Biomonitoring of Fusarium spp. Mycotoxins: Perspectives for an Individual Exposure Assessment Tool

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Fusarium species are probably the most prevalent toxin−producing fungi of the northern temperate regions and are commonly found on cereals grown in the temperate regions of America, Europe and Asia. Among the toxins formed by Fusarium we find trichothecenes of the A−type or B−type, zearalenone, fumonisins or nivalenol. The current exposure assessment consists of the qualitative and/or quantitative evaluation based on the knowledge of the mycotoxin occurrence in the food and the dietary habits of the population. This process permits quantifying the mycotoxin dietary intake through deterministic or probabilistic methods. Although these methods are suitable to assess the exposure of populations to contaminants and to identify risk groups, they are not recommended to evaluate the individual exposition, due to a low accuracy and sensitivity. On the contrary, the use of biochemical indicators has been proposed as a suitable method to assess individual exposure to contaminants. In this work, several techniques to biomonitor the exposure to fumonisins, deoxynivalenol, zearalenone or T−2 toxin have been reviewed.

Key Words: biomarkers, Fusarium, trichothecene, zearalenone, fumonisin

INTRODUCTION

Fusarium species are probably the most prevalent toxin−producing fungi of the northern temperate regions and are commonly found on cereals grown in the temperate regions of America, Europe and Asia (Creppy, 2002). Among the toxins formed are trichothecenes of the A−type, such as T−2 toxin (T−2), HT−2 toxin (HT−2), T−2 triol, T−2 tetraol, neosolaniol (NEO), di− and 15−monoacetoxyscirpenol (DAS, MAS), scirpentriol (SCIRP); trichothecenes of the B−type such as deoxynivalenol (DON), 3− and 15−acetylDON (3− and 15−ADON), nivalenol (NIV), fusarenon−X (FUS−X) as well as zearalenone (ZEA) and fumonisins (FB) (Gelderblom et al., 1988; De Nijs et al., 1996; Glenn 2007).

Fumonisins

Fumonisins (FB) are a group of mycotoxins mainly produced by F. verticillioides and F. proliferatum, which usually contaminate corn. Among them, the most important are fumonisin B_1 (FB_1) and B_2 (FB_2) (Nelson et al., 1992). FB occur mainly in maize and maize−based foods, therefore populations with high maize consumption can be exposed to significant amounts of these mycotoxins through the ingestion of fumonisin contaminated maize (Marasas, 1996; Shephard et al., 1996; Visconti et al., 1996; WHO, 2001).

Toxicity of FB has been widely reviewed by Soriano et al. (2005), Voss et al. (2007), Stockmann−Juvala and Savolainen (2008) and Wan Norhasima et al. (2009). Human exposure to fumonisin contaminated commodities has been linked to esophageal and liver cancer in South Africa and China (Sydenham et al., 1991; Yoshizawa et al., 1994). Acute and chronic toxicity of FB has been largely demonstrated in several animal species, including carcinogenicity and cardiovascular toxic effects (Gelderblom et al., 1988, 1991). FB_1 is a cancer promoter but a poor cancer initiator. It is not genotoxic because FB_1 does not induce unscheduled DNA synthesis in primary rat hepatocytes (Norred et al., 1992). Based on toxicological evidence, the International Agency for Research on Cancer (IARC) has classified FB_1 as possibly carcinogenic (group 2B) to humans (IARC, 1993). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated FB and allocated a provisional maximum tolerable daily intake (PMTDI) of 2 mg/kg body weight/day for FB on the basis of the nonobserved effect level (NOEL) of 0.2 mg/kg body weight/day and a safety factor of 100 (WHO, 2001). This PMTDI for FB has recently been confirmed by the Scientific Committee on Food (ECSCF; European Commission, 2003).

