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## DEOXYNIVALENOL: RATIONALE FOR DEVELOPMENT AND APPLICATION OF A URINARY BIOMARKER

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**Running Title:** Development of a urinary deoxynivalenol biomarker

**Key words:** biomarker, deoxynivalenol, diet, mycotoxin, urine

**ABSTRACT**

Mycotoxins are common dietary contaminants in most regions of the world. The frequency of exposure to the different families of mycotoxins is often dependent upon geographic location, national wealth and related agricultural and regulatory infrastructure, combined with how diverse a diet is available, and the degree of food sufficiency. Deoxynivalenol (DON) is a *Fusarium* mycotoxin that frequently contaminates wheat, corn and barley in temperate regions of the world. A number of acute poisoning incidences have been linked to DON contaminated foods, and chronic exposure to lower levels of DON has been predicted in many regions. DON is a potent animal toxin, and exposure in humans may cause gastroenteritis, growth faltering and immune toxicity. The ability to conduct accurate exposure assessment at the individual level is required in order to fully understand the potential health consequences of human exposure. To date, such exposure biomarkers have been lacking for many important mycotoxins, including DON. To better assess exposure to DON at the individual level we have developed a robust urinary assay, incorporating immunoaffinity column (IAC) enrichment and LC-MS detection. Further refinement of this urinary assay, by inclusion of  $^{13}\text{C}$ -DON as an internal standard was then undertaken, and tested within the UK. Urinary DON was frequently observed in the urine and was significantly associated with cereal intake. A dietary intervention study demonstrated that avoiding wheat in the diet markedly reduced urinary levels of DON. This biomarker requires further validation, but our initial data suggest it may provide a useful tool in epidemiological investigations of the potential health consequences of this common environmental toxin.

## INTRODUCTION

There are around 100,000 fungal species, many of which are beneficial to the environment or useful in medicine and certain food production processes. A number of fungal species can also lead to the production of structurally diverse and highly potent toxins known as mycotoxins (CAST 2003). There are many naturally occurring mycotoxins, with five of major agricultural importance: aflatoxins, deoxynivalenol (DON), fumonisins, ochratoxin A and zearalenone (Miller, 1995). Mycotoxin contamination of food is often heterogeneous (Cirillo et al., 2003) which hinders exposure assessment using classical epidemiological approaches that utilise food diaries and food frequency questionnaires (Wild et al., 2001). With the exception of the extensive developmental and validation work conducted over many years for aflatoxin (reviewed by Wild and Turner 2001, 2002; Groopman et al., 2002, 2005), a lack of biological markers, or biomarkers, still restricts our understanding of exposure at the individual level for the other mycotoxins commonly encountered through diet. As a consequence there is often awareness of frequent exposure to many potent mycotoxins, but a lack of appropriate 'tools' to conduct robust epidemiological studies to assess the potential health consequences.

DON is a *Fusarium* mycotoxin found primarily on cereal crops that are used for human consumption and in feeds for animals (CAST 2003). Due to the stability of DON to processing and cooking (Jackson and Bullerman 1999; Creppy 2002; Sugita-Konishi et al., 2006) human exposure is predicted to occur frequently (Li et al., 1999; Canady et al., 2001, SCOOP 2003). The toxicity of DON has been clearly established in animal studies, and was recently reviewed with a view to understanding the human health risks from natural dietary exposure (Schlatter, 2004; Pestka and Smolinski 2005). DON and other trichothecenes bind to the 60S ribosomal subunit, which inhibits translation and activates a

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2  
3 signaling pathway known as the ribotoxic stress response (Rotter et al., 1996, Pestka et al.,  
4  
5 2004). Feed refusal and vomiting are two of the most common toxic symptoms following  
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7 DON ingestion in several species (WHO, 1990), and the emetic symptom resulted in the  
8  
9 alternative name for DON of 'vomitoxin'. There are species differences in the  
10  
11 susceptibility to DON with swine being the most sensitive, chickens and turkeys  
12  
13 intermediate, and ruminants having a higher tolerance (Pestka and Smolinski, 2005). In  
14  
15 swine, reduced feed consumption and lower weight gain are observed following ingestion  
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17 of feeds containing DON at < 2 ppm, whilst higher doses can cause vomiting, reduction in  
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19 body weight and even death (Rotter et al., 1996, Prelusky et al., 1997). Consumption of  
20  
21 DON contaminated cereals has been associated with several major outbreaks of human  
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23 intoxication in certain parts of the world (Bhat et al., 1989; Luo 1994; WHO, 1990). The  
24  
25 symptoms observed include nausea, vomiting, diarrhea, headache, salivation, dizziness and  
26  
27 convulsions (WHO, 1990). Epidemiological data from food poisoning incidents in China  
28  
29 between 1961 and 1991 imply that DON may have a role in acute human toxicity (Luo,  
30  
31 1994). DON contamination in food was also implicated in a large-scale human toxicosis in  
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33 the Kashmir Valley of India (Bhat et al., 1989) in which ~50,000 people reported vomiting  
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35 and dizziness amongst other symptoms.  
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46 DON also has potent effects on the immune system (Bondy and Pestka, 2000; Meko et al.,  
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48 2001; Gray and Pestka, 2007), including suppression of the normal immune response to  
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50 pathogens. In addition DON exposed mice exhibit elevation of serum IgA-immune  
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52 complexes, mesangial IgA accumulation, and hematuria (reviewed by Pestka, 2003; Pestka  
53  
54 and Smolinski, 2005). These are all hallmarks of IgA nephropathy (Berger's disease), the  
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56 most common type of glomerulonephritis worldwide, which is of unknown etiology.  
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58 Increased levels of serum IgA have also been observed in DON exposed swine (Tiemann et  
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3 al., 2006, Goyarts et al., 2005). In a recent review of human epidemiological data the  
4 possibility of DON-induced gastroenteritis, growth faltering and immunotoxicity, with a  
5 possible increase in susceptibility to infectious disease, were highlighted (Pestka and  
6 Smolinski 2005). The variations in species susceptibility to this toxin may in part reflect  
7 differences in intestinal detoxification to de-epoxy DON (Yoshizawa et al., 1986). A recent  
8 small study suggests that humans lack this putative detoxification route (Sundstol-Eriksen  
9 and Pettersson, 2003). This observation is suggestive that humans could be one of the  
10 species that are more sensitive to DON, though further investigations are warranted.  
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## 24 **EXPOSURE ASSESSMENT**

