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ARTICLE

Determinants of Urinary Deoxynivalenol and De-epoxy Deoxynivalenol in Male Farmers from Normandy, France

Paul C. Turner,*,† Richard P. Hopton,†,‡ Yannick Lecluse,§ Kay L. M. White,† Julie Fisher,‡ and Pierre Lebailly§

†Molecular Epidemiology Unit, Centre for Epidemiology and Biostatistics, Leeds Institute of Genetics, Health and Therapeutics, ‡School of Chemistry, University of Leeds, Leeds, U.K., and §Groupe Régional d'Etudes sur le Cancer EA1772, IFR 146, Centre François Baclesse, Caen, France

Dietary exposure to deoxynivalenol (DON) from contaminated cereal crops is frequent in Europe, and farm workers who handle grain or silage may be at additional risk. In this study we refined a urinary assay for DON and present a novel assay for the DON metabolite de-epoxy-deoxynivalenol (DOM-1). These were applied to a pilot survey of male French farmers (n = 76, aged 23−74). DON was detected in 75/76 samples (range 0.5−28.8 ng/mL) and DOM-1 in 26/76 samples (range 0.2−2.8 ng/mL). In multivariate analysis including creatinine as a covariate, bread consumption, other cereal consumption, and maize acreage contributed to the model, explaining the variation in urinary "DON and DOM-1" concentration combined (R^2 = 0.33). This is the first exposure biomarker survey for DON in a French population, and the first demonstration of urinary DOM-1 in humans. Further investigations into occupational activity, handling, or airborne exposures would be informative.

KEYWORDS: Biomarker; deoxynivalenol; de-epoxy-deoxynivalenol; farmer; France; urine

INTRODUCTION

Agricultural work has been associated with an increased risk of developing certain chronic diseases, including prostate, hematological, and brain cancers, neurological disorders, and reproductive effects (*1*−*4*). In addition to effort in assessing the effects of exposure to pesticides, there remains a need to develop epidemiological or analytical tools for the identification and quantification of other suspected risk factors, such as mycotoxins. Mycotoxins are a diverse group of toxic low-molecular-weight fungal secondary metabolites (*5*) that are common contaminants of food and feedstuffs. A number of mycotoxins are immunosuppressive, including *Fusarium* mycotoxins such as deoxynivalenol (DON), and thus are predicted to be involved in human disease (*6*). DON contaminates cereal crops, including wheat, maize, and barley (*5*), and human exposure within Europe and Asia is predicted to be frequent (*5*, *7*, *8*). Consumption of DON-contaminated cereals has been implicated in food poisoning incidents in China between 1961 and 1991 (reviewed by Pestka and Smolinski (*6*)) and India in 1987 (*9*), in which tens of thousands of people over a wide area were affected. While ingestion of contaminated food is regarded as the more dominant route of human exposure, inhalation is also predicted to be important among certain groups, particularly farm workers handling grains or silage (*5*, *10*−*12*). Our studies in Normandy revealed that farmers were at risk of being exposed to a range of mycotoxins, including aflatoxin, citrinin, zearalenone, and DON, from feeding of animals with maize silage and wheat grains (*13*). DON was detected at the highest level of contamination, peaking at around 200 μg of DON/kg of maize silage.

In order to better understand DON exposure at the individual level, we recently developed a highly sensitive and robust urinary assay for DON (*14*) involving immuno-affinity column (IAC) enrichment, with sample concentration quantified by LC-MS. Both DON and a DON-glucuronide are reported in humans (*15*) and animals (*6*); thus, as DON-glucuronide is not retained by the IAC, a digestion step is performed to convert any DON-glucuronide to the parent DON (*14*−*17*), generating a composite value for urinary DON. Urinary DON has been frequently observed in U.K. adults and was significantly positively associated with cereal intake, in particular bread consumption (*14*, *16*, *18*). Here we report a refinement of the expensive and laborious extraction by use of an immunoaffinity resin with improved flow characteristics and smaller urine volume. A subset of samples previously analyzed by Turner et al. (*16*) was used to compare the two methods across a range of typical exposures. In addition, the application of a novel assay to measure the de-epoxy-deoxynivalenol metabolite of DON (DOM-1) is presented. The formation of this metabolite occurs by intestinal gut microbiota rather than mammalian metabolism (*19*−*23*) and is frequently observed in animals exposed to DON (*6*). The formation of this less toxic metabolite reportedly provides protection against DON toxicity (*6*), though its occurrence in humans remains poorly investigated. The assays developed for DON and DOM-1 were subsequently applied to measure total urinary DON in a novel set of urines from farm workers in Normandy, France, selected from a prospective cohort study (*24*).

