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Aflatoxin, fumonisin, ochratoxin, zearalenone and deoxynivalenol biomarkers in human biological fluids: A systematic literature review, 2001 - 2018

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Abstract

Human exposure to mycotoxins occurs mostly through dietary intake, although exposure through dermal and inhalation routes has also been shown. Depending on the type of mycotoxins, the applied dose and duration of exposure, a particular toxin can cause either chronic or acute illnesses such as kidney failure and cancer. Thus, understanding the biotransformation of mycotoxins and identification of reliable biomarkers in the human body is important for accurate risk assessment of mycotoxin exposure. This review provides a comprehensive overview of worldwide aflatoxins, fumonisins, ochratoxin, zearalenone and deoxynivalenol mycotoxin biomonitoring studies reported in the last 18 years. The studies performed in Africa, Europe, Asia and America are based on the measurement of a limited number of mycotoxin biomarkers and do not provide a comprehensive risk assessment of the mycotoxin exposure. Although the findings represent a small segment of a much larger health risk of mycotoxins exposure, it is acknowledged that a multianalyte approach covering bioconjugated and other metabolites of most often occurring mycotoxins would better reflect the extent of the global exposure problems with these highly toxic compounds.

Keywords: Mycotoxin biomarkers; Aflatoxin; Ochratoxin; Fumonisins; zearalenone; deoxynivalenol

1. Introduction

Mycotoxins, a secondary metabolites of filamentous fungi, found in diverse agricultural crops worldwide, are posing a severe threat to human health. The most toxic fungal genera found in contaminated food are *Aspergillus*, *Fusarium*, *Penicillium* and *Alternaria* (Pitt and Hocking 2009). The fungal contamination may happen during any stage of culturing, harvesting or storage (Marin et al. 2013; Williams et al. 2004). The main factors affecting mycotoxin contamination in human and animal food can be biological or ecological (Bhat and Reddy 2017). Mycotoxin contamination of food is more common in developing countries where poor food quality control, warm climate, poor production technologies and bad crop storage conditions are suitable for fungal growth and toxin formation (Peraica et al. 1999). Food contamination by mycotoxins is considered inevitable and has raised global concerns as mycotoxins cannot be easily destroyed by temperature or by any chemical or physical treatment (Marin et al. 2013). Although, great deal of effort has been devoted to avoid mycotoxin exposure, still human or animal consumption of food contaminated with mycotoxins is a major food safety problem worldwide.

Ingestion of contaminated food is the major route of mycotoxin exposure, while dermal and inhalation routes of mycotoxin exposure are not that common (Zain 2011). Vomiting, nausea, diarrhea, fatigue, hemorrhage, abdominal pain, damage to hematopoietic tissues, skin inflammation and blistering are some of the symptoms of mycotoxicoses. The impact of mycotoxins on human health depends on the toxin type, its conjugation forms and concentration, period of exposure, pharmacokinetics and accumulation of the mycotoxins, age, gender as well as the immune system and health state of the exposed person (Bennett and Klich 2003; Jonathan et al. 2004; Zain 2011). Acute or chronic exposure to mycotoxins might have carcinogenic, nephrotoxic, tremorogenic, immunotoxic, hemorrhagic, teratogenic and dermatological consequences in humans (Bhat and Reddy 2017). Thus, it is of outmost importance to identify the relevant mycotoxin exposure biomarkers in order to detect geographical areas and subpopulation with health impairing, high exposures. Parent mycotoxins, major phase I or phase II metabolites, protein adducts or DNA adducts are the usual biomarkers measured in biological fluids. The selection of biomarkers to be analyzed in certain biological fluid is crucial. The absorption of some mycotoxins, such are aflatoxins and zearalenone, is rather quick after oral ingestion reaching peak concentration in blood within few hours (Devreese 2012). However, their clearance is also rapid unless they have formed adducts with macromolecules. Mycotoxin adducts with macromolecules have longer half-life in blood and can provide crucial information on the cumulative effects of mycotoxins. Another preferred biological fluid used to

analyze mycotoxin biomarkers is urine, which might contain mycotoxin metabolites or the parent compound. The normalization with creatinine or specific gravity is recommended for urine samples (Sauvé 2015) while normalization with albumin for the plasma/serum protein adducts. Also understanding the long-term and short-term biomarkers of exposure is crucial, as well as their sensitivity and dose-response relationship in expression of biomarkers. The mycotoxin metabolism in relation to biomarkers of exposure has been recently reviewed by Vidal and colleagues in a comprehensive review (Vidal 2018).

Beside biomarker studies, the research should also focus to better assess health impacts of mycotoxins and their role in disease onset and development, to study the human, animal, animal and human microbiome and host plant mycotoxin metabolism and determine the efficacy of intervention strategies. The focus of this review is on the metabolism of mycotoxins, on the currently known biomarkers detected in biological fluids and so far reported exposure studies of major mycotoxins with human health concerns: aflatoxins, ochratoxin, fumonisins, zearalenone and deoxynivalenol (Sherif et al. 2009).