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27 In order to understand the health effects of DON it is essential to be able to accurately  
28 assess intake at the individual level. Exposure assessment for dietary toxins has  
29 traditionally been approached by measuring levels of food contamination combined with  
30 estimates of food intake, thus providing population based estimates. A recent scientific  
31 cooperation (SCOOP 2003) task surveyed the frequency of foods, predominantly cereal-  
32 based, that were contaminated by 16 *Fusarium* mycotoxins in EU member states. A total of  
33 44,670 food samples were analysed, including eleven countries providing data on DON.  
34 *Fusarium* mycotoxins were predominantly associated with wheat and maize-based foods.  
35 DON was the most frequently observed *Fusarium* mycotoxin, with 57% of the 11,022  
36 samples tested for DON being found to be contaminated. Wheat and maize were  
37 contaminated with DON most frequently (61% and 89% of samples tested, respectively).  
38 Barley, oats and rye were also contaminated albeit slightly less frequently. France had the  
39 highest recorded level for DON contamination of wheat (50,000µg/Kg), whilst Sweden had  
40 the highest mean level of DON in wheat (1427µg/Kg). For maize the highest level recorded  
41 was again in France (8,850µg/Kg), which also had the highest mean level (1056µg/Kg).  
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6 In the UK more than 600 samples were tested for DON, with around 50% positive, and a  
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8 contamination range from 10 – 2261 $\mu\text{g}/\text{Kg}$  for positive samples (SCOOP 2003). Based on  
9  
10 the national consumption of four major wheat based food items (bread, breakfast cereals,  
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12 biscuits and cakes), both a mean DON intake and a high DON intake (based on consuming  
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14 a mean amount of each food, and an amount of each food corresponding to the 95<sup>th</sup>  
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16 percentile of intake, respectively) were estimated in  $\mu\text{g}/\text{day}$ , using the mean level of  
17  
18 contamination for each item (SCOOP 2003). Data were available for both adult males and  
19  
20 females, and are summarized in Table 1. The majority of exposure in adults was predicted  
21  
22 to come from bread and breakfast cereals, due to their widespread contamination with  
23  
24 DON and the frequent consumption of these items. Additional important sources of  
25  
26 exposure are likely to include products containing wheat flour (excluding the items in  
27  
28 Table 1) for example pasta, corn flour and possibly cereal-based snacks (SCOOP 2003). It  
29  
30 should be noted that it is unlikely that any given individual will consume all of the food  
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32 groups at the high level of consumption, and in the UK, average dietary intakes for DON  
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34 (assuming an average body weight of 70.1kg) were estimated at 176ng/kg(bw)/day and  
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36 142ng/kg(bw)/day, for males and females respectively. Based on a greater food intake per  
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38 unit body mass, higher mean DON intakes were estimated for infants and young children  
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40 (range 400-500ng/kg(bw)/day), with bread, breakfast cereals and cereal based snacks  
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42 predicted to provide the major contributions to exposure.  
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53 These estimates of intake are below the recommended TDI of 1000ng/kg(bw)/day (SCF,  
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55 2002). However, these data represent mean levels, and both the quantity of food and the  
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57 contamination levels of foods were so variable that estimates of exposure may be 10 to 20-  
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59 fold higher in individuals consuming the most contaminated foods. When compared to the  
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3 UK, higher levels of exposure were predicted for some of the other countries within the  
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5 EU, notably France, The Netherlands and Germany (SCOOP 2003).  
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10 In a more global assessment of DON the Joint (FAO/WHO) Expert Committee on Food  
11 Additives (JECFA) also estimated dietary intakes using food consumption and  
12 contamination data (Canady et al., 2001). Five world regions were created, European  
13 (which also includes Australia, Canada, US and New Zealand), Latin America, Middle  
14 Eastern, Far Eastern and African. Data for the exposure estimates were often limited,  
15 particularly outside of Europe, though crude estimates of international exposure are  
16 provided. The JECFA estimates are based on cereal consumers only, whilst it should be  
17 noted that the SCOOP data are for the population as a whole. The estimates range from a  
18 mean of 780ng/Kg(bw)/day in the African region (albeit based on data from South Africa  
19 alone) to 2400ng/Kg(bw)/day in the Middle eastern diet, with the European region having  
20 an estimated intake of 1400ng/Kg(bw)day. These alternative estimates suggest that globally  
21 the TDI for DON will be frequently exceeded. Whilst the TDI is based on a “No Observed  
22 Adverse Effect Level” in animals and incorporates a safety factor, humans may be  
23 particularly sensitive to DON compared to animals as discussed above (Sundstol-Eriksen  
24 and Pettersson 2003). The SCOOP (2003) report additionally highlighted some of the main  
25 limitations in its estimate of DON intake, and suggested that the total dietary intake by  
26 country should generally be considered an underestimate.  
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53 **Biomarker approach to exposure assessment:** As mentioned above, a major obstacle to  
54 studying DON and human health is the heterogeneous nature of cereal contamination,  
55 which makes the estimation of exposure at the individual level difficult. Given the  
56 uncertainties in the assessment of mycotoxin exposure from dietary data, biomarkers have  
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been suggested to offer a better approach (Montesano et al., 1997; Turner et al., 1999; Wild and Turner 2001). Mycotoxin exposure biomarkers potentially provide data at the individual level and may establish the prevalence and range of exposure in a population (Wild and Turner 2002; Turner et al., 1999). In response, the development of a urinary exposure biomarker for DON was initiated based on our understanding of the absorption and clearance of DON in animals. The toxicokinetics of DON in several animal species has previously been reviewed (Pestka and Smolinski, 2005). The absorption of DON following ingestion is species dependant. Swine are particularly sensitive to DON, and following DON ingestion, up to 80% is absorbed (Rotter et al., 1996) and plasma concentrations peak within 15–30 min (Prelusky et al., 1988). In contrast, in ruminants, which are relatively resistant to DON toxicity, a smaller percentage of the parent compound is absorbed; in sheep 6-10% (Prelusky et al., 1986) and in dairy cows <1% (Prelusky et al., 1984). This lower level of uptake may in part reflect the greater capacity for bacterial derived de-epoxidation of DON to DOM-1 within the ruminant gut (Rotter et al., 1996). The elimination half-life for DON in most species is relatively rapid, and the majority of DON and DON metabolites are excreted within about 24 hours (Pestka and Smolinski, 2005). The DON-glucuronide metabolite is observed in the serum and urine of swine (Goyarts and Danicke, 2006), and ruminants (Prelusky et al., 1984; Cote et al., 1986), and the urine of rodents (Meky et al., 2003). In swine the serum clearance profile for DON only and DON + DON-glucuronide are similar (Goyarts and Danicke, 2006). DON and DON-glucuronide excretion in the urine represented 37% and 50% of the ingested DON in rat (Meky et al., 2003) and swine (Goyarts and Danicke, 2006), respectively.