*To whom correspondence should be addressed. Tel: 44-113-343-7770. Fax: 44-113-343-6603. E-mail: p.c.turner@leeds.ac.uk.

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MATERIALS AND METHODS

Immunoaffinity Column Comparison: Single Sample. Initial experiments compared the levels of DON extracted from a single urine sample using two different volumes of urine (4 mL, as used originally (*16*), and a smaller volume of 1 mL) and two types of IAC column (the original DONtest IAC column (Vicam Ltd., Watertown, MA) as used by Turner et al., (*16*, *17*) and the new wide bore DON-IAC column (Vicam Ltd., Watertown, MA) with improved flow characteristics. The columns contain the same antibody, but the modified solid-phase attachment in the latter allows more rapid flow and lower risk of trapping air bubbles. DON was extracted from urine and then quantified as previously described (*16*). In brief, to 4 mL of urine (pH adjusted to 6.8) in triplicate was added $^{13}C_{15}$ DON (125 μL, 160 ng/mL) as an internal standard (IS). Urine was incubated for 18 h at 37 °C with 23 000 units of β-glucuronidase, Type IX-A from *E. coli* (Sigma, Poole, Dorset, U.K.), and following digestion, the samples were centrifuged (2000*g*, 15 min, 4 °C) and the supernatant diluted to 16 mL with phosphate-buffered saline (PBS), pH 7.4. For 1 mL samples the amount of β-glucuronidase was adjusted proportionally to the volume change and, prior to passage through the IAC, digested urines were prediluted to 4 mL rather than 16 mL. After IAC columns were loaded, a wash step with 10 mL of water removed unbound material and DON was eluted with 4 mL of methanol, dried in vacuo, and reconstituted in 250 μL of 10% (v/v) ethanol for analysis. LC-MS was conducted as previously described (*16*) using a Waters 2795 separations module (Milford, MA) connected to a Micromass Quattro Micro triple quadrupole mass spectrometer (Manchester, U.K.). The separation of DON was achieved using a 150 mm × 4.6 mm i.d., 5 μm, Luna C18 column, with a 4 mm × 4 mm i.d. guard column of the same material (Phenomonex, Macclesfield, U.K.). The LC-MS standard curve was constructed using seven concentrations of DON, range 2−250 ng/mL, each spiked with the IS. Selective ion recording was used to quantify the individual DON levels by reference to the IS. Test samples were quantified by reference to a response−ratio calibration curve generated by Quanlynx software.

Immunoaffinity Column Comparison: Multiple Samples. The initial study detailed above used only one concentration of urinary DON. In order to compare data across a range of DON concentrations in naturally contaminated urine, we selected an archived set of urine samples previously described (*16*). Of the original 25 samples, 22 were available. DON was re-extracted from these samples using both the original method (4 mL of urine and DON-IAC columns (Vicam Ltd., Watertown, MA) and from 1 mL of urine using wide bore DON-IAC columns (Vicam Ltd., Watertown, MA). Extractions and LC-MS analysis were conducted as described above.

Urinary De-epoxy Deoxynivalenol (DOM-1) Analysis. *Method Development*. The capacity and efficiency of the wide-bore DON IAC cartridges to retain and enrich this DON metabolite from urine was assessed. Extractions were conducted in triplicate on two separate occasions using 1 mL of both PBS and blank urine spiked with DOM-1 (Sigma, Poole, Dorset, U.K.) at 0, 4, and 20 ng/mL; the urine used was from a celiac volunteer with no wheat consumption and previously demonstrated to have no detectable urinary DON. Each aliquot was digested overnight with β-glucuronidase and extracted using DON IAC cartridges, in a method equivalent to that used for DON extraction. The final eluted methanol fraction was dried in vacuo and reconstituted to 250 uL in 10% ethanol, prior to injection on LC-MS. The DOM-1 LC-MS method used DOM-1 standards (range 0.1−100 ng/mL; LOD 0.2 ng/mL). Two masses of DOM-1 were monitored, DOM-1-H^+ (m/z 281.3) and DOM-1-Na^+ (m/z 303.3), each for 0.25 s and then summed to produce one total ion current peak for DOM-1. All other LC-MS conditions were identical with the DON LC-MS methods described previously. DOM-1 eluted at 14.0 min. The concentration of DOM-1 in test samples was calculated from the standard curve using least-squares regression. The overall LOD for DOM-1 was 0.05 ng of DOM-1/mL of urine.