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Zearalenone

Zearalenone (ZEA) is a nonsteroidal mycotoxin produced by Fusarium spp. that is found commonly in a number of cereal crops, such as maize, barley, oats, wheat, rice and sorghum, being most frequently encountered on corn (Kuiper−Goodman et al., 1987; Tanaka et al., 1988; Krska et al., 2003). ZEA and some of its metabolites have shown to competitively bind to oestrogen receptors. Thus, the toxicity is associated with reproductive problems in specific animals and possibly in humans (Wood, 1992). Fertility problems have been observed in animals such as swine and sheep (Krska et al., 2003). ZEA may be an important etiologic agent of intoxication in infants or fetuses exposed to this mycotoxin, with results in premature thelarche, pubarche and breast enlargement (CAST, 2003). Risk assessment of ZEA by the EC SCF concluded on a PTDI of 0.2 mg/kg body weight whereas the tolerable daily intake (TDI) established by JECFA was 0.5 mg/kg body weight (EFSA, 2004).

Type−A Trichothecenes: T−2 and HT−2 Toxins

T−2 toxin and HT−2 toxin are type−A trichothecene mycotoxins produced by different Fusarium species, that may contaminate a variety of cereal grains, especially in cold−climate regions or during wet storage conditions (Bottalico, 1998; WHO, 2001; SCOOP, 2003). They have been widely studied in animals, but despite their toxic effects, the toxicology has never been assessed in humans. T−2 is a potent inhibitor of protein synthesis and mitochondrial function both in vivo and in vitro and shows immunosuppressive and cytotoxic effects. Moreover, it has been reported that the toxin has extremely toxic effects on skin and mucous membranes (Visconti et al., 1991; Visconti, 2001; Sudakin, 2003; Eriksen and Pettersson, 2004). In poultry, T−2 toxin has been reported to inhibit DNA, RNA and protein synthesis in eukaryotic cells, to affect the cell cycle and to induce apoptosis both in vivo and in vitro (Sokolovic´ et al., 2008). It has been shown that through deacetylation of T−2, HT−2 is obtained as major metabolite; however, little information is available regarding toxicity of HT−2 alone (Visconti, 2001; Sudakin, 2003). JECFA, after assessing the toxic effect of both mycotoxins, has concluded that the toxic effects of T−2 and HT−2 could not be differentiated. Thus, the PMTDI for these toxins, combined or separately, was set at 0.06 mg/kg body weight/day (FAO, 2001).

Type−B Trichothecenes: Deoxynivalenol (vomitoxin)

The mycotoxin deoxynivalenol (DON) is a type−B trichothecene, produced by molds of Fusarium genus, mainly F. graminearum or F. culmorum (Greenhalgh et al., 1986) when grown on various cereals crops (wheat, maize, barley, oat and rye). Although DON is not as toxic as other trichothecenes such as T−2 toxin, HT−2 toxin or fusarenon−X, this mycotoxin is one of the most common contaminants of cereals worldwide (Jelinek et al., 1989; Scott, 1989; IARC, 1993). Upon ingestion it can cause severe toxicosis in humans and farm animals. Acute effects of food poisoning in humans are abdominal pain, dizziness, headache, throat irritation, nausea, vomiting, diarrhoea and blood in the stool (Rotter et al., 1996). The TDI of 1 mg/kg body weight based on a reduction of body weight gain (Iverson et al., 1995) was established by the EC SCF (SCF, 2002).

BIOLOGICAL MARKERS AS AN EXPOSURE ASSESSMENT TOOL FOR MYCOTOXINS

Exposure assessment is the qualitative and/or quantitative evaluation of the likely intake of chemical agents via food as well as exposure from other sources if relevant (WHO, 1997). Thereby, through knowledge of the mycotoxin occurrence in the food and dietary habits of the population, we can quantify the mycotoxin dietary intake. To assess food consumption, four different types of data can be used: food supply data, data from household consumption surveys, data from dietary surveys among individuals and the collection of duplicate diets (Hulshof and Lo¨wik, 1998). The current exposure assessment schemes are largely deterministic and uncertainty and/or variability issues are accounted for by means of cautionary measures which are implicitly embedded in calculation schemes and rules (Verdonck et al., 2005). More recently, probabilistic methods as Monte Carlo simulations have been developed to quantify the sources of uncertainty and variability of human exposure (Verdonck et al., 2006).