2. Metabolism and biomarkers of mycotoxin exposure in humans

2.1. Aflatoxin B1

Aflatoxins are produced by *Aspergillus* species and the main species responsible for aflatoxin production are *A. flavus*, *A. parasiticus* and *A. nomius* (Richard 2007). There are four main types of aflatoxins: aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1) and G2 (AFG2). AFB1 is considered the most toxic aflatoxin and the most potent carcinogenic substance, thus classified as Group 1 human carcinogen by the International Agency of Research on Cancer (IARC 2002). AFG1, AFB2 and AFG2 are less carcinogenic and less mutagenic than AFB1 due to the lack of the presence of double bond in position 8,9 (S. Bbosa et al. 2013; Wild and Turner 2002).

Aflatoxin metabolism differs between children and adults (Dohnal et al. 2014) and the aflatoxin pharmacokinetics is still not completely explored (Dohnal et al. 2014). Liver is the dominant site of aflatoxin metabolism, where aflatoxins are converted to 8,9-epoxide form during the phase I metabolism by primary cytochrome P450 (CYP) enzymes, such as CYP3A4, CYP3A5, CYP3A7 and CYP1A2 (Palacios et al. 2017; Wild and Turner 2002). CYP3A4, CYP1A2 and CYP3A7 in the liver oxidize AFB1 to form AFB1-8,9-exoepoxide and AFB1-endo-epoxide. AFB1-8,9-exoepoxide can bind to DNA forming predominantly 8,9-dihydro-8(N7-guanyl)-9-hydroxy-AFB1 (AFB1–N7-Gua) adduct which is suggested to be responsible for the mutagenic properties of AFB1. However, a positive charge on the imidazole ring of AFB1-N7-Gua makes it

unstable further promoting the release of AFB1-8,9-dihydrodiol, depurination and the opening of the imidazole ring and forming of stable AFB1-formamidopyrimidine adduct (Bedard and Massey 2006; Groopman et al. 1981). The AFB1-formamidopyrimidine lesions are removed less efficiently than AFB1-N7-Gua in mammals, suggesting its role in AFB1-induced toxicity (Bedard and Massey 2006).

AFB1-endo-epoxide is less toxic as it cannot bind to nucleic acids (Dohnal et al. 2014; Palacios et al. 2017; Wild and Turner 2002). However, exo- and endo-epoxide through non-enzymatic hydrolysis can form AFB1-8,9-dihydrodiol which reacts with the ε-amino group of lysine in serum albumin and can be detected in blood (Dohnal et al. 2014; Wild and Turner 2002). Oxidation of AFB1 by CYP3A4 and CYP1A2 also yields other phase 1 metabolites such are hydroxylated AFM1, AFP1 and AFQ1 that can be detected in human urine (Dohnal et al. 2014; S. Bbosa et al. 2013), while the action of cytoplasmic reductase converts it to aflatoxicol. Furthermore, CYP2A13 was found to convert AFB1 to AFB1-8,9-epoxide and AFM1 to AFM1-8,9-epoxide in lungs and to catalyze AFB1-induced DNA damage (Dohnal et al. 2014).

The detoxification process of AFB1-8,9-epoxide mainly involves glutathione S-transferases (GST) that conjugates this metabolite with glutathione (GSH) forming AFB1-8,9-epoxide-GSH, followed by its further biotransformation to AFB1-mercapturic acid. If the GST function is impaired the AFB1-8,9-epoxide can by the action microsomal epoxide hydrolases be converted to AFB1-dihydrodiol (Kensler et al. 2003). AFB1-dihydrodiol in the basic conditions is transformed to ABF1-dialdehyde. Although aldo-keto reductases convert AFB1-dialdehyde to AFB1-dialcohol that is later conjugated to AFB1-glucuronide, protein lysine groups are susceptible to adduction with AFB1-dialdehyde that might affect protein structure and function (Guengerich et al. 2001).

In 2004, an outbreak of acute aflatoxicosis in Kenya was the largest mycotoxin poisoning incident which resulted in 317 intoxication cases with 125 deaths due to contaminated maize and maize products in local food market. Mean values of the measured levels of aflatoxin in homegrown maize were significantly higher compared to kernels sampled from control households, 354.5 μg/kg and 44.1 μg/kg respectively. This was in agreement with the AFB1-lysine serum concentration, for which the mean was 1.2 ng/mg of albumin in the case patients compared to 0.15 ng/mg of albumin of controls. From this incident, serum aflatoxin B1-lysine adduct has been identified as a biomarker for aflatoxins and used to indicate aflatoxin exposure (Azziz-Baumgartner et al. 2005; Probst et al. 2007).

In the mycotoxin exposure study in rats, AFB1, AFP1 and AFM1 were detected in urine by LC-MS/MS (Everley et al. 2007). Urinary AFB1 such as AFM1, AFP1, AFQ1, Aflatoxin glucuronide, AFB1-N7-Gua and AFB1-mercapturic acid were used as biomarkers to assess the exposure of aflatoxins in other studies (Leong et al. 2012; Schleicher et al. 2013; Shirima et al. 2013; Wild and Turner 2002). As AFM1 can be detected in human breast milk it can also serve as a biomarker of maternal and infant exposure to AFB1. Based on human and animal studies, AFB1-N7-Gua adduct in urine is considered the most reliable short term biomarker, with a half-life of 7.5 hours, for evaluating hazards and exposure to carcinogenic AFB1 (Dohnal et al. 2014; Groopman et al. 1993; Jager et al 2016; Wild and Turner 2002). Moreover, AFB1-lysine and aflatoxin-albumin adducts are considered as one of the best biomarkers for long term exposure in blood, due to the albumin half-life of 20 days (Jager et al 2016; Leong et al. 2012). In this study, amongst all the tested markers urinary AFM1 was found to be very sensitive biomarker for monitoring human exposure to food contaminated with AFB1 (Jager et al 2016).

