

ABSTRACT

Title of thesis: A PILOT COMPARISON OF FIRST MORNING VERSUS 24-HOUR URINARY DEOXYNIVALENOL IN UK ADULTS

Natalie T. Boonchaisri, Master of Public Health, 2017

Thesis directed by: Professor Paul C. Turner
Maryland Institute of Applied Environmental Health

Using unpublished data from an original study by Turner et al. (2010a), the relationship between first morning void (FMV) and 24-hour urine collections was examined in UK adults to determine if FMV collections provide a reasonable estimate of DON intake compared to 24-hour collections. The intraclass correlation coefficient (ICC) was computed to evaluate variability in DON concentrations and generalized estimating equation (GEE) models were used to assess the relationship between collection types. Greater between-person variability was observed in 24-hour collections, unadjusted and adjusted for creatinine (ICC=0.78 and 0.56, respectively). GEE models suggest urinary DON concentrations in FMV collections were strongly correlated with 24-hour collections ($r=0.78$, $p<0.0001$), meaning FMV collections may provide just as reasonable an estimate of DON intake compared to 24-hour collections when adjusting for age, sex, and BMI. These results strengthen the methodology behind exposure biomarkers and urinary assays when estimating DON intake.

A PILOT COMPARISON OF FIRST MORNING VERSUS 24-HOUR URINARY
DEOXYNIVALENOL IN UK ADULTS

by

Natalie T. Boonchaisri

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park in partial fulfillment
of the requirements for the degree of
Master of Public Health
in Environmental Health Sciences
2017

Advisory Committee:

Professor Paul Turner, Chair
Professor Robin Puett
Professor Lesliam Quirós-Alcalá

©Copyright by

Natalie T. Boonchaisri

2017

TABLE OF CONTENTS

Chapter 1: Introduction.....	1
Rationale and Research Questions	1
Literature Review	1
Mycotoxins.....	1
Deoxynivalenol and Health.....	2
Deoxynivalenol Biomarkers and Urine Collection Methods	5
Chapter 2: Method	8
Study Design.....	8
Data Analysis.....	9
Chapter 3: Results.....	11
Chapter 4: Conclusion.....	14
Appendix.....	17
Figures	17
Tables.....	18
References.....	22

Chapter 1: Introduction

Rationale and Research Questions

Deoxynivalenol (DON) is a naturally occurring mycotoxin that predominantly contaminates wheat, maize, and barley, and causes adverse health effects in both animals and humans (Pestka, 2007). The development of analytical tools to measure DON and its metabolites have allowed the relationship between exposure to DON and the urinary measure to be examined. Urinary DON was strongly correlated with DON intake and has now been used as a validated exposure biomarker (Turner et al., 2010a). Exposure assessment using urinary biomarkers is best conducted using 24-hour urine collections. In many instances, these remain complex and unreliable to collect in comparison to first morning void (FMV) or spot urine collections. However, it is unclear if FMV collections provide a reasonable estimate of exposure to DON.

Using unpublished data from the same study by Turner et al. (2010a), objectives of this thesis are to: 1) determine the correlation between FMV and 24-hour urine collections within a given study population, and 2) better understand and describe the strength of these measures in estimating DON exposure. The results of this study aim to strengthen the methodology behind exposure biomarkers and urinary assays when estimating DON intake.

Literature Review

Mycotoxins

Mycotoxins are secondary metabolites of certain fungi that produce toxic effects in both animals and humans. Although similar toxic episodes were documented as far back as the 7th and 8th centuries BC, mycotoxins were only identified and research

programs established after a major unexplained toxicosis event occurred in farm animals in England in the 1960s (Peraica et al., 1999). Over the course of a few months, what came to be known as “turkey X disease” was responsible for killing nearly 100,000 turkeys that consumed an unknown contaminant, later identified as a mycotoxin, in contaminated Brazilian peanut meal (de Iongh et al., 1962).

There are many mycotoxins, but only a few are of public health concern. The five major groups of foodborne mycotoxins include trichothecenes, zearalenone, ochratoxin, fumonisins, and aflatoxins. Trichothecenes include deoxynivalenol (DON), nivalenol, diacetoxyscirpenol, and T-2 toxin. Mycotoxin contamination of food and feeds, mostly grains, occurs in both industrialized and developing countries, flourishing where environmental conditions favor mycotoxin growth. Certain mycotoxins such as aflatoxins and fumonisins flourish in hot and humid climates, whereas trichothecenes and ochratoxins flourish in more temperate regions. Industrialized countries are often protected from mycotoxin exposure because of improved farming and storage practices, increased regulation, economic capacity to remove highly contaminated crops from the food chain, and greater dietary diversity, which limits mycotoxin exposure and results in better-nourished populations. By comparison, many developing countries have greater risks following exposure due to dietary monotony and malnutrition. Typically, few regulations exist to protect such populations (Bennett & Klich, 2003).