The rapid clearance of DON from serum did not make this a good matrix to assess dietary exposure in humans. The high level of transfer of DON to urine, combined with the relative

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3 ease of collection however made this a more suitable matrix. In the first stage of  
4 development the clearance of DON plus a DON-glucuronide conjugate were examined in a  
5 rat model, and utilised immunoaffinity enrichment and LC-MS detection (Meky et al.,  
6 2003). Immuno-affinity enrichment resulted in the loss of the DON-glucuronide and thus to  
7 maximize the measure of total DON, the urine samples were pre-incubated with  $\beta$ -  
8 glucuronidase to release the parent toxin.  
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20 In an initial test of a series of 15 female residents in Linxian County, Henan Province or  
21 Gejiu, Yunnan Province, China all urines were positive for DON; median 30ng/ml: range  
22 4-94ng (Meky et al., 2003). To our knowledge, this was the first report of DON in human  
23 urine. The urinary DON levels were consistent with those expected based on the range of  
24 cereal contamination in this region (Luo et al., 1990). The hydrolysis conditions used were  
25 such that several  $\mu$ g of DON-glucuronide in urine would be readily hydrolysed to release  
26 DON (Meky et al., 2003). Given that the Chinese samples contained ng/ml concentrations,  
27 we had considerable confidence that all DON-glucuronide present would be hydrolysed. In  
28 addition, increasing the  $\beta$ -glucuronidase concentration >20-fold gave no significant  
29 increase in DON recovery (unpublished observation). Further refinement of this urinary  
30 assay, by inclusion of  $^{13}\text{C}$ -DON as an internal standard was then undertaken using a UK  
31 population dataset. In the UK, wheat is the main cereal crop responsible for human  
32 exposure to DON (SCOOP 2003). Based on this knowledge we examined firstly, whether  
33 urinary DON could be detected in the UK population and, secondly, whether levels of  
34 urinary DON would be reduced by avoidance of wheat based products in a short-term  
35 intervention study. This intervention study is reviewed below; additional methodological  
36 detail, study design and study results have been recently published (Turner et al., 2007a).  
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3 **Study Design:** The study took place over seven days with 25 healthy adult volunteers, and  
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5 involved two days (Day 1 and 2) of normal diet and four days (Day 3-6) of a wheat  
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7 restricted diet. Suggestions for alternative starch based food items and meals were provided  
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9 for the wheat restriction phase. Food diaries were kept for Days 1 and 2 (normal diet) and  
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11 Days 5 and 6 (intervention diet). A first morning void urine sample was obtained on Day 3  
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13 to represent the 'normal diet', and on Day 7 to represent the 'intervention diet', see Table 2.  
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15 The study design reflects the observation that in animal models urinary DON  
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17 predominantly reflects transfer over the first 24 to 48 hours. The wheat restricted food  
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19 items in this study were primarily bread, breakfast cereal, cakes/biscuits and pasta, and the  
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21 suggested alternatives were rice and potatoes.  
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29 **Urinary Analysis:** Urinary DON was measured as described in detail by Turner et al.,  
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31 (2007a). In brief, urine samples were spiked with <sup>13</sup>C-DON as an internal standard, and  
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33 then digested overnight with 23,000 units β-glucuronidase (Type-IX, Sigma, Poole Dorset),  
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35 to allow assessment of "total DON" (i.e. the amount of DON plus DON-glucuronide).  
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37 Digests were then enriched for DON using immunoaffinity columns (IAC), and analysed  
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39 by HPLC with mass spectrometric detection using selective ion recording. All DON levels  
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41 in the urine samples were quantified by reference to the internal standard. The limit of  
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43 quantification (LOQ) in urine for this analysis was 0.6ng DON /ml urine. Two aliquots of a  
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45 urine sample with previously measured DON concentration were also spiked with the  
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47 internal standard and analysed with each batch of test samples as a quality control (QC).  
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49 The mean QC value was 10.4ng/ml with a CV of 6.1%. As first morning urine samples  
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51 were used the final DON concentration in ng DON/ml of urine was adjusted using a  
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53 measure of creatinine concentration in mg/ml of urine and subsequently data were  
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55 expressed as ng DON/mg creatinine (ng/mg).  
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6 Twenty-five paired samples were available for urine taken corresponding to the normal diet  
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8 and following the four-day intervention trial. During consumption of the normal diet all  
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10 individuals had detectable urinary DON, whilst during the intervention fewer samples were  
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12 positive (9 of 25; 36%) The mean levels of urinary DON in both 'normal' and  
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14 'intervention' samples are shown in Table 3. The geometric mean level during normal diet  
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16 (7.2ng DON/mg creatinine; 95% CI 4.9-10.5ng/mg) was 11 fold higher ( $p < 0.001$ ) than that  
17  
18 during the intervention (0.6ng/mg; 95% CI 0.4-0.9ng/mg). During the consumption of the  
19  
20 'normal' diet bread, breakfast cereals, biscuits, cakes, and pasta were consumed in amounts  
21  
22 consistent with that previously reported for the UK population (Hendersen et al., 2002).  
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24 Overall bread was the most frequent source of wheat-based foods (see Table 4). The  
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26 intervention was implemented between days three and six and the recorded diary  
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28 information on days five and six indicated good compliance with a reduced frequency of  
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30 consumption of wheat-based foods. In order to compensate for this reduction there was a  
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32 marked increase in the consumption of both rice and potatoes (see Table 4). For  
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34 comparison, data from the Chinese urine samples have been included in Table 3. The  
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36 samples from China appear to have slightly higher levels of urinary DON, though we  
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38 should additionally point out that these Chinese data are not adjusted for recoveries, as an  
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40 internal standard was not available at the time of analysis. Creatinine data were also not  
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42 available so this adjustment was not made.  
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53 More recently we have undertaken a larger survey of urinary DON in the UK (Turner et al.,  
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55 2007b). Urine samples were available from the 2000-2001 UK adult National Diet and  
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57 Nutrition Survey (NDNS) (Henderson et al. 2002), permitting comparison of urinary DON  
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59 excretion with specific dietary information collected for each individual. Urine samples  
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3 (24-hour) were collected from 1724 individuals aged 19-64 years of age. In addition  
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5 individuals completed a detailed seven-day weighed food diary (Office for National  
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7 Statistics 2005). The hypothesis was that cereals would provide the major contribution to  
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9 DON exposure, thus in this study individuals were selected following stratification into  
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11 low, medium and high cereal intake groups. Stratification was based on items that provided  
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13 the major sources of cereal in the UK diet; these included breads (wholemeal, white, soft  
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15 grain, other), breakfast cereals (high-fibre and other), pasta, pizza, fruit pies, biscuits and  
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17 buns/cakes. One hundred subjects were identified for each of the following cereal  
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19 consumption groups: low (mean 107g cereal/day; range 88-125), medium (mean 179g/day;  
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21 range 162-195) and high (mean 300g/day; range 276-325). DON was detected in 296 of  
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23 300 (98.7%) urine samples, and the mean and range of urinary DON levels (arithmetic  
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25 mean of 9.0ng DON/mg creatinine; range non detectable to 48.2ng/mg) were similar to that  
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27 reported within the 'normal diet phase' of the intervention study discussed above. Cereal  
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29 intake was significantly associated with urinary DON ( $p < 0.0005$ ), with geometric mean  
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31 urinary levels of 7.2 ng/mg (95%CI: 6.2-8.2ng/mg); 9.2ng/mg (95%CI: 8.1-10.5ng/mg) and  
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33 10.9ng/mg (95%CI: 9.5-12.4ng/mg) for low, medium and high intake groups respectively,  
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35 after adjustment for sex and BMI, see Table 3.  
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46 Crude estimates of daily DON intake in the UK were reported by Turner et al., (2007a,  
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48 2007b). These were based on: (i) the measure of urinary DON, (ii) an assumption that 35-  
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50 50% the ingested DON was being excreted in the urine (Meky et al., 2003; Goyarts and  
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52 Danicke 2006), and (iii) an assumption that the urinary DON originated from DON intake  
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54 in the previous 24 hours. Based on these assumptions the mean intake was calculated to be  
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56 around half of the recommended TDI for DON ingestion which was set at 1000ng/kg(bw)  
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58 (SCF, 2002) with some individuals expected to exceed this intake. However, these values  
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3 should be interpreted with caution, partly because of the uncertainty in the percentage  
4 transfer of DON into human urine and the fact that the kinetics of human urinary excretion  
5 of DON and its metabolites has not been established. In addition, it should be noted that  
6 specific sub-groups in the population, such as vegetarians and children, may have higher  
7 exposures than the general population.  
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## 17 **FUTURE WORK**