2.2. Ochratoxin A

Ochratoxins are common contaminants of food crops, dried nuts, dried fruits and some drinks based on grapes (Bayman and Baker 2006). Ochratoxins (A, B and C, i.e. OTA, OTB and OTC) are produced by *Aspergillus* and *Penicillium* fungi species, mainly *A. ochraceus*, *A. carbonarius*, *A. niger* and *P. verrucosum (*Koszegi and Poor 2016). Among the three ochratoxins, the ochratoxin A (OTA) is mainly responsible for severe adverse effects in both humans and animals (Koszegi and Poor 2016) and is categorized by IARC as possible carcinogen to humans (group 2B) (IARC 1993).

Numerous studies describe detailed OTA metabolism for animals and some of the findings can be translated or can shed light on the metabolic processes of OTA in humans. OTA is metabolized in kidneys, intestines and liver but it also binds to serum proteins such is albumin. OTA half-life in human serum is 35 days and may accumulate in human body tissues or fluids (e.g. plasma and serum) (Koszegi and Poor 2016; Reddy and Bhoola 2010; Studer-Rohr et al. 2000; Wu et al. 2011). OTA biotransformation is triggered by the action of cytochrome P450 enzymes, such as CYP3A4, CYP3A5 and CYP2B6. The main metabolic pathway of OTA includes hydrolysis, which occurs by carboxipeptidases enzyme and results in ochratoxin-α (OTα) during cleavage of the peptide bond (Ringot et al. 2006; Wu et al. 2011). OTα, a metabolite with lower toxicity then OTA, was found in animals and humans (Wu et al. 2011). OTA may be hydroxylated by CYPs or peroxidases to form 4-

(R)-hydroxyochratoxin A (4-OH-OTA) in humans and rats, 4-(S)-hydroxyochratoxin A in pigs and 10-hydroxyochratoxin A in rabbits (Wu et al. 2011). OTC has analogous toxicity as OTA since it can be converted to OTA in the body (Wu et al. 2011). In addition, lactone opened OTA (OP-OTA) is produced through lactone hydrolysis of OTA in rats and is highly toxic. The loss of chlorine at C5 position of OTA yields OTB that is further transformed to 4-OH-OTB and ochratoxin-β (OTβ). OTα and OTβ are less toxic metabolites than the parent compound or OP-OTA metabolite. Nonetheless OTA can be deactivated through sulphate and glutathione conjugation, which will lead to their secretion (Ringot et al. 2006; Wu et al. 2011). Recent studies demonstrated that ß-glucuronidase/arylsulfatase enzymatic hydrolysis of phase II metabolites returned significantly higher values for OTα in urine samples indicating that glucuronidation, and possibly sulfation, is involved in the detoxification process of OTα (Duarte et al. 2011; Klapec et al. 2012; Munoz et al. 2017). Similarly, enzymatic hydrolysis also results in the increased concentration of OTA.

Thus currently used biomarkers of human Ochratoxin A exposure are OTA, OTα, OTβ and 4-OH-OTA. OTA can be detected in human plasma, serum and urine (Assaf et al. 2004; Karima et al. 2010). Recent study assessed the infant exposure to OTA by determining the concentration of OTA in maternal plasma, breast milk and infants' urine samples. All maternal plasma samples analyzed in the period of 2 weeks to 4 months of breastfeeding period were positive for OTA with concentrations that ranged between 0.072 -0.573 ng/ml. The average concentration that was found in breast milk and infants' urine was several times lower than OTA concentration in plasma samples with average values at 4 months of breastfeeding period 0.030 ng/mL and 0.036 ng/mL, respectively (Munoz et al. 2014). Another pilot study, conducted in Belgium, analyzed OTA and 4-OH OTA in 40 human urine samples and identified the presence of both compounds in only one sample, where the concentration of 4-OH OTA was lower than the LOQ concentration (<0.24 ng/ml) and OTA concentration was 0.6 ng/mL. Furthermore, three samples contained OTα concentrations 5.1 ng/mL, 7.0 ng/mL and 15 ng/mL (Ediage et al. 2012).

2.3. Fumonisins

Fumonisins are produced by diverse fungi species such as *Fusarium verticillioides* and *F. proliferatum* (EFSA 2005) as well as *A. niger* (Frisvad et al 2011). Today, 28 fumonisins have been isolated, which are divided into four groups, A, B, C and P (Alberts et al. 2016; Rheeder et al. 2002).