Deoxynivalenol and Health

Trichothecene contamination is becoming an increasingly common problem due to expanded use of no-till farming practices and changing climate patterns (Pestka, 2007). DON, the most frequently occurring trichothecene (Figure 1), is typically produced by

the *Fusarium* species of fungi and can contaminate crops in the field and during storage processes (Peraica et al., 1999; Pestka, 2010). An example of a *Fusarium* species is *F. graminearum*, which is considered a field fungus because it invades crops before harvest. The other *Fusarium* species, *F. crookwellense* and *F. clumorum*, invade after harvest and are referred to as storage fungi (Food and Agriculture Organization of the United Nations, 1997). DON frequently affects commodities like wheat, maize, and barley, and rarely affects oats, rice, and other grains. Occurrences are common in temperate climates in most regions of the world (Zain, 2011). In 2001, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) surveyed food consumption data and dietary intake estimates among five regional diets. Estimates were highest in the Middle East (2.4 µg/kg bw/day), followed by Far Eastern (1.6 µg/kg bw/day), European (1.4 µg/kg bw/day), Latin America (1.2 µg/kg bw/day), and African (0.78 µg/kg bw/day) (Canady et al., 2001).

Adverse health impacts may occur after consumption of DON-contaminated foods. Other less likely exposure routes include skin contact with molded crops and inhalation of mycotoxin spores. All animal species evaluated to date are susceptible to DON, but sensitivity is ranked in the order of pigs being most susceptible, followed by mice, rats, and poultry and ruminants being relatively resistant (Pestka, 2007; Prelusky et al., 1994; Zain, 2011). Differences in toxicokinetics (metabolism, absorption, distribution, and elimination) may account for the differences seen across animal species. The severity of health effects depends on exposure frequency, exposure duration, the exposed subject's age and health, as well as any synergistic effects the mycotoxin may have with other chemicals (Bennett & Klich, 2003; Peraica et al., 1999).

Evidence from animal studies show that acute and extremely high DON exposure can result in abdominal pain, diarrhea, vomiting (pigs only), and even shock-like death or tissue injury, while low DON doses typically just cause vomiting (pigs only) (Pestka, 2007, 2010). Both short-term and long-term exposure can lead to immune system suppression, reduced mean daily food consumption, mean body weight gain, and total body weight (Iverson et al., 1995; Sobrova et al., 2010). Impaired reproduction and development resulting from maternal toxicity has also been observed in rats and mice (Bennett & Klich, 2003; Pestka, 2010; Sobrova et al., 2010). No Observed Adverse Effect Levels (NOAELs) range from 0.04-0.375 mg/kg bw/day (European Commission Scientific Committee on Food, 1999). DON elicits these responses by inducing ribotoxic stress, which disrupts macromolecule synthesis, cell signaling, differentiation, proliferation, and death (Pestka, 2010).

There is historical evidence suggesting that DON causes acute human illness. Between 1946 and 1987, large-scale human toxicosis events were documented in Japan, Korea, China, and India (Pestka, 2010; Zain, 2011). Thousands of people exhibited gastroenteritis with symptoms like nausea, diarrhea, and vomiting after consuming *Fusarium*-contaminated foods. Other documented adverse health impacts include abdominal pain, headache, dizziness, and fever (Sobrova et al., 2010; Zain, 2011). Although primate or human studies on DON-induced health effects have not been reported to date, it is not unreasonable to assume that humans are sensitive to DON. In vitro studies using various human cells resulted in impairment of immune function, as well as decreases in cell proliferation and cellular defense, which closely resembles the mechanistic actions seen in animal studies (Pestka & Smolinski, 2005). Additionally, no

carcinogenic and/or mutagenic properties have been observed from DON contamination. The International Agency for Research on Cancer (IARC) classified DON as a Group 3 carcinogen in 1993, meaning it is “not classifiable as to its carcinogenicity to humans” due to inadequate evidence in experimental animal studies (International Agency for Research on Cancer, 1993). More research must be done to validate DON’s effects in humans.

Advisory limits were set in place to limit exposure to DON. The EU established regulations in 2005 for unprocessed wheat (1,750 ppb), cereals (1,250 ppb), flour and pasta (750 ppb), breads (500 ppb), and processed instant foods (200 ppb) (European Commission, 2005). In comparison to the current FDA advisory level of 1,000 ppb in finished wheat products and 5,000-30,000 ppb in grains and grain byproducts for animal feed (depending on the species), the EU regulations are relatively more conservative (Food and Drug Administration, 2010). This conservative approach may have limitations for other countries because DON is rapidly metabolized, does not accumulate in tissues, and flourishes depending on the climate (Pestka, 2010). These regulations are not generalizable to populations with varying diets and countries with fluctuating climates.