French Farmers' Study Area and Design. Located in Normandy, Calvados is a geographical area of 5500 km^2, with 648 000 inhabitants (1% of the total French population). A total of 80% of the land is devoted to agriculture, mainly open field farming (22% for wheat and barley, 9% for maize) and cattle breeding (430 000 head). A random sample of 8% of the 7991 farms of this area was generated in 1997, and the farm owner of each selected farm was visited (*24*). In case of refusal, a short anonymous questionnaire on the age and sex of the farm owner and on the farm characteristics (including pesticide use) was completed. During the period 1997−2000, 410 farms (74% participation rate) were included and 758 subjects, corresponding to all adults on each participating farm (farm owners, spouses, agricultural workers, and retirees), were surveyed. Biological samples were obtained in more than 90% of these individuals, including the first morning void urine. Urine was stored at −20 °C within 6 h. A face to face comprehensive standardized questionnaire was completed by all enrolled subjects to provide information about sociodemographic characteristics, tobacco and alcohol use, dietary habits, medical histories, and lifetime occupational exposure to pesticides. A questionnaire specifically dedicated to farm characteristics and agricultural activities was also provided from each farm. For this project, ethical approval was obtained from the local ethical committee (*Comité Consultatif Pour les Personnes se Prêtant à la Recherche Biomédicale*) and informed consent was obtained from each subject prior to the beginning of the study.

For the present study, a random sample of 34% (N = 76) of the "never smoking" males from the larger cohort (*24*) were selected for urinary DON and DOM-1 analysis. The mean age subcohort was 43 years (range 23 to 74), the mean height was 1.75 m (range 1.60 to 1.90 m), the mean weight was 77 kg (range 50−105 kg), and the mean BMI was 25.2 kg/m^2 (range 17.1−34.1 kg/m^2). Samples were extracted using wide-bore DON-IAC columns and 1 mL of urine as described above. The extracts from the 22 U.K. samples previously used for DON analysis as part of the improved method development for DON were also injected onto the LC-MS to measure DOM-1. A urinary creatinine measure was additionally made by the Jaffé reaction (*25*) using a Cobas Mira+ device.

DON Extraction Quality Control. Six 1 mL samples of phosphate buffered saline (pH 7.4) were extracted alongside the test samples as a blank quality control; all were below the limit of detection. Four 1 mL urine samples previously demonstrated to be below the limit of detection (< 0.5 ng DON/mL of urine) were analyzed alongside the test samples as a further blank quality control; all were below the limit of detection. Six 1 mL aliquots of a urine sample containing 7.5 ng of DON/mL of urine were extracted alongside the test samples as a positive quality control. All positive quality control samples had detectable DON (mean 7.5 ng of DON/mL of urine (SD 0.4 ng/mL).

Statistical Analysis of the French Farmers' Samples. The concentration of DON and DOM-1 in urine was combined to give a value for total DON (TDON). Normality was tested and rejected because of the skewness of the distribution of TDON. After natural log transformation, normality was not rejected. Parametric statistical tests were then used after log transformation of these data. Eighteen variables were tested, including those related to individual characteristics (age and body mass index (BMI)), cereals within the diet on the day before sampling (beer consumption, bread, breakfast cereals, pasta, pizza, etc.), and occupation (job title, season-related tasks, area devoted to crops, maize or wheat/barley, cattle breeding, specific tasks performed the day before urine sampling). Univariate analyses, using Fisher's exact test or the Student t test, or linear regression analysis, when appropriate, was performed. Significant correlations (Pearson correlations) between variables were identified, and the most meaningful variables were kept for multivariable analysis. Only factors that were identified with $p < 0.20$ in univariate analysis were entered into the multivariable analysis. The multiple linear regression model was used with the backward stepwise method. For the final model, the variable was kept if its p value < 0.05 or if its exclusion from the model changed estimated regression coefficients by more than 30%. Analyses were conducted with log natural DON data with urinary creatinine added in the final model as a separate independent variable, as suggested by Barr et al. (*26*). Any sample below the quantification limit was assigned a value of half the limit of quantification for statistical purposes. Stata version-7 (Stata Corp., College Station, TX) was used throughout.