The most widespread naturally occurring fumonisins are fumonisin B analogues, which includes FB1, FB2 and FB3. Among these, the most poisonous is FB1 and has been classified as a member of group 2B human carcinogen by IARC (IARC 2002). FB2 is a deoxy analogue of FB1, is less abundant than FB1 but has important toxicological effect. FB3 and FB4 are present in lower concentrations and have lower toxicological significance. Fumonisins are similar to sphingoid bases structure and thus interfere with the sphingolipid metabolism (Stockmann-Juvala and Savolainen 2008; Yazar and Omurtag 2008). Metabolic route starts with sphinganine formation, followed by acylation to dihydroceramide and ceramide by the enzyme sphinganine N-acyltransferase (ceramide synthase) (Stockmann-Juvala and Savolainen 2008). The FB1 can inhibit ceramide synthase leading to an increase in highly toxic compounds like intracellular sphinganine and other sphingoid bases (Stockmann-Juvala and Savolainen 2008). This results in increased oxidative stress, impairment of regulation of the cell cycle, cellular differentiation, apoptosis or necrosis (Stockmann-Juvala and Savolainen 2008). These impairments can be responsible for fumonisin-induced toxicity and carcinogenicity. Indeed, studies have proved that FB1 ingestion causes and imbalanced increase in urinary sphinganine (Sa*)* and sphingosine (So) levels in mice and Sa levels in kidneys, liver, and small intestine with consequent increase in the Sa/So ratio. Furthermore, the same study demonstrated that FB1 suppresses ceramide synthase activity in rats (Stockmann-Juvala and Savolainen 2008). Based on this mechanism of action, the Sa/So ratio is the unique indicator of fumonisin exposure and can serve as sensitive biomarker for both blood and urine (EFSA 2005). Level of exposure to FB1 was also correlated with the changes in Sa 1-phosphate (1-P) and the Sa 1-P/So 1-P ratio (Riley et al. 2015). Fumonisin half-life in human serum is approximately 128 minutes and can be detected in urine and feces, possibly as a result of secretion or due to partial adsorption (Persson et al. 2012).

Biomarkers of fumonisin in humans were detected in hair, nails, blood serum, urine and stool (Shephard et al. 2007). Urinary biomarkers of fumonisins are FB1, FB2 and FB3. Many studies have suggested that the increase of Sa:So ratio in biological fluids and tissue can be used as sensitive biomarkers of fumonisin exposure (EFSA 2005; Riley et al. 2015; Voss and Riley 2013).

2.4. Zearalenone

Zearalenone (ZEN), 6-(10-Hydroxy-6-oxo-trans-1-undecenyl)-beta-resorcylic acid lactone, is produced by different species of *Fusarium*, mainly *F. graminearum* and *F. culmorum* (Bhatnagar et al. 2002) and mostly found in corn and grain crops (García-Cela et al. 2012). ZEN is a non-steroidal estrogenic mycotoxin resembling

the structure of 17-β-estradiol, whose biotransformation differs between the fungal species (Binder et al. 2017). The major and the main biologically active and reductive metabolites of ZEN in animals and humans are α-zearalenol (α-ZEL) and β-zearalenol (β-ZEL) (Pfeiffer et al. 2010). The α-ZEL is known as estrogen agonists in mammals. The α-ZEL and β-ZEL metabolites are hydroxylated by 3α- or 3 β-hydroxysteroid dehydrogenase (HSD), and conjugated with glucuronic acid by uridine diphosphate glucuronosyltransferase (UDPGT) yielding α and β-zearalenol-14-glucuronide (α and β-ZEL14GlcA) (Binder et al. 2017; Pfeiffer et al. 2010). ZEN is transformed to zearalenone-14-glucuronide (ZEN14GlcA) by UDPGT enzyme, while zearalenone-14-sulfate (ZEN-14-S) is a bioconjugated form of ZEN, which was detected in swine urine samples (Binder et al. 2017). Free ZEN and ZEN metabolites detected in humans are ZEN, α-ZEL, β-ZEL, α-ZAL β-ZAL, ZEN14GlcA. To date, zearalanone (ZAN), a derivative of zearalenone that is produced by several species of Fusarium, has not been detected in the human body (Binder et al. 2017; Mally et al. 2016; Pfeiffer et al. 2010).

2.5. Deoxynivalenol

Deoxynivalenol (DON), that belongs to trichothecene (TC) group of mycotoxins, is widely found in grains such as wheat, corn and barley while it is less present in rice, oats, sorghum and rye (Creppy 2002). *Fusarium* species, *F. graminearum* and *F. culmorum* are essential in the flora pathogens and are the main source of TC mycotoxins. Among TC mycotoxins, DON, also denoted as vomitoxin, is the most common and has been classified as a group 3 human carcinogen by IARC (Ostry et al. 2017).

The main sites of DON metabolism are liver and intestine. The major metabolic pathway involves conjugation of DON to glucuronic acid and elimination of the bioconjugated form via the urine. The two major metabolites of DON in mammals are DON Glucuronide (DON-GlcA) and de-epoxy deoxynivalenol (DOM-1) (Qing-Hua et al. 2014; Warth et al. 2016; Wu et al. 2014). However, DOM-1 is mostly generated by the intestinal microbiota of mammals, particularly in cattle and is not a major human metabolite (Wu et al. 2014). Free DON and DON-GlcA are the main biomarkers for the assessment of human exposure to DON (Wu et al. 2014). The metabolism of DON in human liver microsomes yields DON-3-Glucuronide (DON-3-GlcA), DON-15-Glucuronide (DON-15-GlcA), DON-7-Glucuronide (DON-7-GlcA) and DON-8-Glucuronide (DON-8-GlcA) (Qing-Hua et al. 2014; Warth et al. 2016; Wu et al. 2014).