Deoxynivalenol Biomarkers and Urine Collection Methods

Traditional mycotoxin detection methods are based off of either reference methods for quantitative analysis or rapid methods for first-level screening of numerous samples (Anfossi et al., 2016). Thin-layer chromatography (TLC) was the first method used for quantitative analysis of mycotoxins. In the developed world, TLC has been phased out and replaced with gas chromatography (GC) and liquid chromatography (LC). Advancements in LC allowed for the largest range of mycotoxins to be determined with

the highest sensitivity, which then became coupled to ultraviolet, fluorescence, or mass spectrometric (MS) detection. Today, the preferred method for mycotoxin detection is LC-MS because not only is it extremely sensitive, but it can determine over 100 mycotoxins in a single run, and can also detect mycotoxins simultaneously with other environmental contaminants (Shephard, 2016).

For first-level screening, immunochemical-based methods are preferred for their simplicity, cost-effectiveness, sensitivity, and selectivity. Enzyme-linked immunosorbent assay (ELISA) kits are commercially available for all regulated mycotoxins, and immunochromatographic test (ICT) technology allows for their visual detection and semi-quantification (Anfossi et al., 2016). An issue surrounding ELISA is the possible response of a structurally related form of the analyzed mycotoxin rather than a single chemical compound (Shephard, 2016). This has been found to be a particular problem for DON analysis (Zachariasova et al., 2008).

Exposure assessment based on dietary analysis has always been problematic due to heterogeneous DON contamination of food items, but biological measures, referred to as biomarkers, have tremendously improved this assessment process. In 2003, the first urinary assay for DON was confirmed using immunoaffinity enrichment, and $^{13}\text{C}_{15}$ -DON was then used as an internal standard for LC-MS detection methods (Meky et al., 2003; Turner et al., 2008a; Turner et al., 2008b). This methodology is robust with high specificity and sensitivity, incorporating an internal standard that allows adjustment for recovery.

Investigators typically collect spot (at any convenient time), FMV, or 24-hour urine collections. For exposure assessment using urinary biomarkers, spot collections are

often the easiest to collect but the least accurate, as they may represent a limited exposure window. When comparing FMV collections versus 24-hour collections, FMV collections provide a time-average for metabolite spikes that occur over a larger window of exposure, biotransformation, and excretion. Also, FMV collections are less affected by hydration/physical status. On the other hand, 24-hour collections tend to be most accurate, providing a time-average for exposure and metabolite spikes that occur over a 24-hour period. 24-hour collections provide the highest-level of sensitivity when assessing very low levels of metabolites, but in many instances, they remain complex and unreliable to collect in comparison to FMV or spot collections. This unreliability stems from the fact that 24-hour collections are more expensive and require participant diligence. It is not uncommon that an individual will forget to provide a urine sample during the 24-hour period. Considering all of this, it is unclear if FMV collections provide a reasonable estimate of exposure to DON.

Chapter 2: Method

Study Design

The original longitudinal study by Turner et al. (2010a) examined the relationship between DON intake and FMV urinary DON over 12 days in 35 adult volunteers from the University of Leeds, UK to validate the latter as a biomarker of human exposure (Turner et al., 2010a). FMV samples were collected during a period of normal diet (eight days) and during a period of either a wheat-restriction intervention diet or a partial wheat-restriction intervention diet (both four days). During the wheat-restriction intervention diet, 10 volunteers were expected to avoid all wheat and corn commodities, whereas the partial wheat-restriction intervention diet allowed bread only for the remaining 25 volunteers.

Validated food diaries were kept for volunteers to document the amount and types of foods eaten prior to urination. If a volunteer during the partial wheat-restriction intervention diet consumed a certain amount of bread, the volunteer was required to cut a representative slice of bread to be sent for DON analysis. Thus, for each day, the urine sample was paired with the dietary information from the previous day. Measurements of urinary DON followed the methods set forth by Turner and colleagues, informed consent was received from all volunteers, and ethical approval was obtained from the Leeds Teaching Hospitals NHS Trust Research Ethics Committee (Turner et al., 2008b, 2010b). Results of the original longitudinal study were published and a strong correlation between DON intake and the urinary biomarker was observed (adjusted $r^2=0.83$, $p<0.001$) in models adjusting for age, sex, and body mass index (BMI).

