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Deoxynivalenol exposure assessment through a modelling approach of food intake and biomonitoring data – a contribution to the risk assessment of an enteropathogenic mycotoxin

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Abstract

Deoxynivalenol (DON), an enteropathogenic mycotoxin produced by *Fusarium* species, is usually associated with adverse health outcomes such as gastrointestinal diseases and immunotoxicity. To estimate DON exposure of the Portuguese population at national level, a modelling approach, based on data from 94 Portuguese volunteers, was developed considering the inputs of the food

consumption data generated within the National Food and Physical Activity Survey and the human biomonitoring data used to assess the exposure to DON. Ten models of association between DON urinary biomarkers and food items (pasta, cookies, biscuits, sweets, bread, rusks, nuts, oilseeds, beer, meat, milk) were established. Applying the most adequate model to the consumption data (n=5,811) of the general population, the exposure estimates of the Probable Daily Intake revealed that a fraction (0.1%) of the Portuguese population might exceed the Tolerable Daily Intake defined for DON. The analysis stratified by age revealed children (3.2%) and adolescents (6.0%) are more likely to exceed the Tolerable Daily Intake for DON. Although the unavoidable uncertainties, these results are important contributions to understand the exposure to this mycotoxin in Portugal, to assess the associated risk and the potential public health consequences.

Keywords: modelling; mycotoxins; food consumption; urinary biomarkers; Public health

1. Introduction

Deoxynivalenol (DON) is a mycotoxin produced by *Fusarium* species, mainly *Fusarium graminearum* and *Fusarium culmorum*. These fungi, commonly found in Europe, grow on cereals cultivated in areas with temperate climates (EFSA, 2017). DON usually occurs in cereal grains such as wheat, barley, oats, rye and maize, and their by-products (Nagl & Schatzmayr, 2015). The adverse health effects related with exposure to DON are related with gastrointestinal diseases and immunotoxicity and consequently this mycotoxin is usually considered an enteropathogenic compound (Assunção, Alvito, Kleiveland, & Lea, 2016; Maresca et al., 2008; Pestka, 2010; Wu, Groopman, & Pestka, 2014). Several outbreaks of human food poisoning with nausea, diarrhoea, and vomiting as symptoms were associated DON-infested food in Japan, Korea, China and United States of America (Wu et al., 2014). Regarding immunotoxicity, DON has an impact on the immune response. Farm animals and mice exposed to DON showed an increase in the plasmatic level of IgA. In human macrophage cell lines (U937 and HL60), DON (500–1,000 ng/mL) upregulated the production of TNF-α, IL-6, IL-8, and the macrophage inflammatory proteins MIP-1α and MIP-1β (Nagashima, Nakagawa, & Kushiro, 2012; Sugita-Konishi & Pestka, 2001).

Based on above mentioned DON outbreaks and additional evidence obtained under experimental animal studies related to hazard health effects, several measures were considered with the aim of protecting the health of consumers. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) recognized DON as a potential cause of acute human illness and established an acute reference dose (ARfD) of 8 µg/kg body weight (bw) (JECFA, 2011). The ARfD was determined based on the lower limit of the benchmark dose required for emesis induction in 10% ($BMDL_{10}$) of pigs, 0.21 mg/kg bw/day, and considering an uncertainty factor of 25 (JECFA, 2011). In 2017, the EFSA Panel on Contaminants in the Food Chain (CONTAM) decided to establish a group Tolerable Daily Intake (TDI) of 1 µg/kg bw/day for the sum of DON, 3-acetyl-deoxynivalenol (3-ADON), 15-acetyl-deoxynivalenol (15-ADON) and deoxynivelnol-3-glucoside (DON-3G) (EFSA, 2017). The European Commission, following the same principles for protection of public health established maximum admissible limits for the occurrence of DON in food commodities, ranging from 200 µg/kg for cereal-based baby foods to 1,750 µg/kg for raw wheat, oat and maize (European Commission, 2006).