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19 Our biomarker data for exposure to DON in the UK are in good agreement with the  
20 estimates from food analysis (SCOOP 2003), in that exposure is both frequent and  
21 predominantly associated with wheat consumption. However, whilst the food analysis data  
22 allows estimates at a population level our biomarker approach allows measures of exposure  
23 at the individual level. Given that a wide range of exposures are predicted within a  
24 population, the assessment of individual exposures within a study population is critical in  
25 order to understand the health consequences. The SCOOP (2003) survey predicts that in  
26 European countries such as France, Germany and The Netherlands, exposure to DON will  
27 be higher than the UK. In a more global consideration, Europe as a region does not have  
28 the highest predicted mean level of DON exposure (Canady et al., 2001). Our biomarker  
29 data suggest DON exposure has been under-estimated by the SCOOP survey, and this  
30 reinforces concerns about the potential health consequences in the UK and elsewhere. This  
31 urinary biomarker may also be useful in providing improved understanding of some of the  
32 acute poisoning incidents which have been potentially associated with DON contaminated  
33 foods (Luo 1994, Bhat et al.,1989). However, there are still a number of outstanding  
34 questions related to fully validating the urinary measure as a reliable exposure biomarker in  
35 order to conduct informative epidemiological studies.  
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3 First, what is the relationship at the individual level between DON intake and the urinary  
4 biomarker? This is probably the most fundamental question to be answered in the further  
5 validation steps. To date we have only sought to compare cereal intake with urinary DON  
6 levels and this limits the ability to interpret biomarker levels in a quantitative manner in  
7 terms of DON consumption. Second, what are the pharmacokinetics of DON and DON-  
8 glucuronide excretion, and, what are the consequent temporal variations in this biomarker?  
9 To date only single time point measures of urinary DON during the consumption of a  
10 'normal diet' have been determined for a given individual. This information is important in  
11 being able to interpret urinary DON biomarker data in relation to recent or typical exposure  
12 levels. It will be necessary to include measures of the temporal variation in both DON  
13 intake and the urinary measure as part of these assessments. Third, does the ratio of DON  
14 to DON-glucuronide in urine vary by individual? The current methodology employed for  
15 measuring urinary DON involves the digestion of any DON-glucuronide present, to the  
16 parent compound, and thus the assay provides a combined measure of free and de-  
17 conjugated DON, or "total" urinary DON. Genetic variation in this conjugation step may  
18 have a bearing on the toxicity of DON, and the DON/DON-glucuronide ratio should be  
19 assessed both between individuals and within individuals at different levels of DON  
20 exposure.  
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Jelinek and colleagues reviewed the occurrence of the most important mycotoxins that naturally contaminate foods for human consumption (Jelinek et al., 1989). Their report details extensive DON contamination of grains, especially wheat, and further highlights the concern of co-contamination of grains by *Fusarium* toxins, especially for DON and nivalenol. They recommended that more information on co-occurrence of *Fusarium* toxins in cereals should be obtained in order to understand the potential additive or synergistic