As provided by the principal investigator of the original study, there exists unpublished data containing urinary FMV & 24-hour DON measurements from 13 adult volunteers from the University of Leeds, UK. Following the same duration of normal dieting (eight days), 12 volunteers were subject to the partial-wheat restriction intervention diet and one volunteer participated in the full wheat-restriction intervention diet (both four days). Each volunteer provided two urine samples for both the FMV and 24-hour collections. Just like the original study design, food diaries were kept, representative bread slices were collected for DON analysis, and urinary DON measurements followed methods set forth by Turner and colleagues (Turner et al., 2008b, 2010b). The University of Maryland Institutional Review Board determined that this thesis did not meet the definition of human subjects research since the data/specimens were collected in 2007, no further data collection was required, and I did not have access to identifiable subject information.

Data Analysis

Using Statistical Analysis Software (SAS) University Edition, normality was first assessed. After looking at all kurtoses and scatterplots, log-transformation was determined unnecessary for these comparisons, as most variables were closer to normal prior to log-transformation. Then, descriptive statistics were summarized for both the study population and outcome variables (unadjusted DON and creatinine-adjusted DON) by collection type (FMV vs. 24-hour).

Since repeated measures were taken on different days for each subject, the intraclass correlation coefficient (ICC) was calculated using mixed effects models. The ICC evaluates within- and between-person variability and reproducibility of DON

concentrations (unadjusted and adjusted for creatinine). An ICC > 0.75 represents excellent reproducibility, between 0.4 and 0.75 represents fair to good reproducibility, and < 0.4 represents modest to poor reproducibility.

Generalized estimating equation (GEE) models were used to evaluate the correspondence between FMV collections and 24-hour collections, using urinary 24-hour DON as the outcome variable and urinary FMV DON as the predictor variable, all adjusted for age, sex, and BMI. Analyses were done using three separate models: Model 1) using unadjusted DON as the outcome variable while keeping all predictors the same, Model 2) using unadjusted DON as the outcome variable while adding creatinine as a predictor, and Model 3) adjusting for creatinine in the outcome variable while keeping all predictors the same.

Pearson correlation coefficients were also computed to measure strength of the relationship between FMV and 24-hour collections. These same comparisons were done on \log_{10} -transformed data because creatinine-adjusted FMV collections exhibited closer normality after log-transforming.

Chapter 3: Results

Among the 13 subjects (8 male, 5 female) in the study, the mean age was 36 years (range 23 – 50) and the mean BMI was 25.2 (range 20.2 – 34.1), as seen in Table 1. Table 1 also displays total mean measurements for the amount of bread consumed (g) as documented in the food diaries, DON ($\mu\text{g}/\text{day}$) from representative bread slices, and urinary DON intake from bread ($\text{ng}/\text{kg bw}$), as well as these measurements when divided between the first and second sample. All of these measurements are based off of the previous day's (24-hours) bread consumption. A higher mean bread consumption and mean DON intake from bread was observed in the second sample compared to the first sample (Mean \pm SD: 148 g (93) vs. 136 g (76) and 179 $\text{ng}/\text{kg bw}$ (160) vs. 154 $\text{ng}/\text{kg bw}$ (114), respectively), but not for DON from bread, which was higher in the first sample compared to the second sample (Mean \pm SD: 19.7 $\mu\text{g}/\text{day}$ (27.8) vs. 13.0 $\mu\text{g}/\text{day}$ (11.0)). There was no statistically significant difference among these measures ($p > 0.05$).

Table 2 displays descriptive statistics for the DON outcome variables (unadjusted and adjusted for creatinine) separated into FMV and 24-hour collections. For FMV collections, the mean urinary DON concentration (ng/ml) was 11.0 ng/ml (SD: 9.3) with a max of 32.8 ng/ml . When adjusted for creatinine (ng/mg), the mean was 9.2 ng/mg (SD: 7.1) with a max of 32.4 ng/mg . Among the 24-hour collections, the mean urinary DON concentration was 11.5 ng/ml (SD: 11.7) with a max of 41.0 ng/ml . When adjusted for creatinine, the mean was 10.0 ng/mg (SD: 6.0) with a max of 21.4 ng/mg . Compared to the 24-hour collections, FMV measurements were all slightly lower.