Regarding the human metabolism of DON, the conjugation with glucuronic acid is the main pathway, leading to the formation of deoxnivalenol-3-glucuronide (DON-3-GlcA) and deoxynivalenol-15-glucuronide (DON-15-GlcA). De-epoxy-deoxynivalenol (DOM-1) has also been identified as a metabolite of DON, being excreted predominantly in faeces but also in urine (Turner, Hopton, et al., 2010; Vidal, Bouzaghnane, De Saeger, & De Boevre, 2020; Vidal et al., 2018). DON excretion occurs within 24 h, with a large amount excreted in the first 6 h after ingestion (Vidal et al., 2018). The recovery of total DON (free DON+DON glucuronides) in urine was determined as 64.0±22.8%, and DON-15-GlcA was identified as the main DON urinary biomarker (constant ratio of 4/1 within 24 h, DON-15-GlcA/DON-3-GlcA) (Vidal et al., 2018). A biokinetic model was recently developed considering these data allowing to determine the preferred urinary biomarker(s) (namely DON-15-glucuronide or total DON), the preferred urinary collection period (24 h), and to estimate the dietary exposure to these mycotoxins (by means of a reversed dosimetry factor) (Mengelers et al., 2019).

The association of DON urinary biomarkers with food consumption has been extensively reported with cereal-based products being identified as main contributors to DON exposure, namely cereals, pasta, white bread, wholemeal bread, high fiber breakfast cereals, buns/cakes, fruit pies, total cereal grain, whole grain, cereal grain from porridge, crispbread, savoury biscuits, pasta, bread (Brera et al., 2015; Hepworth et al., 2012; Srey, Kimanya, Routledge, Shirima, & Gong, 2014; Turner et al., 2008; Turner, White, et al., 2010; A. Vidal, Cano-Sancho, Marín, Ramos, & Sanchis, 2016). Due to the short half-life and fast excretion of DON, a good correlation between food consumption and

urinary biomarkers is expected if the biological sample collection is paired with the application of food questionnaires (Martins, Assunção, Nunes, Torres, & Alvito, 2020). Recently, positive and significant correlations were found between exposure to DON estimated from 24h dietary recalls and exposure determined through measurement of urinary biomarkers along the EFCOVAL cohort (n=600) (De Ruyck et al., 2020).

Considering the above mentioned, the recently obtained food consumption data under the National Food and Physical Activity Survey (IAN-AF) (Lopes et al., 2018) were combined with the data regarding human exposure to mycotoxins obtained through a human biomonitoring (HBM) study (Martins et al., 2019), aiming i) to develop a statistical model relating food consumption and DON urinary biomarkers, and ii) to estimate the exposure to DON of all the participants of IAN-AF, stratified by age, sex and region, based on the developed model.

2. Materials and Methods

2.1. Participants

The sampling strategy of the IAN-AF included two stages: 1) based on the random selection of primary health care units, stratified by the seven Nomenclature of Territorial Units for Statistics (NUTS II) (weighted by the number of individuals registered in each health unit), and 2) based on the random selection of registered individuals in each health unit, according to sex and age groups (Lopes et al., 2018). A convenience sub-sample of 94 participants was recruited to participate in the biological samples collection for human biomonitoring studies. These participants collected first-morning urines and 24h urine paired samples following a standardized protocol, at the previous and the day itself of the second interview and in the conditions previously described by Martins et al., 2019 and Lopes et al., 2018. Ethical approval was obtained from the National Commission for Data Protection (Authorization numº 4940/2015) and the Ethical Committee of the Institute of Public Health of the University of Porto (Decision numº CE 16053). All participants provided their written informed consent according to the Ethical Principles for Medical Research involving human subjects expressed in the Declaration of Helsinki and the national legislation. Data collection was performed

under pseudo-anonymization, and all documents with identification data were treated, and stored in a different dataset (Lopes et al., 2018).

Considering the sampling strategy presented above, for the present study two groups of participants were considered. The first group, used to model food consumption and exposure to DON, included the 94 participants from whom HBM and food consumption data was obtained. The second group, for whom exposure to DON was estimated using the modelling tools generated in this study, included 5811 participants who reported consumption data.

2.2. Food and sociodemographic questionnaires

Participants performed two non-consecutive 24h recalls, 8-15 days apart from each other, and an attempt was made to schedule the second interview for a day different from the first interview (n=5,811). All foods, including beverages and composite dishes/recipes consumed during the 24-h period, were quantified as eaten. Food categories comprised three levels of aggregation (Lopes et al., 2017, 2018). For the present study, seven food categories in the 1st and 2nd levels of aggregation were considered for the modelling approach: “fruits and vegetables” (fruits, vegetables, pulses, nuts and oilseeds), “dairy products” (milk, cheese, yoghurt, milk cream), “cereals” (pasta, rice and other grains, flours and bakery powders, breakfast cereals and bars), “meat, fish & eggs”(meat, fish, eggs), “cookies, biscuits & sweets” (sweets, cakes, cookies and biscuits), “non-alcoholic drinks” (tea, coffee, water), and “alcoholic drinks” (wine, beer, other drinks).