Although these methods are suitable to assess the exposure of populations to contaminants and to identify risk groups, they are not recommended to evaluate the individual exposition due to a low accuracy and sensitivity. The use of biochemical indicators has been proposed as a suitable method to assess individual exposure to contaminants. The WHO defined in 1993, a biomarker as ‘any parameter that can be used to measure an interaction between a biological system and an environmental agent, which may be chemical, physical or biological’. This method allows effective exposure considering variability among food contamination levels, cooking effect, individual consumption, variations in toxicokinetics or toxicodynamics (WHO, 1993; Paustenbach and Galbraith, 2006). Among the potential valuable application of biomarkers in epidemiologic studies and in clinical trials, there is the possibility of measuring them earlier than the observed true endpoint

of interest, given their property of relating the effect of exposures or treatments on cellular and molecular changes to the true endpoint/outcome (Merlo et al., 2006).

Interpretation of biomarkers of effect is hampered by lack of knowledge on the metabolism of most non-nutrients and their mechanisms of action in humans in vivo. Before a biochemical indicator can be used as a measure of dietary intake, it must be evaluated with respect to its sensitivity to the intake of those contaminants. If these indicators are to be used as measures of dietary exposure, however, the epidemiologist is obviously responsible for ensuring that the exposure measure is a valid representation of long-term intake. Several strategies are available to define the relationships between long-term dietary intake and biological levels: (i) animal studies; (ii) geographic correlation of intake and biological marker; (iii) correlation with individual intake; (iv) dietary manipulation in humans and (v) repeated measures (Walter, 1998).

Regarding biomonitoring of mycotoxin intake, successful results have been reported about biomarkers of ochratoxin A and aflatoxins. Higher levels of ochratoxin A have been found in blood samples from people with kidney or urinary disorders than in healthy people, showing good correlation among dietary intake and blood levels of this toxin (Scott, 2005). The use of serum aflatoxin B_1-albumin adducts as biomarkers of aflatoxin exposure has been validated in experimental and human sample analyses (Wild et al., 1990a, b, 1992). The use of urinary aflatoxin B_1-N^7-guanine adduct validated in the laboratory with human samples, provides a measure of acute exposure to aflatoxin B_1 (AFB_1) and reflects a relatively short-term (24..48 h) exposure (Groopman et al., 1992a, b, 1993).

Another problem to conduct an accurate exposure assessment is the presence of conjugated forms of mycotoxins, known as 'masked' mycotoxins. More important 'masked' mycotoxins have been reported to be produced by Fusarium species. For example, more common mycotoxin conjugation products in mammals are glucuronides, as found in ZEA-4-glucoside and DON-3-glucoside. These conjugated metabolites are usually stable under extraction conditions, maintaining the capability to produce toxic effects (Berthiller et al., 2009). Unfortunately they cannot be detected through routine analysis making necessary alternative methods.

Biomonitoring Exposure to Fumonisins

Methods to biomonitor the exposure to FB have been reviewed previously in several cases (Turner et al., 1999; Shephard et al., 2007). Mainly, two analytical procedures have been reported as fumonisin biomarker: fumonisin B_1 and sphingoid bases ratios.

Fumonisin B_1 as Biomarker

Absorption, distribution and excretion of FB_1 have been widely studied in several animal species including rats, laying hens, vervet monkeys, swine or piglets (Prelusky et al., 1994; Shephard et al., 1994a, b; Fodor et al., 2006, 2008) and FB_2 toxicokinetics has been studied in rats and vervet monkeys (Shephard et al., 1995; Shephard and Snijman, 1999). These toxicokinetics studies have shown that FB_1 had low oral bioavailability, with values ranging from 3% to 6% (Prelusky et al., 1994; Fodor et al., 2008) and short half-life when dosed intraperitoneally or intravenously. Half-life in rats has been reported at 18 min and 40 min in vervet monkeys and estimated by regression analysis in 70 kg human as 128 min giving an animal's weight to fit the prediction (Shephard et al., 1992; Delongchamp and Young, 2001).

As FB_1 is mainly excreted in feces, HPLC with fluorescence detection method was initially developed as suitable tool to exposure assessment to FB. Determination of FB_1 in faeces was applied on exposure assessment of rural and urban populations from South Africa with mean fumonisin levels in maize for consumption of 2.2 and 0.3 mg/kg, respectively. Results showed significant differences among FB_1 concentrations in rural and urban feces ($p = 0.014$). Considering fecal samples were taken 24 h after maize consumption, FB_1 could be expected to be a suitable short-term biomarker of this toxin exposure (Chelule et al., 2000).