RESULTS AND DISCUSSION

Column Comparison. The original DONtest IAC columns and the newer wide bore DON-IAC columns were compared for their ability to extract DON from the normal volume of urine (4 mL) and a smaller volume of urine (1 mL). There were no significant differences between the DON concentrations obtained from any of the four extraction methods ($p > 0.30$ for all comparisons).

For the DONtest IAC, mean urinary DON levels were 5.8 ng/mL (SD 0.2 ng/mL) and 5.7 ng/mL (SD 0.2 ng/mL), for 4 and 1 mL, respectively, while for the wide-bore DON-IAC columns urinary DON levels were 6.1 ng/mL (SD 0.2 ng/mL) and 6.0 ng/mL (SD 0.1 ng/mL), for 4 and 1 mL, respectively. The percent difference between the original methodology (4 mL of urine) with DON-IAC columns and other tests was ≤5% for all comparisons. This is within the normal variation of the assay. In comparison to the original method (DONtest IAC using 4 mL of urine), the percentage reductions in IAC extraction times were ∼15%, ∼60%, and ∼70% for the DONtest IAC using 1 mL of urine, the wide-bore DON-IAC using 4 mL, and the wide bore DON-IAC using 1 mL, respectively.

Subsequently DON was extracted from the 22 archived urines using DONtest IAC columns and 4 mL of urine (original methodology (*16*)) and from the wide-bore DON-IAC columns using 1 mL of urine. DON was detected in all 22 samples, using both methods. For DONtest IAC columns the mean was 9.2 ng of DON/mL of urine, range 1.0−58.8 ng/mL, while for the wide-bore DON-IAC columns the mean was 9.2 ng/mL, range 0.9−57.4 ng/mL. A scatter plot to compare these two data sets revealed an excellent correlation between the two methods ($R^2 >$ 0.99, best line of fit $y = 0.96x - 0.60$). Removal of the one high outlier in the set had no strong effect on the comparison of the data ($R^2 = 0.98$). The mean percentage difference for all pairs of measurements was 4.2% (95% CI: −1.5, 7.9%). These data provide considerable confidence to adopt the more rapid extraction approach in subsequent studies.

There was 22 months of cryo-storage (−40 °C) between the original analysis of samples (*16*) and the subsequent analysis using both the original method and the modified method. A similarly strong correlation between the samples measured by the same extraction process but separated by nearly 2 years was apparent ($R^2 > 0.99$, best line of fit $y = 0.95x - 0.51$). The mean percentage difference for all pairs of measurements was 10.6% (95%CI: 2.1, 18.1%). A further test of storage stability on this set was conducted by extracting DON using the original method 36 months after the first analysis. Again a strong correlation remained when comparing the original measure (*16*) and the measure after 3 years of storage ($R^2 > 0.99$, best line of fit $y = 1.05x - 0.38$). The mean percentage difference for all pairs of measurements was 6.3% (95%CI: 0.4, 12.2%). It would appear that urinary DON was stable when samples were stored for up to 3 years at −40 °C. Longer term storage trials are in progress, but these data suggest that archived urines may provide a valuable resource for epidemiological investigations.

DOM-1 Spiking Experiment. For the spiking experiment no peak was observed for blank PBS and blank urine, while DOM-1 was evident in the LC-MS chromatograms for all spiked samples. The mean and standard deviation for each set of replicates was 3.5 ± 0.2, 3.2 ± 0.3, 18.2 ± 0.7, and 17.4 ± 0.9 ng/mL for PBS 4 ng/mL, urine 4 ng/mL, PBS 20 ng/mL, and urine 20 ng/mL, respectively, for extraction 1 and 3.6 ± 0.2, 3.5 ± 0.2, 18.4 ± 1.2, and 16.9 ± 1.0 ng/mL, respectively, for extraction 2. Overall the percentage recovery of DOM-1 was slightly higher from PBS than from urine. The percentage recovery in urine was 87.5, 80.0, 87.0, and 83.5% for 4 ng/mL (extraction 1, extraction 2), 20 ng/mL (extraction 1, extraction 2), respectively; the overall mean recovery of DOM-1 from urine was 84.5% (SD 3.5%). These data indicate that the DON-IAC columns were able to enrich for urinary DOM-1, using conditions identical with those for urinary DON extraction, and with high recovery and little variation at the concentrations tested. To date a ^{13}C-labeled variant of DOM-1 is not available to serve as an internal standard, as used in the DON assay. However, these data provide confidence that both DON and DOM-1 can be isolated from urine and detected by LC-MS using the same methodology.