Socio-demographics data included: sex and age (calculated using the first interview date and birth date), automatically imported from datasets obtained from the National Health Registries and checked during the first contact with the participants; information on marital status, number of completed years of education, professional situation, household structure and household monthly income, collected in a format of closed questions (Lopes et al., 2017, 2018).

2.3. Exposure data to DON using HBM data

DON urinary biomarkers were used to estimate the exposure of the Portuguese population, considering the results obtained by Martins et al. (2019). Data regarding urinary biomarkers were obtained for 24h urine and first-morning urine paired samples using a QuEChERS-based procedure (Quick, Easy, Cheap, Effective, Rugged, Safe) for sample preparation followed by identification and

quantification by liquid chromatography with mass spectrometry detection (LC-MS/MS) and was performed at Ghent University (Ghent, Belgium). The analytical method was previously optimized by Vidal et al., (2018) and is described in detail by Martins et al., (2019). Further details are included in Supplementary Material (S1).

The Probable Daily Intake (PDI) was estimated considering the excretion rate of 64% (Vidal et al., 2018). A multiple imputation procedure was applied based on 20 simulation and with a maximum of 100,000 for case and parameter to treat the left-censored data of urinary biomarkers results (Martins et al., 2019). This procedure allowed to keep variability within the results below the limit of detection (LOD) (H. Chen, Quandt, Grzywacz, & Arcury, 2013) and the use of the complete dataset for the modelling approach.

2.4. Modelling of food consumption and HBM data

Data obtained by Martins et al. (2019) for urinary levels of DON, DOM-1, DON-3G, DON-3-GlcA, DON-15-GlcA and Total DON (Sum of DON, DOM-1, DON-3G, DON-3-GlcA and DON-15-GlcA, considering the mass ratio between the parent compound and the metabolites), expressed as volume weighted concentrations (µg/L), creatinine adjusted concentrations (µg/g crea) and daily excretion (µg/day) were used for the modelling approach. These data were compared with food consumption data (1st and 2nd level of aggregation, in a total of 30 variables) obtained from food questionnaires. Considering that significant associations between food consumption of last 24h and urinary biomarkers are expected for mycotoxins with short half-lives (Martins et al., 2020), only consumption data from the 2nd interview was considered for this modelling. At first, both variables (biomarkers and food consumption) were compared as continuous variables by bivariate analysis (Spearman's correlation coefficient) (n=94). Food consumption variables associated with urinary biomarkers concentration ($p < 0.2$) were retained for the multivariate analysis. For the multivariate analysis, the Generalized Linear Model (GLM) was chosen due to the non-normality of urinary biomarkers' distributions. For the model, food consumption variables were considered as independent variables and urinary biomarkers levels were considered as dependent variables. Three types of GLM were tested: i) normal distribution, ii) gamma distribution, and iii) normal distribution with dependent variable log transformed. Variables were retained and considered to contribute significantly to the GLM if $p < 0.1$. The criteria considered for assessing the adjustment of models were the Spearman correlation coefficient and Omnibus test. Residuals analysis was performed.

The models developed were used to derive HBM and PDI data for the group of 5,811 participants of IAN-AF study. For estimation of usual exposure, the models were applied to consumption data of both interviews using SPADE software (Statistical Program to Assess Dietary Exposure, implemented in R software as package SPADE.RIVM) (Dekkers, Verkaik-Kloosterman, van Rossum, & Ocké, 2014), and an overall analysis weighted for the Portuguese population was performed, presenting mean, median and percentiles 75 and 95 for HBM (µg/g creatinine) and PDI (µg/kg bw/day). For estimation of exposure stratified by sex (male; female), age (children 0-9 years; adolescents 10-17 years; adults 18-64 years; elderly > 64 years) and region (North, Centre, Lisbon Metropolitan Area, Alentejo, Algarve, Madeira, Azores), descriptive (mean, median, and percentiles 75 and 95) and inferential analysis (Mann-Whitney and Kruskal Wallis non-parametric tests) were performed. The estimates of PDI were achieved considering the derived HBM data and the individual body weight. For daily urinary volume the following values were considered: 48 mL/kg for participants ≤ 5 years, 36 mL/kg for participants > 5 years and ≤11 years, and 24 mL/kg for participants ≥ 12 years (Hazinski, 1992). Statistical analysis was performed with SPSS v.24 and R software.