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3 effects of such exposure mixtures. We envisage that our initial development of a urinary  
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5 marker for DON will add impetus to the development of additional mycotoxin exposure  
6  
7 biomarkers. It is possible that as this field continues to expand, additional matrices may be  
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9 investigated including sera, saliva or faeces. It is also envisaged that biomarkers of the  
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11 effects of mycotoxin exposures will follow from this development. It is likely that one or  
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13 more of the 'omics' approaches will provide a wealth of data to further understand and  
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15 develop such biomarkers and point to new avenues of research involving mechanisms of  
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17 action. These latter markers of biological effect may be more generic with respect to  
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19 similar *Fusarium* mycotoxins such as DON and nivalenol, and thus have the utility of  
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21 providing important information on the effect of co-exposures.  
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29 In cereals, mixtures of different classes of mycotoxin naturally occur, and these may  
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31 include deoxynivalenol (and other tricothecenes), fumonisins, ochratoxin A, zearalenone  
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33 and aflatoxin (reviewed by Jelinek et al., 1989; Speijers and Speijers 2004). Understanding  
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35 the risks associated with exposure to mixtures of mycotoxins is still in its infancy even in  
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37 animal studies. In humans such studies require validated methods of exposure assessment  
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39 in order to compare exposure to toxins both individually and in combination with health  
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41 outcomes. In this context accurate analytical determination of a mycotoxin in a biological  
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43 sample does not translate to a valid marker of exposure. Rather, the biomarker must be  
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45 shown to correlate with mycotoxin intake at the individual level as was originally  
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47 demonstrated for aflatoxins (Wild et al., 1992) and is currently being addressed in our own  
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49 group for deoxynivalenol (Turner et al., 2007a,b) and fumonisin B1 (Gong et al., 2008).  
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51 Eventually methodological advances such as applied to the simultaneous measurement of  
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53 mycotoxin mixtures from cereals (Lattanzio et al., 2007a,b) may be of value for more rapid  
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55 multi-toxin bio-monitoring.  
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## SUMMARY

To date there is overwhelming evidence that certain dietary staples based on cereal crops worldwide are contaminated with DON. In order to better estimate exposure we have developed a urinary biomarker assay (Meky et al., 2003; Turner et al., 2007a). The methodology is robust, with high specificity and sensitivity, incorporating immunoaffinity isolation of DON and LC separation with mass spectrometric detection. Furthermore, the incorporation of an internal standard allows adjustment for recovery. Preliminary data from the UK (Turner et al., 2007a,b) reveal an almost ubiquitous exposure to DON. The specific activities outlined above indicate the research required to more fully validate this biomarker as a quantitative measure of exposure. This will provide an important foundation to further investigate the possible health effects of DON exposure. These latter investigations need to consider that there are likely to be sub-groups of people who experience particularly high exposures, warranting future targeted surveys of these potentially 'high risk' groups. One of these sub-groups may be children for whom exposure is predicted to be higher than adults, and in whom there may be an increased sensitivity to exposure. Finally, given the reported poisoning episodes in China and India, and the higher average exposure levels in the Middle and Far East, it will be important to target future collaborative research which focuses on potential health concerns within these higher exposure regions.

**Table 1: Predicted intake of DON from four specific food items (adapted from SCOOP 2003)**

Food item	Mean* DON intake µg / day		High** DON intake µg / day	
	Male	Female	Male	Female
<b>Breakfast cereal</b>	3.99	2.89	10.58	7.37
<b>Bread</b>	7.46	4.69	14.50	8.77
<b>Biscuits</b>	0.55	0.47	1.54	1.27
<b>Cakes</b>	0.55	0.43	1.41	1.10

\*Mean based on mean intake and mean contamination of specific foods (see SCOOP 2003)

\*\*High based on 95<sup>th</sup> percentile intake and mean contamination of specific foods (see SCOOP 2003).

**Table 2: Design of wheat restriction intervention (Turner et al., 2007a)**

Day	Diet	Food Diary	First morning urine*
One	Normal	Yes	/
Two	Normal	Yes	/
Three	Intervention	/	Yes
Four	Intervention	/	/
Five	Intervention	Yes	/
Six	Intervention	Yes	/
Seven	Normal	/	Yes

Lighter shaded area represents 'normal' dietary period and urine collection.

Darker shaded area represents 'intervention' dietary period and urine collection.

\* First morning urines are predicted to reflect intake in the previous 24-48hours.