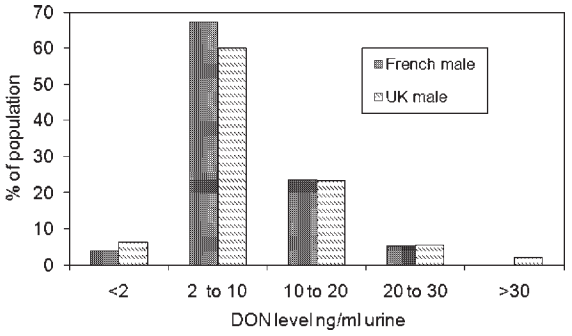

Figure 1. Comparison of the distribution of urinary DON between male French farmers and U.K. (*16*) male adults.

Urinary DON and DOM-1 in French Farmers. All but one of the samples from French farmers (75/76) (>99%) had detectable urinary DON (median level 6.8 ng/mL; range 0.8−28.8 ng/mL). The frequency and levels of DON were similar to those observed in U.K. male adults (*16*), though the latter were collected over 24 h rather than first morning voids (**Figure 1**). DOM-1 was detected in 26/76 (34%) of urine samples (median level among positive samples 0.2 ng/mL, range 0.2−2.8 ng/mL). A typical LC-MS chromatogram of a sample from a French farmer, a DOM-1 standard, and a blank are presented in **Figure 2**. DOM-1 was detected in samples with slightly higher urinary DON (geometric mean 8.1 ng/mL (95% CI: 6.0, 10.9 ng/mL) compared to samples with no detectable DOM-1 (geometric mean DON 5.5 ng/mL (95% CI: 4.5, 6.9 ng/mL; $p = 0.043$)). For individuals who were positive for urinary DOM-1, DOM-1 represented on average 4.7% (95% CI: 1.1, 8.3%) of the DON concentration. DOM-1 was observed in urine from 21/26 individuals who worked on farms involved in handling cattle. To our knowledge, this is the first survey reporting the presence of DOM-1 in human biofluids. DOM-1 was not observed in the 22 U.K. adult samples (limit of detection 0.05 ng/mL).

In animals a number of DON metabolites are reported in biological samples. These include the parent compound DON, DON-glucuronide, a de-epoxide of DON known as DOM-1, and DOM-1-glucuronide (*6*, *15*, *19*−*22*, *28*). In our analysis all samples underwent β-glucuronidase digestion; thus, the respective ratios of the glucuronide components were not investigated. The toxicokinetics and metabolism of DON strongly influence species susceptibility (*6*). The microbiota in cattle rumen are able to transform >95% of ingested DON to DOM-1 (*6*, *23*, *27*) and are relatively resistant to DON toxicity, while swine are least able to produce DOM-1 (*6*, *28*) and are particularly sensitive to DON. On average DOM-1 represented <5% of the total urinary DON in the farmers that had detectable DOM-1, a ratio similar to that observed in swine (*28*). A small survey of human fecal samples ($n = 10$) previously reported a lack of ability to form the de-epoxy metabolite of DON (*29*), while our survey suggests a limited capacity may be apparent in certain groups. However, overall the majority of individuals either had low levels or were not positive for DOM-1 (French and U.K.), data suggestive that humans may be particularly sensitive to DON toxicity in comparison to other species. The presence of DOM-1 in French farmers but not U.K. non-farmers remains intriguing. The formation of DOM-1 occurs through the activity of gut microbiota rather than mammalian metabolism (*19*−*22*). In a laboratory setting swine display negligible DOM-1 formation, while swine raised in a farm setting produce DOM-1 more readily. It is notable that when swine refractive to DOM-1 production were