3. Results and Discussion

3.1. Sociodemographic characterization of participants

Participants in HBM study (n=94) were similarly distributed by sex, with 51.1% of males and 48.9% of females, and were from two regions of Portugal North (78.7%) and Centre (21.3%). Regarding the educational level, about half of the participants (51.1%) reported 9 years or less of education. Only 13.8% reported a monthly income above 1,941€ and 55.3% of the participants are workers for remuneration or profit.

Participants in IAN-AF study (n=5,811) were similarly distributed by sex, with 48.1% of males and 51.9% of females, and presented a distribution across the country with similar percentages from all regions. This group included participants from all age groups. Regarding the educational level, 44.5% of the participants reported 10-12 years of education. Regarding the monthly income, the range of 485-1,455€ was reported by almost half of the participants (49.0%). More than half of the participants (55%) are workers for remuneration or profit. Both groups of participants presented

similar sociodemographic characteristics. The group of 5,811 participants is representative of the Portuguese population at regional level.

3.2. DON urinary biomarkers and food consumption data

DON exposure levels (n=94) were used according to the urinary biomarkers determined by Martins et al. (2019) and are presented in Table 1. The authors reported the results of the urinary biomarkers of DON in a human biomonitoring study where 24h urine and FMU paired samples of participants from North and Centre regions of Portugal were analysed. All the analysed metabolites of DON were detected in both types of urine samples. Regarding 24h urine samples, positive samples (>LOD) were reported for DON (63%), DOM-1 (41%), DON-3G (20%), DON-3-GlcA (44%) and DON-15-GlcA (52%). Regarding FMU samples, positive samples (>LOD) were reported for DON (30%), DOM-1 (32%), DON-3G (11%), DON-3-GlcA (24%) and DON-15-GlcA (39%) (Martins et al., 2019) (Supplementary Material 2).

~~<Table 1>~~

The food consumption of across the different categories considered under the present study are reported in Table ~~2~~1. The food category with the highest reported consumption was “non-alcoholic drinks”, mainly due to the consumption of water (data not shown). Regarding the remaining food groups, “fruits and vegetables” was the group presenting the highest median consumption, followed by “cereals”, “dairy products”, “meat, fish and eggs” and “cookies, biscuits, sweets”. The consumption reported for all food categories did not present statistically significant differences between the 1st and the 2nd interview ($p > 0.05$).

< Table ~~2~~1>

3.3. Link between food consumption and exposure levels of deoxynivalenol

The results presented in Table ~~3~~ 2 summarize the statistically significant associations between the consumption of some food items and the DON urinary levels of biomarkers. From the different GLMs

tested, it was considered the log-transformed urinary biomarkers as dependent variables and the food consumption data (from the seven categories considered) of 2nd interview as independent variables.

<Table ~~3~~2>

Several models of association between DON urinary biomarkers and food consumption data were obtained (Table ~~3~~2). Regarding the food items, there is a predominance of cereal-based products such as pasta, cookies, biscuits, bread, and beer. Martins et al., 2020 reviewed the available literature regarding the association of mycotoxins' urinary biomarkers and food consumption and the following food items were found as related with DON urinary excretion: bread, breakfast cereals, pasta, pizza, fruit pies, buns/cakes (Turner et al., 2008), wheat- and maize-based foods (Turner, White, et al., 2010), cereal products (Hepworth et al., 2012; Wallin et al., 2013), maize products (Srey et al., 2014), breakfast cereals and bread (Brera et al., 2015). These studies assessed this association considering different levels of aggregation of food consumption data as well as different types of food surveys.

For the remaining food items considered in the models (Table ~~3~~2), some available literature may validate the results obtained, namely for the occurrence of DON in nuts (Cunha, Sá, & Fernandes, 2018) and animal products (Pralatnet et al., 2015).

The present study considered data obtained through a detailed food survey (24h dietary recall) and a paired biological samples collection of 24h urine and FMU. Considering the excretion pattern of DON, it was expected to find good correlations for 24h urine (Mengelers et al., 2019). The correlations found for FMU can be explained by an exposure occurring in the last meals of the day before the sample collection. Regarding the DON urinary biomarkers of exposure, models of association were determined for all of them, confirming the potential use of these metabolites as valid biomarkers of exposure.