Moreover, FB have been detected in human hair using LC-MS-MS analytical method. Hair samples were obtained from South African population highly exposed to fumonisin with probable daily intake for 70 kg individuals of 13.8 mg/kg body weight per day. Results showed that mean values of FB_1 ranged from 33.0 to 22.2 mg/kg hair, with maximum values of 93.5 mg/kg hair, concluding that human hair analysis could be an useful tool to measure the cumulative exposure to FB (Sewram et al., 2003).

Urinary FB_1 have been reported recently as a sufficiently sensitive tool to assess the human exposition to FB. A liquid chromatograph-mass spectrometry method and extraction on Oasis MAZ cartridges was performed to determine urinary FB_1. Urinary FB_1 was correlated with maize intake ($p = 0.001$) and the correlation remained significant after adjusting for age, education and place of residence (Gong et al., 2008).

Sphingoid Base Levels and Ratios in Plasma

Due to rapid elimination and low bioavailability of FB, an indirect indicator of human exposition to these toxins has been required. FB have a remarkable structural similarity to sphingolipids (Merrill et al., 1996; Riley et al., 2001). This group of mycotoxins, especially FB_1 potently inhibits the enzyme ceramide (CER) synthase, which catalyzes the acylation of sphinganine

and reacylation of sphingosine. The inhibition of CER synthase by FB increases the intracellular sphinganine concentration, process described as the main contributor to the toxicity and carcinogenicity of FB_1 (Wang et al., 1991; Merrill et al., 1993; Yoo et al., 1996; Riley et al., 2001). Based on this biological perturbation, particularly elevation of sphinganine (Sa) to sphingosine (So) or Sa 1−phosphate to So 1−phosphate ratios in tissues, urine and blood, have been proposed as potential biomarkers of fumonisin exposure in various animal species (Wang et al., 1992; Riley et al., 1993; Morgan et al., 1997; Wang et al., 1999; Van der Westhuizen et al., 2001; Kim et al., 2006; Tran et al., 2006; Cai et al., 2007). This biomarker was validated initially in Wistar rats by Solfrizzo et al. (1997) and recently in F344 rats by Cai et al. (2007), obtaining more sensitive results in urine than in serum for acute and sub−chronic exposure to FB_1. Furthermore, several studies have been conducted to assess the effectiveness of this biomarker on human population without successful results to obtain an accurate validation due to the low sensitivity when applied to individuals (Van der Westhuizen et al., 1999, 2008; Abnet et al., 2001; Qiu and Liu 2001; Solfrizzo et al., 2004; Missmer et al., 2006; Nikiema et al., 2008).

Van der Westhuizen et al. (1999) initially conducted a study to assess Sa : So ratio in human plasma and urine from three different populations from Africa (Centane, n ¼ 154; Bomet, n ¼ 29 and KwaZulu−Natal, n ¼ 27) with mean fumonisin intake of 3.8, 0.06 mg/kg body weight/day and nondetected levels, respectively. Despite these differences among exposures, nonsignificant differences in Sa : So ratios were found, showing mean levels of serum ratios of 0.34, 0.43 and 0.28 in Centane, Bomet and KwaZulu−Natal population and urinary ratios of 0.41 and 0.38 in Centane and Bomet.

More recently, they conducted a cross−sectional study in two areas from the same region of South Africa (Bizana, n ¼ 150 and Centane, n ¼ 152), concluding that although significant and contrasting differences in plasma and urinary sphingoid base levels in the areas were observed, there was no significant difference in the mean total fumonisin levels in the maize consumed, mean plasmatic ratio and urinary ratio from Bizana population (Van der Westhuizen et al., 2008).