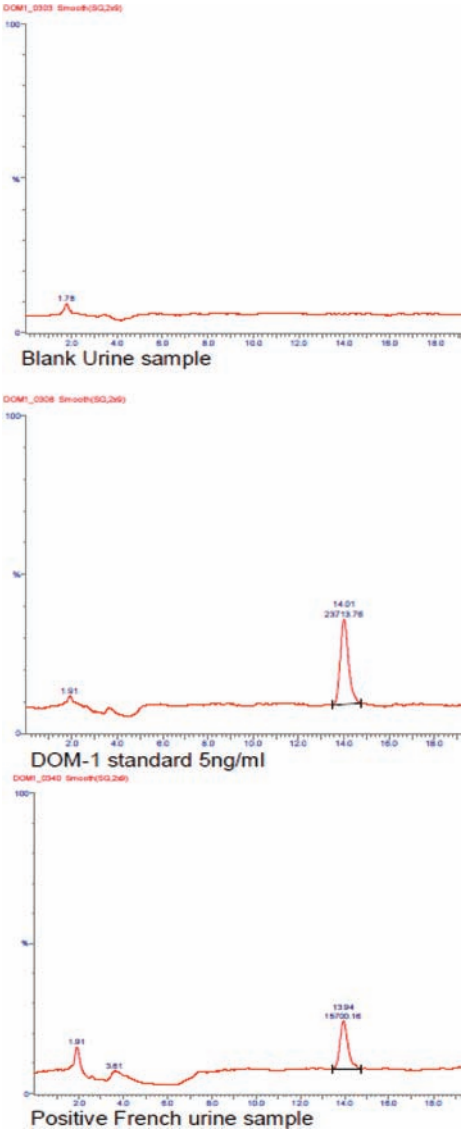

Figure 2. LC-MS chromatograms of DOM-1: blank, DOM-1 standard 5 ng/mL, and positive French sample (2.8 ng/mL). The DOM-1 standard was eluted at 14.0 min.

transferred to a farm setting in which swine fecal matter from animals that produce DOM-1 was spread, the refractive animals were stimulated to produce DOM-1 (*30*). In our study of French farmers DOM-1 was detected more frequently in farmers handling animals, while no DOM-1 was observed in the U.K. adults, for whom no connection to farming activities are recorded. While the numbers of samples remain low, one plausible explanation for the difference in the French and U.K. observations of DOM-1 could be the accidental transmission of animal microbiota to farm workers, which may provide some, albeit modest, capacity for DON conversion to DOM-1. This hypothesis warrants further investigation. DON is also rapidly converted to DOM-1 in the rumen of dairy cows (*27*), and DOM-1 but not DON itself is transferred to the milk in dairy cows ingesting feed heavily contaminated with DON. In our survey of French farmers DOM-1 was prevalent in individuals with higher urinary DON, and U.K. adults had no DOM-1, suggesting that DOM-1 may more likely be linked to DON intake and subsequent metabolism in farmers rather than direct DOM-1 intake through milk. However, we cannot exclude either possible route for the presence of urinary DOM-1.

Table 1. Consumption of Food Containing Cereals Potentially Contaminated by DON

beer consumption the day before	
no	84%
yes	9%
missing	7%
bread the day before (g/day)	
0	0
>0−200	12%
200−399	32%
400	36%
>400	12%
missing	9%
breakfast cereals	
no	84%
once or twice a week	2%
every day including the day before	5%
missing	9%
other cereals (pasta, pizza)[a]	
usual consumption	
less than 5 days/week	28%
consumption 5 days/week or more	60%
missing	12%
amount of food based on pictures of plates	
low intake	3%
medium intake	59%
high intake	16%
missing or not applicable (less than 2 days/week)	22%
consumption the day before	
none	40%
yes	52%
missing	8%
combined variables	
none the day before and less than 5 days/week	8%
none the day before and more than 5 days/week	28%
the day before and less than 5 days/week	19%
the day before and more than 5 days/week and middle quantity/meal	20%
the day before and more than 5 days/week and large quantity/meal	8%
missing data for one of the 3 variables	17%

[a] $N = 76$.