Human exposure to DON is monitored by analyzing the free or bioconjugated forms of DON such as DON-GlcA, DON-15-GlcA, DON-3-O-glucoside (DON3GlcA), DON-7-GlcA, DOM-1 (Qing-Hua et al. 2014) and DON-3-GlcA (Qing-Hua et al. 2014; Warth et al. 2016; Wu et al. 2014).

3. Biomonitoring of human mycotoxin exposure

The aim of the risk assessment is to provide an overview of the potential dangerous exposure to mycotoxin. As previously mentioned, the main cause of contamination with mycotoxin, for humans and animals, is through consumption of contaminated food. In light of that, the strategy of estimation of exposure can separated in two: (i) *risk assessment of dietary exposure to mycotoxins* – uses statistical data on the food consumption of the population, average body weight of the population and concentration estimation of the contaminant in food products (ii) *risk assessment using biomarker quantification* – uses the excreted levels of the contaminant in urine or the level of the contaminant in blood and aims to estimate the intake level. Several possible sources of uncertainty can affect the estimation of the actual intake of mycotoxin and consequently can lead to erroneous risk assessment. For the first strategy, dietary exposure risk assessment, the uncertainties can occur due to inaccurate consumption recall, and often, snack foods – a potential source of contamination – are not declared. In case of the former strategy, if only a low fraction of the mycotoxin is excreted through urine then the estimation will be erroneous (IARC 2012). Furthermore, a selection of matrix is crucial, as consumption of contaminated food may have strong correlation with the biomarkers in one matrix compared to the other (Gilbert et al. 2001). The second approach of risk assessment can lead to bias when biomarker in biofluid does not correlate well with exposure. This correlation depends on multiple factors such as the type of the sampled biofluid, the applied sensitivity of the analytical method to quantify mycotoxin, the pharmacokinetic of the mycotoxin, time elapsed between exposure and bodyfluid sampling, which parameters are often not available in biomarker based exposure studies.

Molecular biomarkers of mycotoxins such as mycotoxin metabolites or mycotoxin bioconjugated forms are used to measure human exposure and were used to assess the relationship between mycotoxin exposure and development of disease (Sangare-Tigori et al. 2006; Grosso et al. 2003; Hassen et al. 2004; Zaied et al. 2011; Domijan et al. 2009; Ozcelik et al. 2001; Brewer et al. 2013).

3.1 Risk assessment of dietary exposure to mycotoxins

Human exposure to mycotoxin should be monitored with accurate analytical approaches that enable quantification of a wide range of mycotoxins. Several international organizations such as JECFA (Joint Expert Committee on Food Additives), FAO/WHO and European Commission have outlined hazardous limits for the level of mycotoxins in human food and animal feed expressed as tolerable daily intake (TDI) and tolerable weekly intake (TWI). On the other hand, there are some countries that have no monitoring limits of mycotoxin in foodstuff such as South Korea, Mexico and Pakistan. Mycotoxin exposure levels in dietary intake can be calculated via Estimated Daily/Weekly Intake (EDI, EWI) exposure levels (Adhikari et al. 2017). The national regulations in some cases may be different from the toxicity levels determined by WHO. More specifically, India has a limit of mycotoxin based on Food Safety and Standards Authority of India (FSSAI).

$$EDI(g/kgbw/day) = \sum_{i=1}^{n} \frac{Amount \times \overline{Rt}}{Body\ Weight} \quad (1)$$

Here, EDI is estimated as a daily intake in g/kg body weight/day for all food type consumed, $Amount$ signifies the quantity of one particular type of food, index i takes values between 1 and n, where n defines the number of consumed food type, $\overline{Rt}$ is the concentration of a specific mycotoxin ingested from one particular type of food.

Table 1. Tolerable daily/weekly intake of mycotoxin in human body determined by the different food safety/control authorities.

Mycotoxin	**Tolerable Daily intake (TDI)**	**Reference**
Aflatoxin	<1ng/kg of body weight	(Leblanc et al. 2005)
Fumonisins B1, B2 and B3	Maximum TDI is 2 µg/kg	(WHO 2002)
Ochratoxin A	TDI is 14 ng/kg bw Tolerable weekly intake (TWI) is 100 ng/kg of body weight	(WHO 2002)
Zearalenone	TDI is 0.25 µg/kg body weight per day	(EFSA 2014)

Deoxynivalenol	Maximum TDI is 1 µg/kg of body weight. This value was extended to its acetyl derivatives and 15-acetyl-DON by JECFA.	(WHO,SCF 2002) (JECFA 2010)

FAO/WHO and EFSA have determined hazardous quotient for the daily and weekly consumption of several mycotoxins. Table 1 includes TDI and TWI for the major mycotoxins described previously in section 2. Due to the lack of data on DON and DON metabolites absorption and toxicity the regulatory limits have not been set yet, although they are considered by EFSA (EFSA 2013).

For aflatoxins, a small amount detected, such as 1 ng/kg or even less, is considered to exceed the TDI, as this compound is categorized as class 1 carcinogen (Bennett and Klich 2003; Leblanc et al. 2005; Oyedele et al. 2017; Raad et al. 2014; Van de Perre et al. 2015). Moreover, aflatoxin amounts that are tolerable for adults can be fatal for children (Williams et al. 2004).