Croatia is a country located in the region affected by endemic nephropathy (EN), chronic renal disease geographically restricted to several European Eastern countries. Ribar et al. (2001) conducted a study to determine the possible modifications in the concentrations of urinary and serum Sa, So and Sa : So ratio of healthy subjects and EN patients from EN endemic area in Croatia. Eighty−nine serum samples and 30 urine samples were obtained from men and women affected (n ¼ 1), suspected (n ¼ 7) or at risk (n ¼ 12) to EN as well as healthy (n ¼ 27) and control from nonendemic area (n ¼ 20). Sphingolipids were extracted from serum and urine according to the method of Riley et al. (1994). Results showed nonstatistically significant difference in the serum Sa : So ratio in either men or women from the endemic area as compared with the control group of subjects. While urinary Sa : So ratio was found to differ significantly in the male group of healthy, suspected and affected people, among women it differs significantly in subjects at risk and suspected to EN. The authors did not report conclusions but suggested that study subjects could be presumed to have been exposed to FB and sphingolipid metabolism impairment could be postulated as an early indicator of EN (Ribar et al., 2001).

Mexico is one of the most important countries regarding maize consumption. Human consumption is approximately 300 g/day providing 56% of the calories. Population of 38 Mexican volunteers (categorized with different maize based food consumption level: high, medium and low) participated in a trial to determine urinary Sa : So ratio. Urine samples were collected at three stages: A) at the beginning of the experiment with normal diet, B) after two weeks without consumption of any type of maize based food and C) one week after the re−assumption of normal maize based food consumption. Urine samples were analyzed according to methodology described by Solfrizzo et al. (1997). Results showed that there were no significant differences among the groups in the estimated mean fumonisin intake and the Sa : So ratio. Sa : So ratio was significantly higher during exposed stage A and C, with respective mean fumonisin intake of 6 and 5.1 mg/kg bw/day than Sa : So ratio obtained during nonexposed period B (Landeros et al., 2005).

Other study was performed in China with 15 females and 13 males exposed to FB_1 in corn diets over 1 month to analyze So and Sa in human urine and monitor the Sa : So ratio. The estimated daily FB_1 intake was ranged between 0.4 and 457 mg/kg body weight/day in females and between 0.5 and 740 mg/kg body weight/day in males. Urinary Sa : So ratio did not change over the month in females (0.2 initially and 0.18 at the end), while mean urinary ratio increased from 0.11 to 0.21 in males. However, it could be ascribed to a single participant with a high value, as they reported (Qiu and Liu, 2001).

Solfrizzo et al. (2004) assessed urinary sphingoid bases of population from northern Argentina (n ¼ 74) and southern Brazil (n ¼ 116) as exposed population with mean fumonisin intake of 0.56 g mg/kg body weight/day and urinary sphingoid bases of population from southern Italy (n ¼ 66) and central Argentina (n ¼ 20) with low or no fumonisin exposure (control group). Mean Sa : So ratio in regions with exposure to FB was 1.24, significantly higher than regions without exposure, where the Sa : So ratio was 0.36. However, mean Sa : So ratio from northern Argentina was 0.69, not significantly different from the control population and significantly lower than the value 1.57 showed in the southern

in southern Brazil cannot be associated to fumonisin exposure, existing with other confounding factors.

Moreover, the ratios have been assessed in simultaneous matrices as buccal cells, urine and serum in population from Burkina Faso, without showing any association between urinary Sa : So ratios and fumonisin intake, but suggesting a positive trend between fumonisin intake and Sa : So ratios in serum (Nikiema et al., 2008).

Latest study was performed to assess Sa : So ratio and frequency of detection in urine samples from urban and rural population from Portugal. A total of 68 urine samples were collected from male and female adult healthy volunteers from urban (n ¼ 38) and rural (n ¼ 30) zone. Optimized extraction method, based on the procedures described by Castegnaro et al. (1996, 1998) and Qiu and Liu (2001), followed by derivatization with naphtalene-2,3-dicarboxyaldehyde (NDA) and injection to HPLC-FD system, was carried out to detect and quantify urinary Sa and So. Sa : So ratio was between 0.11 and 0.95, with a mean value of 0.43±0.22. Significant differences were not found when the results of Sa, So and Sa : So ratio of males, females as well as combined (males and females together) were compared between rural and urban population (Silva et al., 2009).