**Table 3: Assessment of urinary DON in UK and Chinese samples (modified from Meky et al., 2003 and Turner et al., 2007a,b)**

	Detection rate	DON (ng/ml)* Mean (range)	DON (ng/mg)** GM (95% CI)	Reference
<b>UK adults - normal diet</b>	<b>25/25 (100%)</b>	<b>10.8 (0.7 – 61.3)</b>	<b>7.2 (4.9, 10.5)</b>	<b>Turner et al., 2007a</b>
<b>- intervention diet</b>	<b>9/25 (36%)</b>	<b>1.0 (nd – 8.4)</b>	<b>0.6 (0.4, 0.9)</b>	
<b>Chinese adults #</b>	<b>15/15 (100%)</b>	<b>30 (4 - 94)</b>	<b>/</b>	<b>Meky et al., 2003</b>
<b>UK adults## - low cereal <sup>1</sup></b>	<b>97/100</b>	<b>5.4 (nd-19.8)</b>	<b>7.2 (6.2, 8.2)</b>	<b>Turner et al., 2007b</b>
<b>- medium cereal <sup>2</sup></b>	<b>99/100</b>	<b>7.8 (0.4-56.4)</b>	<b>9.2 (8.1, 10.5)</b>	
<b>- high cereal <sup>3</sup></b>	<b>100/100</b>	<b>9.3 (2.1-44.6)</b>	<b>10.9 (9.5, 12.4)</b>	

nd - non detected

\* ng DON /ml urine (mean and range)

\*\* ng DON/mg creatinine; GM - geometric mean and 95%confidence intervals

# no adjustment for DON recoveries

## data adjusted for age, sex and BMI

low cereal <sup>1</sup> 107g; range 88-125g

medium cereal<sup>2</sup> 179g; range 162-195g

high cereal<sup>3</sup> 300g; range 276-325g

**Table 4 – Average frequency of consumption of dietary staples in 25 individuals consuming their normal diet (average of two days), and during the wheat reduction intervention diet (average of two days) (modified from Turner et al., 2007a).**

<b>FOOD GROUP</b>	<b>Normal Diet Average Frequency (%)</b>	<b>Intervention Diet Average Frequency (%)</b>	<b># p - value</b>
<b>Bread</b>	22 / 25 (88)	1.5 / 25 (6)	<0.001
<b>Breakfast Cereal*</b>	9 / 25 (36)	0 / 25 (0)	<0.001
<b>Cakes / Biscuits</b>	18 / 25 (72)	4.5 / 25 (18)	<0.001
<b>Pasta</b>	6 / 25 (24)	0 / 25 (0)	<0.001
<b>Potatoes</b>	9 / 25 (32)	15 / 25 (60)	0.016
<b>Rice**</b>	3 / 25 (12)	17.5 / 25 (70)	<0.001

# T test for difference in frequency of consumption for Days 1 & 2 combined compared to Days 5 & 6 combined

\*Excluding Rice Crispies breakfast cereal and porridge

\*\* Including Rice Crispies breakfast cereal

## References

Bhat R.V., Beedu S.R., Ramakrishna Y., Munshi K.L. (1989) Outbreak of trichothecenes mycotoxicosis associated with consumption of mould-damaged wheat production in Kashmir Valley, India. *Lancet* 1(8628): 35-37.

Bondy, G.S., and Pestka, J.J. (2000) Immunomodulation by fungal toxins. *J. Toxicol. Environ Health-Part B- Crit. Rev.* 3: 109-143.

Canady, R.A., Coker, R.D., Egan, S.K., Krska, R., Kuiper-Goodman, T., Olsen, M., Pestka, J., Resnik, S., and Schlatter, J. (2001) Deoxynivalenol. In: Safety evaluation of certain mycotoxins in food, pp. 420–529. Geneva: World Health Organization.

CAST. (2003). Potential economic costs of mycotoxins in the United States. In *Mycotoxins: Risks in plant, animal, and human systems*, pp. 136–142. Task Force Report No. 139. Ames, IA: Council Agri. Sci. Tech.

Cote, L. M., Dahlem, A. M., Yoshizawa, T., Swanson, S. P., and Buck, W. B. (1986). Excretion of deoxynivalenol and its metabolite in milk, urine, and feces of lactating dairy cows. *J. Dairy Sci.* 69:2416–2423.

Creppy E.E. (2002) Update of survey, regulation and toxic effects of mycotoxins in Europe. *Toxicol Lett.* 127(1-3) 19-28



1  
2  
3 Cirilloy T., Ritieniy A., Galvanoz F., Amodio-Cocchieriy, R. (2003) Natural co-  
4  
5 occurrence of deoxynivalenol and fumonisins B1 and B2 in Italian marketed  
6  
7 foodstuffs. *Food Add. Cont.* 20(6): 566-571.  
8  
9

10  
11  
12 Gong Y. Torres-Sanchez L. Lopez-Carrillo L. Peng J.H. Sutcliffe A.E. White K.L.M.  
13  
14 Turner P.C. Humpf H. Wild C.P. (2008) Detection of fumonisin B1 in Mexican urine  
15  
16 samples using liquid chromatography-mass spectrometry - a possible biomarker of dietary  
17  
18 fumonisin exposure. *Cancer Epi. Biomarkers Prev.* (in press).  
19  
20  
21

22  
23  
24 Goyarts T. and Danicke S. (2006) Bioavailability of the Fusarium toxin deoxynivalenol  
25  
26 (DON) from naturally contaminated wheat for the pig. *Toxicol Lett.* 163(3):171-82.  
27  
28  
29

30  
31 Goyarts T., Danicke S., Rothkotter H.J., Spilke J., Tiemann U., Schllenberger M.  
32  
33 (2005) On the effects of a chronic deoxynivalenol intoxication on performance,  
34  
35 haematological and serum parameters of pigs when diets are offered either for ad  
36  
37 libitum consumption or fed restrictively. *J Vet. Med. A Physiol. Pathol. Clin. Med.*  
38  
39 52(6): 305-314.  
40  
41  
42

43  
44  
45 Gray JS., and Pestka J.J. (2007). Transcriptional regulation of deoxynivalenol-induced  
46  
47 IL-8 expression in human monocytes. *Toxicol Sci.* 99(2):502-11  
48  
49  
50

51  
52  
53 Groopman, J.D., Jackson, P.E., Turner, P.C., Wild, C.P. and Kensler, T.W. (2002).  
54  
55 Validation of Exposure and Risk Biomarkers: Aflatoxin as A Case Study. *Biomarkers*  
56  
57 *of Environmentally Associated Disease* (Ed S.H.Wilson, W.A.Suk). CRC Press  
58  
59 pp307-318.  
60