Results of the ICC computations are displayed in Table 3, unadjusted and adjusted for creatinine. For the FMV collections, negative values were observed: -0.37

unadjusted and -0.30 when adjusted for creatinine. Typically, negative values are not reported for ICCs, but after much trouble shooting, a positive value could not be obtained. According to Taylor (2009), “negative estimates are possible and can be interpreted as indicating that the true ICC is low.” Essentially, “two members chosen randomly from any class vary almost as much as any two randomly chosen members of the whole population” (Taylor, 2009). These negative values can also be driven by the fact that there exists a large exposure/intake difference between the first and second samples. For example, a measurement from the second sample may contain DON remaining from the first sample if the exposure/intake was large enough. This depends on exposure/intake amount, individual kinetic clearance, and time of exposure versus time of urinary collection. By comparison, 24-hour collections resulted in an ICC of 0.78 unadjusted and 0.56 when adjusted for creatinine. This means that 78% variability in unadjusted 24-hour DON measurements is due to between-person variability, while 22% is due to within-person variability. For creatinine-adjusted 24-hour DON, 56% variability is due to between-person variability and 44% is due to within-person variability.

Table 4a presents results of the GEE models examining the relationship between FMV collections and 24-hour collections (adjusted for age, sex, and BMI). For each model (using unadjusted DON as the outcome variable while keeping all predictors the same, using unadjusted DON as the outcome variable while adding creatinine as a predictor, and adjusting for creatinine in the outcome variable while keeping all predictors the same), the beta coefficients (β) show a small increase (0.2, 0.1, and 0.2, respectively) for every unit increase of the outcome variable. The Pearson correlation coefficient (r) for each model was 0.63 ($p=0.0006$), 0.63 ($p=0.0006$), and 0.47

($p=0.0143$), respectively. This indicates that all models possess statistically significant, and moderate to strong correlations. Using log-transformed data (Table 4b), the beta coefficients (β) show a larger increase (0.4, 0.4, and 0.5, respectively) for every unit increase of the outcome variable. The Pearson correlation coefficient (r) for all models was 0.78 ($p<0.0001$), indicating that the log-transformed models exhibit even stronger and more statistically significant correlations.

Chapter 4: Conclusion

Mycotoxin contamination occurs worldwide, with nearly 25% of agricultural commodities affected (Turner et al., 2010a). Results of an assessment of dietary intake by populations of EU member states show that average level intakes for the population as well as for adults are low and do not exceed 46.1% of the recommended TDI of 1 µg/kg bw/day for DON, but due to DON's resistance and growth in temperate regions, DON exposure is expected to increase in the UK (SCOOP, 2003). Additionally, biomarker data is suggestive that 5-10% of EU adults are exceeding the recommended TDI, and this may be predicted to be higher in infants and young children (Turner et al., 2012). There is a general lack of understanding regarding how DON health effects in animals translates to humans, but the use of urinary biomarkers has greatly improved DON exposure assessment (Turner et al., 2008a). Results of the original longitudinal study by Turner et al. (2010a) showed a strong correlation between DON intake and the urinary biomarker observed (adjusted $r^2=0.83$, $p<0.001$) in models adjusting for age, sex, and BMI. Still, it remained unclear which type of urinary collection best measured DON intake.

Using unpublished data from that original study, this study aimed to determine the correlation between FMV and 24-hour urine collections within a small subset of the original study population, and to better understand and describe the strength of these measures in estimating DON exposure. Results of this study show that urinary DON concentrations in FMV collections are strongly correlated with levels in same-day 24-hour collections. Greater between-person variability than within-person variability was observed in urinary 24-hour DON collections. The unadjusted ICC (0.78) indicated excellent reproducibility and the creatinine-adjusted ICC (0.56) indicated fair

reproducibility. Thus, 22%-44% of the observed variability was due to within-person variability. As for the FMV collections, results were unreliable due to the negative values obtained. Urinary DON concentrations in FMV collections were strongly correlated with 24-hour log-transformed data ($r=0.78$, $p<0.0001$) and moderately to strongly correlated in non log-transformed data ($r=0.47-0.63$, $p<0.05$), meaning urinary DON concentrations in FMV collections are strongly correlated with levels in same-day 24-hour collections when adjusted for age, sex, and BMI. Ultimately, the results of this study strengthen the methodology behind exposure biomarkers and urinary assays when estimating DON intake and show that FMV collections may provide just as reasonable of an estimate as 24-hour collections.

There are several limitations within this study, the first one being such a small population size. A larger population would provide more statistical power and strength in the results. Additionally, the samples were pooled, meaning the regression combined the first and second sample per individual for each urinary collection type. Another issue with the population at hand is that all participants were white Caucasian adults and residents of the UK, meaning that results of this study are not generalizable to other populations based on age, race, ethnicity, culture, and diet. It would be useful to conduct a pilot study to examine DON intake among different races and ethnicities because it is expected that age, culture, and diet play a large role in these differences.