In our latest study (unpublished data), performed with two exposure groups from the same region (exposed and nonexposed group from Catalonia, Spain), significant differences were observed among mean plasma Sa : So ratios. Results showed significant differences in sphingosine levels in groups considering both sexes combined or among males ($p < 0.05$), while no significant differences were observed in females between groups ($p > 0.05$). Thus these results suggest that the decrease of the ratios could be due to a decrease of sphingosine level, as should be expected according to the mechanism of action. However, wide ranges of Sa : So ratios and bad correlation coefficients were observed when linear regression was fitted, which suggests that this biomarker is low sensitive and imprecise to apply over individuals.

Further studies are required to better understand all physiological factors that lead to Sa : So ratios variations as reported by Abnet et al. (2001) as well as biochemical processes that can modify sphingoid metabolism as extensive cell death, metabolization by other bioactive molecules or alteration by other components of the diet (Merrill et al., 2001). Other main problem is the lack of information about sphingoid bases basal levels in tissues, urine and blood of healthy human population.

Biomonitoring Exposure to DON

Absorption, distribution, accumulation, metabolism and elimination of DON have been reported in a wide range of animal species. Toxicodynamic studies have shown low absorption in poultry (<1%), in sheep ranged 6..10% and at least 29% was absorbed by dairy cows when DON toxin was administered; on the other hand, high absorption has been estimated in swine (47..82%). Plasma elimination of DON tended to be slower in pigs, taking approximately 7 times longer than sheep, 2 times longer than cow and slightly longer than laying hens to clear the toxin after a single oral dose. Numerous studies have reported that swine is very sensitive to DON in contaminated feedstuffs. The distribution characteristics of DON in swine are also different than in other species. Only a small proportion of the dose can be found in the blood, although the toxin is extensively absorbed (Yoshizawa et al., 1981; Prelusky et al., 1985, 1986, 1988, 1994; Friend et al., 1986).

DON and DON-glucuronide excretion in the urine represented 37% and 50% of the ingested DON in rats (Meky et al., 2003) and swine respectively (Goyarts and Danicke, 2006). Goyarts and Danicke (2006) have confirmed that not all animals are able to detoxify DON to the metabolite de-epoxy-DON and that this metabolism occurs principally in the large intestine, where unlikely absorption proceeds. Furthermore, it was shown that quantitative urinary recovery of DON can be considered as an indicator for its systemic absorption, as it approximates the bioavailability as estimated by the kinetic study. Assuming a high comparability of digestion and excretion in humans and swine, they concluded that although DON is poorly detoxified, it is rapidly excreted and so is not found in remarkable concentrations in serum after 24 h.

Regarding human population, an earlier study was performed to develop, to validate and to measure urinary concentrations of DON and its metabolites in 15 females from Henan (Linxian) region, where the staple diet was based on corn and wheat (high-risk region of esophageal cancer, n ¼ 11), or rice (low-risk region of esophageal cancer, n ¼ 4). The mean levels of DON detected in the samples from high-risk and low-risk areas were 37 ng/mL and 12 ng/mL, respectively. Through these values and specific assumptions regarding excretion, urine production and recoveries, the authors estimated a daily exposure ranged from 1.9 to 13.0 and 0.6..2.5 mg/kg/day for high- and low-risk population respectively, in the line of previous studies that have been estimated this exposure through classic methods (Meky et al., 2003).

Urinary DON was widely surveyed in a large-scale study conducted in UK and compared with cereal-based food intake (Turner et al., 2008a). Three population groups were selected according to low, medium or high cereal intake, estimated previously through 7-day weighted food diary. From each group, 100 individuals were selected and urinary samples were collected during the period on the basis of available data in the 7-day diary that was provided. DON was detected in 296 of 300 (98.7%) urine sample, with geometric mean of 9.42 mg DON/day (nd-65.97 mg/day). Cereal intake was

