1016/j.fct.2015.05.023

Wallin, S., Hardie, L., Kotova, N., Lemming, E. W., Nalsen, C., Ridefelt, P., Turner, P. C., White, K., & Olsen, M. (2013). Biomonitoring study of deoxynivalenol exposure and association with typical cereal consumption in Swedish adults. *World Mycotoxin Journal, 6*(4), 439–448. https://doi.org/10.3920/WMJ2013.1581

Warth, B., Sulyok, M., Fruhmann, P., Berthiller, F., Schuhmacher, R., Hametner, C., Adam, G., Frohlich, J., & Krska, R. (2012). Assessment of human deoxynivalenol exposure using an LC-MS/MS based biomarker method. *Toxicology Letters, 211*(1), 85–90. https://doi.org/10.1016/j.toxlet.2012.02.023

Warth, B., Sulyok, M., Fruhmann, P., Mikula, H., Berthiller, F., Schuhmacher, R., Hametner, C., Abia, W. A., Adam, G., Frohlich, J., & Krska, R. (2012). Development and validation of a rapid multi-biomarker liquid chromatography/tandem mass spectrometry method to assess human exposure to mycotoxins. *Rapid Communications in Mass Spectrometry, 26*(13), 1533–1540. https://doi.org/10.1002/rcm.6255

Wells, L., Hardie, L., Williams, C., White, K., Liu, Y., De Santis, B., Debegnach, F., Moretti, G., Greetham, S., Brera, C., Papageorgiou, M., Thatcher, N. J., Rigby, A., Atkin, S. L., & Sathyapalan, T. (2017). Deoxynivalenol Biomarkers in the Urine of UK Vegetarians. *Toxins (Basel), 9*(7). https://doi.org/10.3390/toxins9070196