Another limitation is that the 24-hour collections contained a few milliliters of urine from the FMV collections, which may account for part of the correlation seen in the results. Generally, FMV collections are not ideal for assessing daily exposure because they are best used to measure the previous day's intake, but according to our results,

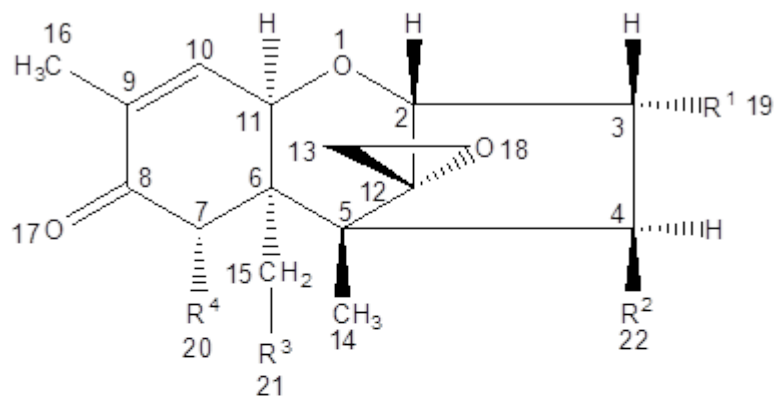
urinary DON concentrations in FMV collections may be just as reliable as 24-hour collections when estimating DON intake. More studies should be conducted to validate that this is true. Some of the correlation seen may also be due to potential DON intake from non-restricted diet sources. During the partial wheat-restriction intervention diet, soups and sauces were not restricted, which may be a potential source of DON, especially if they contained corn. Caution in this study data being applied elsewhere is also required in populations where the range of exposures may be higher.

Lastly, variability and reproducibility calculations were unreliable due to the negative ICC values obtained from the FMV collections. Reasons for this indicate that the true ICC is low, but it is also possible that although most urinary DON is affected by the previous day (24-hours), the previous 48 hours may have affected this as well, suggesting that the ICC values are not absolute due to timing of consumption and timing of urine collection. For this reason, it is recommended that the future of this study looks at the relationship between timing of food consumption and urine collection and examine how these variables influence urinary DON levels over several consecutive days.

Appendix

Figures

Figure 1. Generic structure of Type B-trichothecenes, including deoxynivalenol



	R ¹	R ²	R ³	R ⁴
Nivalenol	OH	OH	OH	OH
Deoxynivalenol	OH	H	OH	OH
3-Acetyl-Deoxynivalenol	OCOCH ₃	H	OH	OH
15-Acetyl-Deoxynivalenol	OH	H	OCOCH ₃	OH
Fusarenon X	OH	OCOCH ₃	OH	OH

Tables

Table 1. Descriptive statistics for study population and for previous day's bread and DON intake (n=13, 26 observations total (2 per individual))

<u>Characteristic</u>	<u>No. (%) or Mean (SD)</u>	<u>Range</u>
Gender		
Male	8	
Female	5	
Age (years)	35.8 (10.0)	23.0-50.0
Height (m)	1.7 (0.1)	1.6-1.9
Weight (kg)	76.5 (15.8)	61.0-108.0
BMI (kg/m ²)	25.2 (4.0)	20.2-34.1
Bread consumed* (g)		
Total	146 (79)	0-344
Sample 1	136 (76)	0-292
Sample 2	148 (93)	0-344
DON from bread* (µg/day)		
Total	12.2 (9.7)	0-36.6
Sample 1	19.7 (27.8)	0-108.0
Sample 2	13.0 (11.0)	0-36.6
DON intake from bread* (ng/kg bw)		
Total	167 (136)	0-502
Sample 1	154 (114)	0-394
Sample 2	179 (160)	0-502

*Measurements analyzed bread consumption from the previous day (24-hrs)

Table 2. Descriptive statistics for DON outcome variables, unadjusted and adjusted for creatinine, separated by urinary collection type (n=13, 26 observations total (2 per individual))

<u>Sampling Type and Outcome Variables</u>	<u>Mean (SD)</u>	<u>p25</u>	<u>p50</u>	<u>p75</u>	<u>Max</u>
<i>First Morning</i>					
DON (ng/ml)	11.0 (9.3)	3.0	8.2	18.8	32.8
Creatinine (mg/ml)	1.2 (0.5)	0.7	1.2	1.5	2.2
DON/Creatinine (ng/mg)	9.2 (7.1)	4.4	8.0	13.0	32.4
<i>24-hour</i>					
DON (ng/ml)	11.5 (11.7)	4.0	6.4	17.4	41.0
Creatinine (mg/ml)	1.0 (0.5)	0.6	0.8	1.3	2.1
DON/Creatinine (ng/mg)	10.0 (6.0)	5.4	10.9	14.3	21.4