significantly associated with urinary DON ($p < 0.0005$), showing mean levels of 6.55, 9.63 and 13.24 mg/day in low, medium and high cereal intake groups, respectively. The food groups associated with urinary DON were predominantly wheat based, particularly the three main bread groups (white, wholemeal and other bread). A crude estimation was made based on: (i) the amount of urinary DON, (ii) an assumption that 50% of the ingested DON was being excreted in the urine (Meky et al., 2003; Goyarts and Danicke, 2006) and (iii) the urinary DON originated from DON intake in the previous 24 h. For the 300 individuals the mean intake was estimated as 319 ng/kg body weight/day, below TDI for DON ingestion of 1 mg/kg body weight (SCF, 2002) and slightly higher than previous estimation that showed DON daily intakes of 176 and 142 ng/kg body weight/day for males and females respectively (SCOOP, 2003). Briefly, more detailed analysis of these data will be published. In this report, food diary information ($n ¼ 255$) for (a) the day of urine collection, (b) the previous 24-h period and (c) the day of urine collection plus the previous 24 h combined, were further examined to assess whether the recent intake of cereal correlated more strongly with urinary DON, compared with (d) the longer term assessment of usual cereal intake from 7-day food diaries. Results suggest that the inter-individual variation in urinary DON was somewhat better explained by recent cereal intake compared with usual cereal intake assessed over 7 day (Turner et al., 2009).

An intervention study was conducted to assess the effect of wheat-restricted diet over DON urinary levels. The study was performed with 25 healthy adult volunteers and involved 2 days of normal diet and 4 days of a wheat-restricted diet. Food diaries were kept for normal diet days and for the two latest days of intervention diet. Initial morning urinary samples were collected the following day of each period. Samples were analyzed and adjusted using a creatinine concentration in mg/mL of urine and subsequent data were expressed as ng DON/mg creatinine. Results showed that during intervention diet period there was a low percentage of detected samples (36%) while during normal diet all samples had detectable levels of DON. Mean levels and ranges were 1.0 (nd-8.4) and 10.8 (0.7..61.3) ng/mL for intervention and normal diet respectively .. results in the line of previous study conducted in UK (Turner et al., 2008b).

Recently, another intervention trial conducted with 22 urine samples from UK volunteers to correlate urinary DON level with one or more metabolite in the urine was published. A ^{1}H-Nuclear Magnetic Resonance-based chemometrics approach (metabolomics) was utilized to examine samples from individuals eating a normal diet. Urinary DON was determined using an in-house immunoaffinity-LC-MS assay (Turner et al., 2008b). Model was built on 16 individuals, eight with low urinary DON and eight with high urinary DON level; and validated with a further six urine samples of which there were three in each category of DON level. Through statistical analysis two possible biomarkers were identified: hippurate and mannitol, the first one being the more interesting candidate (Hopton et al., 2010).

Turner et al. (2008c) emphasized urinary DON as a good tool to assess exposure to this contaminant at the individual level. In contrast, they reported several uncertainties to resolve the full validating of this biomarker to apply in epidemiological studies. Their questions were: (a) what is the relationship at the individual level between DON intake and the urinary biomarker?; (b) what are the pharmacokinetics of DON and DON-glucuronide excretion and what are the consequent temporal variations in this biomarker?; (c) Does the ratio of DON to DON-glucuronide in urine vary by individual? Therefore, they concluded that in humans, such studies require validated methods of exposure assessment to compare exposure to toxins both individually and in combination with health outcomes (Turner et al., 2008c).

Biomonitoring the Exposure to ZEA

Absorption of ZEA has been reported as extensive and quick in rats and rabbits (Kuiper-Goodman et al., 1987; Ramos et al., 1996), being estimated in the range of 80-85% in pigs (Biehl et al., 1993). In mammals, ZEA is mainly metabolized into a-zearalenol (a-ZEA) and b-zearalenol (b-ZEA), while the first is the most predominant in pigs, the second is the most predominant metabolite in cows (Jodlbauer et al., 2000; Kleinova et al., 2002; Zo¨llner et al., 2002). Earlier studies of Ueno et al. (1983) showed that there are two types of ZEA reductase differing in optimum pH. In humans as in pigs, ZEA probably can be absorbed after oral administration and can be metabolized in intestinal cells into a-ZEA and b-ZEA and would be excreted significantly in bile and urine (Do¨ll et al., 2003).

In a previous study conducted with one male volunteer, 100 mg of ZEA were administered and a-ZEA and b-ZEA concentrations were determined in the urine at 6, 12 and 24 h after the administration. The respective concentrations of ZEA, a-ZEA and b-ZEA were 3.7, 3 mg/mL and not detected after 6 h; 6.9, 6 and 2.7 mg/mL after 12 h; and 2.7, 4 and 2 mg/mL after 24 h (Mirocha et al., 1981).