1  
2  
3  
4  
5  
6 Groopman J.D. Johnson D. and Kensler T. (2005) Aflatoxin and hepatitis B virus  
7  
8 biomarkers: a paradigm for complex environmental exposures and cancer risk. *Cancer*  
9  
10 *Biomarkers* 1(1) 5-14.  
11

12  
13  
14  
15 Henderson L, Gregory J, Swan G. (2002) The National Diet and Nutrition Survey: adults  
16  
17 aged 19 to 64 years. HMSO: Norwich  
18  
19  
20 <http://www.food.gov.uk/multimedia/pdfs/ndnsprintedreport.pdf>  
21

22  
23  
24 Jackson L.S and Bullerman L.B. (1999) Effect of processing on *Fusarium*  
25  
26 mycotoxins. *Adv. Exp. Med. Biol.* 459: 243-261.  
27  
28

29  
30  
31 Jelinek C.F., Pohland A.E. Wood G.E., (1989) Worldwide occurrence of mycotoxins  
32  
33 in foods and feeds--an update. *J. Assoc. Off. Anal. Chem.* 72(2): 223-30  
34  
35

36  
37  
38 Lattanzio V.M., Solfrizzo M., Powers S., Visconti A. (2007a) Simultaneous  
39  
40 determination of aflatoxins, ochratoxin A and *Fusarium* toxins in maize by liquid  
41  
42 chromatography/tandem mass spectrometry after multitoxin immunoaffinity cleanup.  
43  
44 *Rapid Commun Mass Spectrom.* 21(20):3253-61.  
45  
46  
47

48  
49  
50 Lattanzio V.M., Solfrizzo M., Visconti A. (2007b) Determination of trichothecenes in  
51  
52 cereals and cereal-based products by liquid chromatography-tandem mass  
53  
54 spectrometry. *Food Addit. Contam.* [Epub ahead of print]  
55  
56  
57  
58  
59  
60

1  
2  
3 Li, F., Luo, X., Yoshizawa, T. (1999) Mycotoxins (trichothecenes, zearalenone and  
4 fumonisins) in cereals associated with human red mold intoxications stored since  
5  
6  
7  
8 1989 and 1991 in China. *Nat. Toxins* 7: 93–97.  
9

10  
11  
12  
13 Luo, X. 1994. Food poisoning caused by *Fusarium* toxins. *Proc. Second Asian Conf.*  
14  
15 *Food Safety*, pp. 129–136.  
16

17  
18  
19  
20 Luo, Y., Yoshizawa, T., Katayama, T., 1990. Comparative study on the natural  
21  
22 occurrence of *Fusarium* mycotoxins (trichothecenes and zearalenone) in corn and  
23  
24 wheat from high- and low-risk areas for human esophageal cancer in China. *Appl*  
25  
26 *Environ. Microbiol.* 56, 3723–3726.  
27

28  
29  
30  
31  
32 Meko, F.A., Hardie, L.J., Evans, S.W., and Wild C.P. (2001). Deoxynivalenol-  
33  
34 induced immunomodulation of human lymphocyte proliferation and cytokine  
35  
36 production. *Food Chem. Toxicol.* 39, 827-836.  
37

38  
39  
40  
41 Meko, F.A., Turner, P.C., Ashcroft, A.E., Miller, J.D., Qiao, Y-L., Roth, M.J., Wild,  
42  
43 C.P. (2003) Development of a urinary biomarker of human exposure to  
44  
45 deoxynivalenol. *Food Chem. Toxicol.* 41(2): 265-273.  
46  
47

48  
49  
50  
51 Miller, J.D. (1995) Fungi and mycotoxins in grain: Implications for stored product  
52  
53 research. *J. Stored Prod. Res.* 31: 1-16  
54

55  
56  
57  
58 Montesano R, Hainaut P, Wild CP. (1997) Hepatocellular carcinoma: From gene to  
59  
60 public health. *J. Natl. Cancer Inst.* 89:1844-1851

1  
2  
3 Office for National Statistics. 2005. Social and Vital Statistics Division and Food  
4 Standards Agency, National Diet and Nutrition Survey: Adults Aged 19 to 64 Years,  
5  
6 2000-2001. UK Data Archive: Colchester, Essex SN: 5140.  
7  
8  
9

10  
11  
12 Pestka JJ (2003) Deoxynivalenol-induced IgA production and IgA nephropathy-  
13 aberrant mucosal immune response with systemic repercussions. *Toxicol Lett.* 140-  
14 141:287-95.  
15  
16  
17  
18  
19

20  
21  
22 Pestka, J.J. and Smolinski A.T. (2005) Deoxynivalenol: toxicology and potential  
23 effects on humans. *J Toxicol Environ Health B Crit Rev.* 8(1): 39-69.  
24  
25  
26  
27

28  
29 Pestka J.J., Zhou H.R., Moon Y., Chung Y.J., (2004) Cellular and molecular  
30 mechanisms for immune modulation by deoxynivalenol and other trichothecenes:  
31 unraveling a paradox. *Toxicol. Lett.* 153(1):61-73  
32  
33  
34  
35  
36

37  
38 Prelusky, D.B., 1997. Effect of intraperitoneal infusion of deoxynivalenol on feed  
39 consumption and weight gain in the pig. *Nat. Toxins.* 5, 121-125.  
40  
41  
42  
43

44  
45 Prelusky, D.B., Trenholm, H.L., Lawrence, G.A., and Scott, P.M. (1984).  
46 Nontransmission of deoxynivalenol (vomitoxin) to milk following oral administration  
47 to dairy cows. *J. Environ. Sci. Health B* 19:593–609.  
48  
49  
50  
51  
52

53  
54 Prelusky, D.B., Veira, D.M., Trenholm, H.L., and Hartin, K.E. (1986). Excretion  
55 profiles of the mycotoxin deoxynivalenol, following oral and intravenous  
56 administration to sheep. *Fundam. Appl. Toxicol.* 6:356–363.  
57  
58  
59  
60