Table 3. Intraclass Correlation Coefficients for FMV and 24-hour collections, unadjusted and adjusted for creatinine (n=13, 26 observations total (2 per individual))

<u>Sampling Type and Outcome Variables</u>	<u>ICC</u>
<i>First Morning</i>	
DON (ng/ml)	-0.37
DON/Creatinine (ng/mg)	-0.30
<i>24-hour</i>	
DON (ng/ml)	0.78
DON/Creatinine (ng/mg)	0.56

Table 4a. Modeling of 24-hr urinary DON using FMV collections as predictors, adjusted for age, sex, and BMI; unadjusted and adjusted for creatinine (n=13, 26 observations total (2 per individual))

<u>Model type</u>	<u>Regression model</u>		<u>Pearson correlation</u>	
	β (95% CI)	Intercept	r	p-value
<i>Model 1</i> Unadjusted (ng/ml)	0.2 (-0.2, 0.5)	5.9	0.63	0.0006
<i>Model 2</i> Creatinine added as predictor (ng/ml)	0.1 (-0.1, 0.3)	6.2	0.63	0.0006
<i>Model 3</i> Adjusted for creatinine (ng/mg)	0.2 (-0.02, 0.4)	10.5	0.47	0.0143

Table 4b. Modeling of log-transformed 24-hr urinary DON using FMV collections as predictors, adjusted for age, sex, and BMI; unadjusted and adjusted for creatinine (n=13, 26 observations total (2 per individual))

<u>Model type</u>	<u>Regression model</u>		<u>Pearson correlation</u>	
	β (95% CI)	Intercept	r	p-value
<i>Model 1</i> Unadjusted (log-ng/ml)	0.4 (0.1, 0.8)	2.1	0.78	<0.0001
<i>Model 2</i> Creatinine added into model (log-ng/ml)	0.4 (0.02, 0.7)	2.2	0.78	<0.0001
<i>Model 3</i> Adjusted for creatinine (log-ng/mg)	0.5 (0.2, 0.8)	1.9	0.78	<0.0001

References

- Anfossi, L., Giovannoli, C., & Baggiani, C. (2016). Mycotoxin detection. *Current Opinion in Biotechnology*, 37, 120–126.
<https://doi.org/10.1016/j.copbio.2015.11.005>
- 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>
- Canady, Richard, Coker, Raymond, Egan, Kathleen, Krska, Rudolf, Kuiper-Goodman, Tine, Olsen, Monica, ... Schlatter, Josef. (2001). DON Evaluation by Joint FAO/WHO Expert Committee on Food Additives. Retrieved May 27, 2017, from <http://www.inchem.org/documents/jecfa/jecmono/v47je05.htm>
- de Jongh, H., Beerthuis, R. K., Vles, R. O., Barrett, C. B., & Ord, W. O. (1962). Investigation of the factor in groundnut meal responsible for “turkey X disease.” *Biochimica et Biophysica Acta*, 65(3), 549–551. [https://doi.org/10.1016/0006-3002\(62\)90471-7](https://doi.org/10.1016/0006-3002(62)90471-7)
- European Commission. (2005). Commission Regulation (EC) No 856/2005 regarding Fusarium Toxins. Retrieved May 27, 2017, from <http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32005R0856>
- European Commission Scientific Committee on Food. (1999). *Opinion on Fusarium Toxins*.
- Food and Agriculture Organization of the United Nations. (1997). Mycotoxins in Grain. Retrieved March 8, 2017, from <http://www.fao.org/wairdocs/x5008e/x5008e01.htm>

- Food and Drug Administration. (2010). Advisory Levels for Deoxynivalenol (DON) in Finished Wheat Products for Human Consumption and Grains and Grain By-Products used for Animal Feed. Retrieved May 27, 2017, from <https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ChemicalContaminantsMetalsNaturalToxinsPesticides/ucm120184.htm>
- International Agency for Research on Cancer. (1993). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 56. Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins. Retrieved March 8, 2017, from <http://monographs.iarc.fr/ENG/Monographs/vol56/>
- Iverson, F., Armstrong, C., Nera, E., Truelove, J., Fernie, S., Scott, P., ... Gunner, S. (1995). Chronic Feeding Study of deoxynivalenol in B6C3F1 male and female mice. *Teratogenesis, Carcinogenesis, and Mutagenesis*, 15(6), 283–306. <https://doi.org/10.1002/tcm.1770150606>
- Meky, F. A., Turner, P. C., Ashcroft, A. E., Miller, J. D., Qiao, Y.-L., Roth, M. J., & Wild, C. P. (2003). Development of a urinary biomarker of human exposure to deoxynivalenol. *Food and Chemical Toxicology*, 41(2), 265–273. [https://doi.org/10.1016/S0278-6915\(02\)00228-4](https://doi.org/10.1016/S0278-6915(02)00228-4)
- Peraica, M., Radić, B., Lucić, A., & Pavlović, M. (1999). Toxic effects of mycotoxins in humans. *Bulletin of the World Health Organization*, 77(9), 754–766.
- Pestka, J. J. (2007). Deoxynivalenol: Toxicity, mechanisms and animal health risks. *Animal Feed Science and Technology*, 137(3-4), 283–298. <https://doi.org/10.1016/j.anifeedsci.2007.06.006>