The risk assessment in case of AB1 is typically based on the margin of exposure (i.e. MoE). MoE is the ratio between the no-observed-effect-level (NOEL) and EDI previously described. Alternatively, MoE can be calculated using benchmark dose lower confidence limit, i.e. $BMDL_1$,$BMDL_{05}$ or $BMDL_{10}$, obtained from animal studies, divided by EDI. Here, subscript 1, 5 and 10 represent the percentage of the confidence level of the dose response curve. In other words, MoE indicates how large is a "safe level" (i.e. a level at which cancer did not occur) of a carcinogenic compound, with respect to the estimated intake of the carcinogen (EFSA 2007). The larger is the MoE, the lower is the risk. Benford et al. 2010 used the dose response curve from the study of Wogan et al. 1974 to assess the risk of dietary exposure to Aflatoxin (Benford et al. 2010; Wogan et al. 1974). The levels of MoE beyond which the risk is insignificant, are still a matter of debate. However, a widely accepted value is 10.000 (Nugraha et al. 2018).

It has been suggested by Codex Committee that the maximum concentration for AFM1 are 0.05 and 0.5 µg/kg for additives and contaminants of food respectively, since at this concentration, the risk of carcinogenic potential would be very low (WHO 2002). The assessment of the risk exposure consist of calculation of EDI or EWI (eq. 1) and comparison with TDI or TWI respectively. In analytical studies of mycotoxin exposure, EDI is calculated for recruited population and compared to TDI/TWI to determine, which mycotoxin exceed the established limit of TDI and TWI (Gerding et al. 2014).

Alternatively, Tresou and colleagues (Tressou et al. 2004) expressed the probability ($r(d)$) of exceeding a specific hazardous quotient (i.e. TDI or TWI) as follows:

$$r(d) = \frac{n(K_i \geq d)}{N} \quad (2)$$

In equation 2, d represents TDI or TWI, depending on the study; $n(K_i \geq d)$ is the number of food sources (e.g. food types) that has exceeded the toxicity limit, N is the total number of food intake sources for a person. To exemplify, if from 3 different types of food that a person consumes, 2 exceeded TDI for Ochratoxin, then the probability to have a high exposure risk to Ochratoxin is $r\left(EDI > 2000\ {}^{ng}/_{kg\ bw}\right) = {}^{2}/_{3}$.

3.2. Risk assessment using biomarker quantification

The study made by Solfrizo and co-workers estimates the so-called Probable Daily Intake (PDI) using the following expression:

$$PDI = C \cdot \frac{V}{W} \cdot \frac{100}{E} \quad (3)$$

where C, in the above equation, is the concentration of the mycotoxin biomarker in urine, V is the mean of the urine volume excreted in 24 hours, W is mean human body weight and E is the mean urinary excretion of mycotoxin in 24 hours post intake.

The main difference between EDI and PDI is that the latter is based on the measurements of the concentration of the biomarkers linked to mycotoxins and represents the value which is used to provide an approximate estimation of the level of consumption. Further, PDI is often compared with TDI to estimate the potential risk of exposure of the subject to the mycotoxins. More specifically, if PDI exceeded TDI, further enquiries must be made in order to establish the source of contamination.

The daily intake of OTA can be estimated from plasma analysis using Klassen equation as demonstrated in two recent studies (Miraglia et al. 1996; Woo and El-Nezami 2016):

$$k_0 = Cl_{renal} \cdot {}^{C_p}/_{A} \quad (4)$$

Here, k_0 is the estimated daily intake of OTA (ng/kg bw/day), C_p is the concentration of OTA in plasma (ng/mL), while A represents the bioavailability of the toxin, Cl_{renal} is the renal daily clearance (mL/kg bw/day).

Commonly used values for renal clearance are 0.67 (Hagelberg et al. 1989), which is derived from the clearance of insulin or 0.99, which is a value determined based on a single human experiment using OTA kinetics (Miraglia et al. 1996; Schlatter et al. 1996).

Numerous studies worldwide have evaluated human exposure to mycotoxins. In this review, we discuss chronic exposure to mycotoxins based on previous studies that targeted small cohorts of healthy population of different age groups.

3.3 Mycotoxin biomonitoring studies in Africa

In the last 18 years, eighteen studies assessed the occurrence of aflatoxin, fumonisin, ochratoxin, zearalenone and deoxynivalenol biomarkers in human biological fluids (Table 2). Most of the biomonitoring studies originate from Tunisia and Nigeria in that period. Although studies covered the research of the presence of mycotoxin biomarkers in urine, blood, blood components and maternal breast milk were the most commonly investigated matrix. Even though the cohort of the majority of the studies were healthy adults, a significant number of studies focused on the incidence of mycotoxin biomarkers in children and patients.