Considering all the models obtained for DON biomarkers, it was decided to proceed with the model for Total DON (24h urine), considering pasta and biscuits consumption, for the estimation of exposure to DON for the Portuguese population. The food items considered in this model are supported by available literature as determinants of exposure (Turner et al., 2008) and the biomarker "total DON" corresponds to the sum of all biomarkers of DON (considering the mass ratio between

the parent compound and the metabolites) thus allowing a direct comparison with the TDI for DON (group TDI of 1 µg/kg bw/day) after estimation of exposure (EFSA, 2017).

3.4. Estimation of exposure of Portuguese population by sex, age group and region to deoxynivalenol

Using the data collected under the IAN-AF and the model developed in the present study, the estimation of exposure to DON for a representative sample of Portuguese population (n=5,811) stratified by region, sex and age groups was performed.

Considering the consumption data generated using SPADE software, the usual exposure to DON for the 5811 participants was determined and is summarized in Table 43.

< Table 4 3 >

The median estimate of PDI applying modelling was 0.372 µg/kg bw/day. The estimation of the percentage of participants that would exceed the TDI for Total DON of 1 µg/kg bw/day (0.1%) is lower than the percentage determined by Martins et al., 2019 (9-10%), with considerably lower estimates of intake for the high percentiles of exposure, meaning that the results obtained by modelling were conservative.

Results for the estimated exposure of 5,811 participants, stratified by sex, age and region, and the percentage of participants from each category that exceeded the TDI established for DON are presented in Table 54.

< Table 5 4 >

Regarding sex, males and females presented similar patterns of estimated exposure, with no statistically significant differences between them, and with a reduced percentage of participants estimated to exceed the TDI for DON (1.8% for males and 3.0% for females).

Regarding the age group, higher concentrations of total DON are estimated for children and adolescents than for adults and elderly, and this fact is maintained when calculating the PDI. This is

in accordance with what was previously reported. Data from HBM studies performed in UK, Italy and Norway and Belgium also revealed the exceedance of TDI by children and adolescents in higher percentages than adults (Brera et al., 2015; Heyndrickx et al., 2015). The higher DON urinary concentration in children was referred as being caused by the immature liver function (in comparison with adults), resulting in a lower expression of the UGT-enzyme and in a lower ability to detoxify and metabolize potentially harmful substances (Chen et al., 2017). Overall, in the present study the percentage of participants estimated to exceed the TDI for DON ranged from 0.4 – 6.0% considering all the age groups.

Regarding the region of Portugal, the percentage of participants estimated to exceed the TDI for DON ranged from 1.2% to 3.0%. Alentejo and Algarve showed statistically significant differences with estimated urinary DON concentrations and PDI lower than the remaining regions. These differences may be explained by the lower consumption of pasta, cookies, and biscuits in Alentejo and Algarve when compared with the other regions of Portugal (data not shown).

Results obtained by Martins et al. (2019) showed that 9-10% of participants exceeded the TDI for DON. These data were obtained considering data for daily urinary volume, body weight and concentrations of urinary DON at individual level, thus providing results for PDI with a reduced level of uncertainty. In the present study, results were obtained for 5811 participants through a modelling approach based on food consumption data, where data at individual level was available for food consumption (24h recall) and body weight. The urinary volume was imputed according to body weight, as detailed in Materials and Methods section. The interpretation of these estimates should be considered carefully since they are also affected by a degree of uncertainty. Additionally, these estimates considered only the food consumption variables that remained significant in the statistical models, leaving aside other possible sources of exposure.

Nevertheless, the results obtained under this study are the first estimates of exposure to DON for a representative sampling of the Portuguese population. It was also possible to estimate the percentage of participants that may exceed the reference intake value and whose exposure could potentially represent a health concern. The over-exposure of children and adolescents is again demonstrated when compared to other age groups, meaning that this is an issue requiring further assessments.

Despite the uncertainties referred, these results are important contributors to the risk assessment of Portuguese population exposed to DON and consequently, an important contribute to the potential establishment of public health preventive measures. The study highlights the importance of properly

and periodically assessing the exposure of Portuguese population to mycotoxins, with the development of epidemiological studies including collection of blood paired with urine samples, for a broader view on exposure and consequently a more accurate risk characterization. These assessments will make possible the continuous identification of vulnerable population groups and the evaluation of time trends regarding exposure. If needed, and using the precautionary principle, the implementation of control strategies for the contamination levels of food products should be put in place.