1  
2  
3  
4  
5  
6 Prelusky, D.B., Hartin, K.E., Trenholm, H.L., and Miller, J.D. (1988).  
7  
8 Pharmacokinetic fate of 14C-labeled deoxynivalenol in swine. *Fundam. Appl. Toxicol.*  
9  
10 10:276–286.  
11

12  
13  
14  
15 Rotter B.A., Prelusky D.B., Pestka J.J. (1996) Toxicology of deoxynivalenol  
16  
17 (vomitoxin). *J. Toxicol Environ Health.* 48(1): 1-34.  
18  
19

20  
21  
22 SCF (2002) Opinion of the Scientific Committee on Food on Fusarium toxins. Part 6:  
23  
24 Group evaluation of T-2 toxin, HT-2 toxin, nivalenol and deoxynivalenol:  
25  
26 SCF/CS/CNTM/MYC/27 Final.  
27  
28 [http://europa.eu.int/comm/food/fs/sc/scf/out123\\_en.pdf](http://europa.eu.int/comm/food/fs/sc/scf/out123_en.pdf).  
29  
30  
31

32  
33  
34 Schlatter J. (2004) Toxicity data relevant for hazard characterization. *Toxicol. Lett.*  
35  
36 153(1):83-9  
37

38  
39  
40  
41 SCOOP (2003) Collection of occurrence data of Fusarium toxins in food and  
42  
43 assessment of dietary intake by the population of EU member states. SCOOP Task  
44  
45 3.2.10 Final Report. <http://europa.eu.int/comm/food/fs/scoop/task3210.pdf>  
46  
47  
48

49  
50  
51 Speijers G.J.A. and Speijers M.H.M. (2004) Combined toxic effects of mycotoxins.  
52  
53 *Toxicol. Lett.*, 153(1):91-8 .  
54

55  
56  
57 Sundstol-Eriksen, G. and Pettersson, H. (2003) Lack of de-epoxidation of type B  
58  
59 trichothecenes in incubates with human faeces. *Food Add. Contam.* 20: 579–582.  
60

1  
2  
3  
4  
5  
6 Sugita-Konishi Y., Park B.J., Kobayashi-Hattori K., Tanaka T., Chonan T.,  
7  
8 Yoshikawa K., Kumagai S. (2006) Effect of cooking process on the deoxynivalenol  
9  
10 content and its subsequent cytotoxicity in wheat products. Biosci. Biotechnol.  
11  
12 Biochem. 70(7): 1764-8.

13  
14  
15  
16  
17 Tiemann U., Bruusow K.P., Jonas L., Pohland R., Schneider F., Danicke S. (2006)  
18  
19 Effects of diets with cereal grains contaminated by graded levels of two Fusarium  
20  
21 toxins on selected immunological and histological measurements in the spleen of  
22  
23 gilts. J Anim. Sci. 84(1): 236-245.  
24  
25

26  
27  
28  
29 Turner P.C., Burley V.J., Rothwell J.A., White K.L.M., Cade J.E., Wild C.P. (2007a)  
30  
31 Dietary wheat reduction decreases the level of urinary deoxynivalenol in UK adults. J.  
32  
33 Exposure Sci. Env. Epidemiol. (Epub on line *doi:10.1038/sj.jes.7500611*).  
34  
35

36  
37  
38 Turner P.C., Nikiema P., Wild C.P. (1999) Fumonisin Contamination of food:  
39  
40 progress in development of biomarkers to better assess human health. Mut. Res., 443:  
41  
42 81-93.  
43  
44

45  
46  
47  
48 Turner P.C., Rothwell J.A., White K.L.M., Cade J.E., Wild C.P. (2007b). Cereal  
49  
50 intake is correlated with urinary deoxynivalenol in individuals from the UK Environ.  
51  
52 Health Persp. 36: 1119-1125.  
53  
54

55  
56  
57 WHO (1990). In: Selected Mycotoxins: Ochratoxin, Tricothecenes, Ergot.  
58  
59 Environmental Health Criteria No 105. Geneva: WHO.  
60

1  
2  
3  
4  
5  
6 Wild C.P., Andersson C., O'Brian N.M., Wilson L., Woods J.A., (2001) A critical  
7  
8 evaluation of the application of biomarkers in epidemiological studies on diet and  
9  
10 health. *Br. J. Nutr.* 86 Suppl 1:S37-53.  
11

12  
13  
14  
15 Wild C.P., Hudson G.J., Sabbioni, G., Chapot B., Hall A.J., Wogan G.N., Whittle H.,  
16  
17 Montesano R., Groopman J.D. (1992) Dietary intake of aflatoxins and the level of  
18  
19 albumin-bound aflatoxin in peripheral blood in The Gambia, West Africa. *Cancer*  
20  
21 *Epidemiol Biomarkers Prev.* 1(3):229-34.  
22  
23

24  
25  
26  
27 Wild C.P. and Turner P.C. (2001). Exposure biomarkers in chemoprevention studies  
28  
29 of liver cancer. Eds: Miller, A.B., Bartsch, H., Bofetta, P., Dragsted, L., and Vanio,  
30  
31 H. *Biomarkers in Cancer Chemoprevention* (IARC Scientific Publications No 154)  
32  
33 International Agency for Research on Cancer, Lyon. pp 215-.222.  
34  
35

36  
37  
38  
39 Wild C.P. and Turner P.C. (2002) The Toxicology of Aflatoxins as a basis for Public  
40  
41 Health Decisions. *Mutagenesis* 17(6): 471-481.  
42  
43

44  
45  
46 Yoshizawa, T., Cote, L. M., Swanson, S. P., and Buck, W. B. (1986). Confirmation of  
47  
48 DOM-1, a de-epoxidation metabolite of deoxynivalenol in biological fluids of  
49  
50 lactating cows. *Agric. Biol. Chem.* 50: 227–229.  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60