Studies performed in Tanzania and Nigeria have shown that more than 70% of children are exposed to aflatoxin (Chen et al. 2018; McMillan et al. 2018; Shirima et al. 2013). The children in Tanzania were found to be mainly exposed to fumonisin with more than 80% of positive samples (Chen et al. 2018; Shirima et al. 2013) compared samples collected and measured in Cameron (Njumbe Ediage et al. 2013) and Nigeria (Ezekiel et al. 2014), where less than 15% samples were positive for either FB1 or FB2. The presence of aflatoxins or fumonisins in children samples raised particular health concerns as many studies detected high concentration of aflatoxin in urine, blood, stool, liver, lungs and brain of kwashiorkor in children. This disease causes uncontrolled behavior, pneumonia and may lead to death (Cherkani-Hassani et al. 2016; Peraica et al. 1999). Furthermore, one third of children urine samples were positive for OTA while the presence of DON and ZEN biomarkers of exposure was found in less than 18% and 10% of the analyzed samples, respectively (Ezekiel et al. 2014; Njumbe Ediage et al. 2013).

Based on the studies covered with this survey, more than half of the mothers in Africa with young children are exposed to aflatoxins, which is class I carcinogens. Namely, a high incidence of aflatoxin biomarker AFM1 was found in 54% and 82% of the maternal breast milk in mothers originating from Sudan and Nigeria, respectively

(Adejumo et al. 2013; Elzupir et al. 2012). Furthermore, all analyzed samples from Kenya women showed the presence of AFB1-lysine conjugate (Leroy et al. 2015). The high level of aflatoxins in maternal breast milk present a serious threat to infants. Indeed, the mean concentration of AFM1in urine was higher in partially weaned infants than fully weaned infants in Cameron, which may indicate the transfer of AFM1 from the mother's milk to the child (Njumbe Ediage et al. 2013).

In Tunisia, the OTA levels have been assessed in different geographical areas in healthy volunteers and patients with diseased of Nephropathy (Grosso et al. 2003; Hassen et al. 2004; Karima et al. 2010; Zaied et al. 2011). Majority of the samples analyzed in Tunisia were positive for OTA, however the OTA levels were significantly higher in the nephropathy patients without bladder tumor (Grosso et al. 2003) and chronic interstitial nephropathy patients of known etiology (Hassen et al. 2004). Indeed, the high levels of OTA in Tunisia have already been associated with chronic nephritic disease in this area at the end of last century (Maaroufi et al. 1995). High levels of OTA (7.8 and 11.76 ng/ml) were also reported in two samples of healthy adults, compared to the mean OTA concentration of the rest of the samples (0.49± 0.04 ng/mL). Interestingly, higher incidence of OTA was observed in healthy population (44%, range 0.01-5.81 ng/mL) compared to the nephropathy patients (20%, range 0.17-2.42 ng/mL) living in Ivory Coast (Sangare-Tigori et al. 2006). Similar amount of OTA was also reported for the healthy population of Morocco (Filali et al. 2002) where 60% of analyzed samples were positive for OTA.

OTA has also been analyzed in 98 serum samples from Egyptian pregnant women. EDI for pregnant women was calculated based on the serum OTA level by using the Klaassen equation and the EDI for fetal exposure was evaluated based on maternal data (Woo and El-Nezami 2016). However, the highest exposure of OTA in pregnant women was 3.26 ng/kg BW /day, which is lower than the estimated daily intake for Negligible Cancer Risk Intake (NCRI, 3.38 ng/kg BW /day) while the highest exposure group of OTA for fetal exposure was nearly double the NCRI number and the margin of exposure (MOE) is $2.5 \cdot 10^3$. The OTA concentration in pregnant women was in the range of 0.20 to 1.53 ng/ml and the estimated OTA range in fetus was 0.40 to 3.06 ng/ml. For this reason, essential solutions to reduce the risk of mycotoxin contamination are recommended e.g. by monitoring food mycotoxin contamination and removing food with high mycotoxin concentration from the food market in Egypt (Woo and El-Nezami 2016). A South African study examined the exposures of farmers to mycotoxins with the occurrence of esophageal cancer (Shephard et al. 2013). In this area, 90 % of the population consumes maize daily. Therefore, the study involved collection of raw and cooked maize samples

to measure contamination level of mycotoxin in food samples and in urine samples of 54 female individuals. Urine samples have been analyzed by single and multi-biomarker detection as shown in Table 4. The mycotoxins ZEN, DON, FB1 and FB2 were positively identified in 90% of the samples, which may have originated from maize. OTA however, although not detected in maize samples, it was detected in urine samples with the mean urinary level for OTA of 0.024 ng/ml. Furthermore, the concentration of FB1 was high in urine, indicating considerable exposure of the population to FB1.

In northern Nigeria, Ezekiel and coworkers have assessed mycotoxin exposure in rural areas from different age groups (19 children, 20 adolescents, and 81 adults). Population of Nigeria rely significantly on crops such as groundnuts, maize, sorghum and millet. For this reason, besides urine samples, samples from consumed meals have been collected and analyzed to assess mycotoxins exposure and to identify the source of human intoxication (Ezekiel et al. 2014). Mycotoxins were detected in all measured human samples grouped by age yielding 50.8% positive detection rate. However, OTA had the highest incidence, affecting 28.3 % of the subjects with a mean concentration of 0.2 ng/ml followed by AFM1 and FB1 with mean concentration of 0.3 ng/ml and 0.6 ng/ml respectively. Aflatoxins and FB1 (both potent carcinogens) were detected in most of the samples which may consequently lead to considerable health risk, (Ezekiel et al. 2014) and must be communicated in order to raise awareness among consumers and health authorities. Reanalysis of the same samples by the improved mycotoxin detection method showed the presence of mycotoxin biomarkers in all samples, and the co-occurrence of AFM1 and FB1 in 57% of samples analyzed (Sarkanj et al. 2018).