- 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>
- Pestka, J. J., & Smolinski, A. T. (2005). Deoxynivalenol: Toxicology and Potential Effects on Humans. *Journal of Toxicology and Environmental Health, Part B*, 8(1), 39–69. <https://doi.org/10.1080/10937400590889458>
- Prelusky, D. B., Gerdes, R. G., Underhill, K. L., Rotter, B. A., Jui, P. Y., & Trenholm, H. L. (1994). Effects of low-level dietary deoxynivalenol on haematological and clinical parameters of the pig. *Natural Toxins*, 2(3), 97–104.
<https://doi.org/10.1002/nt.2620020302>
- SCOOP. (2003). *Collection of occurrence data of Fusarium toxins in food and assessment of dietary intake by the population of EU Member States*.
- Shephard, G. S. (2016). Current Status of Mycotoxin Analysis: A Critical Review. *Journal of AOAC International*, 99(4), 842–848.
<https://doi.org/10.5740/jaoacint.16-0111>
- Sobrova, P., Adam, V., Vasatkova, A., Beklova, M., Zeman, L., & Kizek, R. (2010). Deoxynivalenol and its toxicity. *Interdisciplinary Toxicology*, 3(3), 94–99.
<https://doi.org/10.2478/v10102-010-0019-x>
- Taylor, P. J. (2009). *An Introduction to Intraclass Correlation that Resolves Some Common Confusions*. University of Massachusetts, Boston, MA. Retrieved from http://www.faculty.umb.edu/peter_taylor/09b.pdf
- Turner, P., Burley, V., Rothwell, J., White, K., Cade, J., & Wild, C. (2008a). Deoxynivalenol: Rationale for development and application of a urinary

biomarker. *Food Additives & Contaminants: Part A*, 25(7), 864–871.

<https://doi.org/10.1080/02652030801895040>

Turner, P. C., Burley, V. J., Rothwell, J. A., White, K. L. M., Cade, J. E., & Wild, C. P. (2008b). Dietary wheat reduction decreases the level of urinary deoxynivalenol in UK adults. *Journal of Exposure Science and Environmental Epidemiology*, 18(4), 392–399. <https://doi.org/10.1038/sj.jes.7500611>

Turner, P. C., Flannery, B., Isitt, C., Ali, M., & Pestka, J. (2012). The role of biomarkers in evaluating human health concerns from fungal contaminants in food. *Nutrition Research Reviews*, 25(01), 162–179.

<https://doi.org/10.1017/S095442241200008X>

Turner, P. C., Hopton, R. P., Lecluse, Y., White, K. L. M., Fisher, J., & Lebailly, P. (2010b). Determinants of Urinary Deoxynivalenol and De-epoxy Deoxynivalenol in Male Farmers from Normandy, France. *Journal of Agricultural and Food Chemistry*, 58(8), 5206–5212. <https://doi.org/10.1021/jf100892v>

Turner, P. C., White, K. L. M., Burley, V. J., Hopton, R. P., Rajendram, A., Fisher, J., ... Wild, C. P. (2010a). A comparison of deoxynivalenol intake and urinary deoxynivalenol in UK adults. *Biomarkers*, 15(6), 553–562.

<https://doi.org/10.3109/1354750X.2010.495787>

Zachariasova, M., Hajslova, J., Kostelanska, M., Poustka, J., Krplova, A., Cuhra, P., & Hochel, I. (2008). Deoxynivalenol and its conjugates in beer: A critical assessment of data obtained by enzyme-linked immunosorbent assay and liquid chromatography coupled to tandem mass spectrometry. *Analytica Chimica Acta*, 625(1), 77–86. <https://doi.org/10.1016/j.aca.2008.07.014>

Zain, M. E. (2011). Impact of mycotoxins on humans and animals. *Journal of Saudi Chemical Society*, 15(2), 129–144. <https://doi.org/10.1016/j.jscs.2010.06.006>