After DON and DOM-1 levels were pooled, 75/76 farmers had detectable total DON (TDON; median level 6.8 ng/mL, range 0.5−28.8 ng/mL). Among the demographic characteristics, only age was negatively associated with TDON level ($r^2 = -0.21$, $p = 0.06$), though it did not reach statistical significance. Dietary information on the day before urine sampling is provided in **Table 1**. Data were mainly qualitative and include the frequency of consumption by week or day prior to sampling with information on quantity per meal based on pictures of portion sizes provided at the time of interview. Only 9% of individuals drank beer the day before urine sampling. Almost half of the individuals had bread consumption greater or equal to 400 g per day, and few individuals (5%) were breakfast cereal consumers. For other cereals, 36% of individuals did not eat other cereals the day before and 8% were frequent cereal consumers. Beer ($p = 0.95$), breakfast cereals ($p = 0.54$), and other cereal consumptions ($p = 0.13$) were not significantly associated with TDON levels in univariate analysis. However, bread consumption was positively associated with TDON level ($r^2 = 0.27$, $p = 0.03$).

Farm organization and agricultural activities are presented in **Table 2**. Most individuals (93%) were still active at enrolment and were mainly farm owners (82%). The enrolment lasted three years and the calendar year was divided into four seasons based on crop activity; winter (November to March) involving little crop activity, spring (April to May) involving full time crop activity, summer (June to August) involving wheat/barley harvesting and fall (September to October) involving maize harvesting. Urine

samples were obtained from 56%, 11%, 18% and 15% of the study population in each season, respectively. The mean farm area was 72 ha range (0 to 235 ha). Most farms (85%) grew crops and a small number of them (11%) grew only meadows. Wheat or barley was grown by 79% farms (average area 23 ha) and 73% grew maize (average area 14 ha). Most farms bred beef cattle (91%) and 64% produced milk. Most individuals (64%) were in contact with animals, during their occupational activities, the day before sampling and 5% of these had harvest tasks. Retired farmers had lower TDON ($p = 0.0007$) levels than active farmers. Season was not significantly associated with TDON levels even though median levels were slightly lower during the fall season (4.8 ng/mL 95%CI: 2.8, 9.3 ng/mL) than during other seasons (6.9 ng/mL 95%CI: 5.5, 8.9 ng/mL). The number of cattle, the acreage of maize and of wheat and barley combined were associated with TDON level ($r^2 = 0.18$ and $p = 0.13$; $r^2 = 0.24$ and $p = 0.04$; $r^2 = 0.17$ and $p = 0.15$, respectively). Maize and wheat/barley acreages, as well as the number of beef cattle, were correlated with each other thus as maize acreage was the most highly correlated parameter with TDON level it was kept for multivariable analysis. When maize acreage was subdivided into quartiles, only farmers with the largest acreage had significantly higher levels of urinary TDON compared to farmers with no maize crops ($p = 0.02$). The type of task performed the day before urine sampling was not associated with TDON level (data not shown).

Table 2. Description of the Characteristics of Agricultural Activities

job title	
active farm owner	82%
active farm worker	8%
retired	7%
other	3%
season of urine sampling	
fall (Sept−Oct), harvest time for maize	15%
spring (April−May), full time crop activity	11%
winter (Nov−March), less crop activity	56%
summer (June−Aug), harvest time for hay and wheat/barley	18%
mean farm area in ha (SD, range)[a]	72 (40, 0−235)
area devoted to crops	
no	11%
missing	4%
yes	85%
mean farm area devoted to crops in ha (SD, range)	49 (43, 1−208)
wheat or barley	
yes	79%
mean area in ha (SD, range)	23 (24, 1−108)
maize	
yes	73%
mean area (SD, range)	14 (9, 1−36)
cattle	
dairy product (yes)	
yes	64%
beef cattle	
yes	91%
mean number (SD, range)	96 (46, 10−200)
occupational activities the day before sampling	
with cattle	
no	29%
yes	64%
missing	7%
harvest (hay, maize or wheat)	
no	88%
yes	5%
missing	7%

[a] 4% missing values.

Multivariable Analysis. Five individuals were retired farmers and had low levels of urinary TDON. They also had lower levels of bread consumption, and thus, as job status was a covariate of interest, these individuals were excluded from multivariable analysis. The final model included creatinine level, maize acreage, and bread and other cereal consumptions (**Table 3**). All variables contributed to the model, but only contributions of bread intake ($p = 0.05$) and other cereal intake ($p = 0.02$) reached statistical significance. Overall, the variables included in the model predicted 33% of the variation in urinary DON ($p = 0.0025$). The explanation of the variation in urinary DON is similar to, though slightly greater than, that reported recently by Turner et al. (*16*, *18*), in which diet alone was compared to the biomarker. In a farm setting, exposure to DON may come from dietary as well as grain handling activities. The known heterogeneity of DON contamination of cereals and silage, combined with a relatively small sample size, probably reflects this modest, albeit significant, statistical explanation of the variation in urinary DON.