4. Conclusions

The health effects of DON, an enteropathogenic mycotoxin, represent a potential threat from a public health and economic perspective. Through mathematical modelling of HBM and food consumption data, it was possible to estimate the exposure of the Portuguese population to DON for a representative sampling. These estimates revealed that the Portuguese population is exposed to DON, with a low fraction (0.1%) estimated to exceed the TDI established for this mycotoxin. The stratified analysis by age revealed that children and adolescents are estimated to exceed the TDI in higher percentages than adults, thus highlighting the importance of measures aiming to reduce the exposure in vulnerable population groups.

The importance of the development of biomonitoring studies linked with food and health surveys is highlighted in this study, since a more complete analysis become possible. The acquisition of data from participants in these three domains opens the possibility of designing tailored public health interventions aiming to reduce exposure levels and the potential associated toxic effects.

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Highlights

A mathematical modelling was performed to estimate exposure to deoxynivalenol (DON).

The exposure of Portuguese population to DON was estimated for all age groups.

Children (3.2%) and adolescents (6.0%) exceeded the Tolerable Daily Intake for DON.

Cereal-based products were confirmed as contributors for exposure to DON.

Table 1 - Food consumption reported in edible grams per day (g/day) by the two groups of IAN-AF: n=94 and n=5811.

		HBM study (n=94)			IAN-AF (n=5811)		
	Interview	**Median (g/day)**	**IQR (g/day)**	**P95 (g/day)**	**Median (g/day)**	**IQR (g/day)**	**P95 (g/day)**
Fruits and vegetables	1st	297.4	186.2 - 528.7	699.1	272.6	159.4 – 408.0	662.7
	2nd	311.9	177.9 - 465.8	783.9	263.4	153.7 – 393.2	655.4
Dairy products	1st	193.0	27.6 - 384.7	744.8	272.8	118.9 – 462.1	794.7
	2nd	222.8	100.9 - 326.3	528.4	268.8	109.0 – 460.1	789.7
Cereals	1st	287.5	186.8 - 393.6	661.8	264.4	169.1 – 379.2	623.9
	2nd	278.1	179.8 - 416.7	720.0	256.1	167.6 – 366.4	606.2
Meat, Fish and Eggs	1st	188.7	117.8 - 284.4	535.9	133.7	69.5 – 220.8	391.1
	2nd	165.9	94.5 - 275.3	457.2	134.2	68.9 – 221.7	405.5
Cookies, biscuits, sweets	1st	39.6	10.4 - 121.8	209.3	29.0	6.0 – 101.2	240.0
	2nd	38.9	9.5 - 102.5	259.4	28.0	6.0 – 100.0	242.4
Non-alcoholic drinks	1st	1273.0	726.2 - 1822.8	2884.3	899.1	412.1 – 1551.0	2351.6
	2nd	1183.1	742.0 - 1811.8	2548.7	866.1	410.0 – 1514.2	2329.4
Alcoholic drinks	1st	8.3	0.0 - 251.1	921.9	0.0	0.0 – 27.7	582.2
	2nd	7.6	0.0 - 238.4	979.9	0.0	0.0 – 25.3	591.3

HBM = Human Biomonitoring; IAN-AF = National Food, Nutrition and Physical Activity Survey; IQR = Interquartile range; P95 = Percentile 95

Table 2 - Effect of consumption of food categories and the urinary levels of DON, DOM-1, DON-3G, DON-3-GlcA and DON-15-GlcA.