Furthermore, ZEA and its metabolites were studied in serum from 32 girls affected by central precocious puberty (CPP) and in 31 healthy female. Results showed increased serum levels of ZEA and a-ZEA in 6 girls with CPP. ZEA levels correlated with patient height and weight. The authors concluded that ZEA is suspected to be a triggering factor for CPP development in girls and may also represent a growth promoter in exposed patients (Massart et al., 2008).

ZEA dietary intake was estimated by JECFA to European region, reporting ranges of 0.004..0.029 and 0.006..0.055 mg/kg body weight per day for adults and

infants respectively (CAST, 2003). Despite the high consumption of cereals in European countries, few studies have been conducted to assess the exposure to this mycotoxin, neither through conventional method nor biomarkers. Thus more studies are required to accurately characterize the risk of this endocrine disruptor and confirm it as a dangerous problem for human health (Minervini et al., 2005).

Biomonitoring the Exposure to T-2 and HT-2 Toxins

T-2 toxin is more rapidly absorbed than DON after its ingestion by most species, its plasmatic half-life being less than 20 min. The fraction of T-2 toxin eliminated as parent compound in the urine was showed as negligible. In spite of administration of a lethal oral dose in swine (2.4 mg/kg) and toxic oral doses (up to 3.6 mg/kg) in calves, no parent T-2 toxin was detected in plasma or urine (Beasley et al., 1986; Larsen et al., 2004). T-2 toxin can be detected in pig blood before 30 min after their ingestion (Eriksen et al., 2004). The main reactions in trichothecene metabolism are hydrolysis, hydroxylation and deep oxidation. Typical metabolites of T-2 toxin in an organism are HT-2 toxin, T-2-triol, T-2-tetraol, 3-pm-hydroxy-T-2 and 3^0-hydroxy-HT-2 toxin. There are significant differences in the metabolic pathways of T-2 toxin between ruminants and nonruminants. Ruminants are more resistant to the adverse effects of T-2 toxin due to microbial degradation within rumen microorganisms (Dohnal et al., 2008). The patterns of distribution and excretion suggest that T-2 toxin and/or its metabolites are excreted into the intestine through the bile and that the liver is a major organ for excretion of the toxin (Chi et al., 1978). No studies have been conducted with humans until now to assess the presence of this toxin or its metabolites in biological fluids.

CONCLUSIONS

Conventional methods of exposure assessment are based on the combination of food analysis data with dietary intake data. That combination of data can be deterministic or probabilistic; however, both cases have been proven to be limited, due to low sensitivity and accuracy. A useful method to assess effective exposition of human populations to contaminants could be through the study of the effect on biological molecules or monitoring these toxins directly on biological fluids. These biological markers, known as biomarkers, allow the assessment of exposure of human populations to mycotoxins considering the variability within dietary intake, cooking effect, intestinal absorption, metabolism or distribution over individuals. Thus, the understanding of mechanism of action, toxicokinetics and toxicodynamics of the mycotoxins, is required to develop useful biomarkers.

Successful methods have been developed to biomonitor exposure to ochratoxin A and aflatoxins. However, few studies and unsuccessful results have been obtained with biomarkers of Fusarium toxins exposure. Urinary levels of FB have been reported as an effective method to assess short-term intake of this toxin. Despite the ratio Sa : So has been validated as biomarker of fumonisin exposure in animal species, unsuccessful results have been reported among human populations. Further studies are required to understand accurately basal levels of these contaminants, interactions with other contaminants or variability sources.

Regarding DON, several studies have reported data on absorption, toxicokinetics, toxicodynamics and metabolism in animals, but few studies have been conducted in human populations. Urinary level of DON has been used as biomarker to assess the exposure of human populations showing successful results, with positive correlations among estimated dietary intake of the toxin and urinary levels. Inspite of the interest of researchers in ZEA contamination of food and its toxicity in animal species, very few studies have been conducted to assess the real impact on human population. Finally, no studies have been conducted to assess possible biomarkers to assess the exposition to T-2 toxin or HT-2 toxin.

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