Humans are frequently exposed to a range of highly potent mycotoxins, yet the adverse health effects of exposure remain poorly explored. While some progress is being made using mycotoxin exposure biomarkers (*15*−*17*, *31*−*35*), a lack of well-validated biomarkers continues to hamper our understanding of the health consequences of both acute and chronic mycotoxin exposures, with research on aflatoxin as the exception (*36*−*39*). Here we present the refinement of an IAC-LC-MS urinary assay for DON and the development of a urinary assay for DOM-1; the use of IAC enrichment and specific m/z measures imparts a high level of specificity to these measures. All but one of the male farmers had detectable urinary TDON. Positive correlations were observed not only with covariates associated with cereal consumption but also with occupational agricultural activities. The correlation observed between TDON level and the maize acreage

Table 3. Multiple Linear Regression Modeling [a] on TDON Level[b]

		multivariate analysis[d]	
dependent variable: Ln (total DON)	univariate analysis regression coefficient (SE[c])	regression coefficient (SE[c])	model
creatinine (mg/mL)	0.38 (0.15) $p = 0.01$	0.26 (0.15) $p = 0.08$	
maize (hectares)			
none			$p = 0.0025$, $R^2 = 0.33$
>0−10 ha	0.26 (0.22) $p = 0.23$	0.29 (0.20) $p = 0.15$	
11−20 ha	0.05 (0.22) $p = 0.81$	0.07 (0.20) $p = 0.74$	
>20 ha	0.59 (0.25) $p = 0.02$	0.38 (0.23) $p = 0.11$	
quantity of bread (g/day)	0.25 (0.10) $p = 0.01$	0.18 (0.09) $p = 0.05$	
quantity of other cereal (g/day)	0.19 (0.16) $p = 0.01$	0.16 (0.07) $p = 0.02$	

[a] Urinary DON and DOM-1 combined. [b] For definition of variables, see Materials and Methods. [c] Standard error. [d] Retired farmers ($n = 5$) were excluded from multivariate analyses, and 15 observations were also excluded from the final model because of missing data for one or more variables ($n = 1$ for maize acreage, $n = 8$ for quantity of bread, $n = 13$ for cereal consumption, $n = 1$ for job title, and $n = 2$ for creatinine).

was significant in univariate analysis but did not remain significant after adjustment for diet. Few data are available from occupational exposures to mycotoxins in general, especially for the trichothecene mycotoxins (*11*). DON was also one of the six mycotoxins (aflatoxin B1, citrinin, zearalenone, gliotoxin, ochratoxin A, DON) which has been found both as a regular component and at the highest level in previous studies in maize silage in this region (*13*, *40*, *41*). Total daily DON intake was roughly estimated on the basis of a number of assumptions: first, the amount of urinary TDON, second, an assumption that about 50% of the ingested DON was being excreted in the urine, on the basis of data from animal models, particularly swine, third, the estimated creatinine excreted during 24 h on the basis of body weight and age, and fourth, the TDON/creatinine ratio in the urine. The mean estimated daily intake in this population was 264 ng/kg bw/day (range 27−1088); thus, 1 of 76 (1.3%) was predicted to exceed the tolerable daily intake set at 1000 ng/kg bw/day (*42*). A major caveat in generating this estimate is the transfer of DON to urine in humans.

To our knowledge this is the first biomarker survey for DON in a French population. This is also the first to observe DOM-1 in urine from humans. It was notable that DOM-1 was present in French farmers but not in U.K. adults. It will be important to understand the potential contribution of exposure sources to this and other mycotoxins, including those posed by farming activities.

ABBREVIATIONS USED

BMI, body mass index; CI, confidence interval; DOM-1, de-epoxy-deoxynivalenol; DON, deoxynivalenol; IAC, immunoaffinity column; IS, internal standard; LC-MS, liquid chromatography−mass spectrometry; PBS, phosphate-buffered saline; SD, standard deviation; TDON, total DON (deoxynivalenol and de-epoxy-deoxynivalenol).

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