Urine sample	Urinary biomarker	Food category	Regression coefficients	*p* value	R	Omnibus
24h urine	DON (µg/g crea)	Milk Cream	0.044	0.031	0.400	0.000
		Pasta	0.002	0.010		
		Cookies, biscuits & sweets	0.003	0.003		
	DOM-1 (µg/g crea)	Pasta	0.002	0.004	0.300	0.005
	DON-3G (µg/g crea)	Pasta	0.001	0.004	0.294	0.005
		Cookies, biscuits & sweets	0.001	0.040		
	DON-3-GlcA (µg/g crea)	Pasta	0.002	0.036	0.242	0.014
		Cookies & Biscuits	0.006	0.035		
	DON-15-GlcA (µg/L)	Pasta	0.003	0.023	0.250	0.009
		Cookies & Biscuits	0.007	0.031		
	TOTAL DON (µg/L)	Pasta	0.002	0.033	0.311	0.008
		Cookies & Biscuits	0.006	0.008		
First Morning Urine	DON (µg/g crea)	Bread & Rusks	0.001	0.035	0.476	0.000
		Nuts & Oilseeds	0.011	0.042		
		Pasta	0.002	0.025		
		Meat	0.001	0.058		
		Cookies, biscuits & sweets	0.001	0.073		
	DOM-1 (µg/g crea)	Bread & Rusks	0.001	0.089	0.286	0.004
		Nuts & Oilseeds	0.012	0.012		
		Beer	0.001	0.071		
	DON-3-GlcA (µg/g crea)	Nuts & Oilseeds	0.022	0.000	0.361	0.000
		Meat	0.001	0.032		
		Cookies & Biscuits	0.005	0.024		
	DON-15-GlcA (µg/L)	Nuts & Oilseeds	0.021	0.002	0.250	0.011
		Milk	0.001	0.054		

R = Spearman's correlation coefficient; DON = Deoxynivalenol; DOM-1 = deepoxy-deoxynivalenol; DON-3G = deoxynivalenol-glucoside; DON-3-GlcA = deoxynivalenol-3-glucuronide; DON-15-GlcA = deoxynivalenol-15-glucuronide.

Table 3 - Estimated usual exposure to deoxynivalenol of 5811 participants of IAN-AF, **weighted** for the Portuguese population distribution.

	Distribution					Reference Intake	
	P25	*Median*	*Mean*	*P75*	*P95*	*RVI*	*% ≥ RVI*
Total DON							
Exposure (µg/L)	5.37	6.72	7.10	8.41	11.62	-	-
PDI (µg/kg bw/day)	0.30	0.37	0.39	0.45	0.62	1.0	**0.1**

RVI=Reference Value for Intake; DON=Deoxynivalenol; P25=Percentile 25; P75=Percentile 75; P95=Percentile 95; PDI=Probable Daily Intake

Table 4 - Estimated exposure to DON for participants of IAN-AF (n=5811), stratified by sex, age and region.

	DON - Distribution Exposure (µg/g crea); PDI (µg/kg bw/day)				RVI
	Median	Mean	P75	P95	% ≥ RVI
Sex					
Male	4,99; 0.242	8.27; 0.371	7.33; 0.398	16.56; 0.802	1.8
Female	5.52; 0.242	7.31; 0.330	7.22; 0.367	13.29; 0.650	3.0
Age *					
Children (0-9 years)	5.91; 0,431	7.08; 0.495	7.53; 0.535	12.21; 0.867	3.2
Adolescents (10-17 years)	6.45; 0.275	10.39; 0.445	9.84; 0.419	24.75; 1.099	6.0
Adults (18-64 years)	4.89; 0.183	7.93; 0.297	7.14; 0.268	15.54; 0.583	1.8
Elderly (>64 years)	4.89; 0.183	6.13; 0.230	6.49; 0.244	9.74; 0.365	0.4
Region **					
North	5.56; 0.268	7.57; 0.343	7.57; 0.391	15.44; 0.741	2.4
Centre	5.65; 0.255	8.25; 0.371	7.88; 0.398	16.64; 0.777	2.8
Lisbon Metropolitan Area	5.31; 0.240	7.47; 0.339	7.14; 0.370	14.07; 0.727	2.8
Alentejo	4.89; 0.216	6.92; 0.311	6.73; 0.367	12.58; 0.611	1.5
Algarve	4.89; 0.222	6.81; 0.309	6.58; 0.367	11.84; 0.632	1.2
Madeira	5.78; 0.248	8.86; 0.395	8.01; 0.412	19.33; 0.839	3.0
Azores	5.14; 0.240	8.32; 0.373	7.21; 0.367	14.42; 0.801	2.8

DON=deoxynivalenol; P75=percentile 75; P95=percentile 95; PDI=probable daily intake; RVI=reference value for intake (TDI for DON = 1 µg/kg bw/day);

* Kruskal-Wallis test ($p<0.05$); significant differences were found for all the categories of age;

** Kruskal-Wallis test ($p<0.05$); significant differences were found for the regions of Algarve and Alentejo.