A study in Cameroon explored the presence of mycotoxin biomarkers in HIV-sera positive samples (Abia et al. 2013). Among all the biomarkers analyzed, aflatoxins, fumonisin, ochratoxin, ZEN and DON metabolites, majority of the samples (>60%) showed the presence of DON biomarkers of exposure, followed by the presence of OTA (25%) and AFM1 (15%), while ZEN and fumonisin biomarkers were found in less than 6% of the samples.

Table 2. Summary of studies measuring mycotoxin levels in human samples from Africa.

Country	Sample type	No. of samples	Positive samples (%)	Mycotoxin type (LOD / LOQ) ng/mL	Mean (Range) ng/mL	Method of detection	Reference
Cameroon	Children (1-5yrs) Urine	220	14	AFM1 (0.01 / n.s.)	0.33 (0.06 -4.7)*	HPLC	Njumbe Ediage et al. 2013 (#)
			32	OTA (0.03 / n.s.)	0.20 (0.04-0.2.4)*		
			17	DON (0.04 / n.s.)	2.22 (0.1-77)*		
			11	FB1 (0.01 / n.s.)	2.96 (0.29 -0.53)*		
			4	ZEN (0.1 / n.s.)	0.97 (0.06-48)*		
			8	β-ZOL (0.01 / n.s.)	1.52 (0.02-12.5)*		
			4	α-ZOL (0.31 / n.s.)	0.98 (0.26 -1.3)*		
Cameroon	Adults (18-58 yrs) HIV sero-positive Urine	145	10	AFM1 (0.05 / 0.17)	0.05 (< LOQ -1.38)	LC-MS/MS (ESI)	Abia et al. 2013
			3	FB1 (0.5 / 1.7)	0.63 (< LOQ-14.8)		
			1	FB2 (0.5 / 1.7)	< LOQ (n.a.)		
			17	OTA (0.05 / 0.17)	0.08 (< LOQ-1.87)		
			6	DON (4 / 13)	< LOQ (n.a.)		
			43	DON-15-GlcA (3 / 11)	5.49 (< LOQ-96.2)		
			11	DON-3-GlcA (6 / 10)	3.93 (< LOQ-22.5)		
			3	ZEN (0.4 / 1.3)	0.22 (< LOQ-1.42)		
			3	ZEN-14-GlcA (1 / 3.3)	0.81 (3.38-31)		
			1	a-ZEL (0.5 / 1.7)	< LOQ (n.a.)		
Egypt	Pregnant women Serum	98	82	OTA (0.2 / 0.2)	0.26 (0.20-1.53)	HPLC-FLUO	Woo et al. 2016
Ivory Coast	Healthy people Blood	63	43.9	OTA (n.s. / n.s.)	0.83 (0.01 – 5.81)	HPLC	Sangare-Tigori et al. 2006
	NEPH patients Blood	39	20.5		1.05 (0.167–2.42)		
Kenya	Women Serum	844	100	AFB1-lysine (0.2 pg/mg ALB / n.s.)	7.82 (6.04-8.90)* pg/mg ALB	HPLC-FLUO	Leroy et al. 2015
Morocco	Adults plasma	309	60	OTA (n.s. / 0.4)	0.29 (0.08 -6.59)	HPLC-FLUO	Filali et al. 2002
Nigeria	Maternal breast milk	50	82	AFM1 (0.01 / 0.05)	n.s. (0.00349–0.035)	HPLC	Adejumo et al. 2013 (#)
		120	14.2	AFM1 (0.05 / 0.15)	0.34 (0.08-1.54)	UHPLC	

Nigeria (North)	Children, adolescents and adults		0.8	DON (4 / 4)	5 (0.94-6.84)		Ezekiel et al. 2014 (#)
			5	DON-15-O-GlcA (4 / 6)	3.5 (n.s.)		
			13.3	FB1 (2 / 2)	4.56 (2.08-12.77)		
	Urine		1.7	FB2 (2 / 0.7)	1 (n.s.)		
			28.3	OTA (0.05 / 0.15)	0.2 (0.08-0.56)		
			6.7	ZEN (0.4 / 0.6)	3.13 (0.94-6.84)		
			6.7	ZEN-14-GLcA (1 / 1)	9.5 (n.s.)		
Nigeria	Children (6-48 mos) Plasma	58	100	AFB1-lysine (0.022 / 0.022)	2.6 (0.2 - 59.2 pg/mg ALB)	LC-MS/MS with IDMS	McMillan et al. 2018
Nigeria (North)	Children, adolescents and adults	120	72.5	AFM1 (0.0003 / 0.001)	0.04 (0.001-0.62)	UHPLC-MS/MS	Sarkanj et al. 2018 (#)
			19.2	DON (0.05 / 0.15)	2.37 (0.08-6.22)		
			70.8	FB1 (0.001 / 0.01)	1.09 (0.08-14.88)		
			78.3	OTA (0.0003 / 0.001)	0.05 (0.003-0.31)		
	Urine		81.7	ZEN (0.001 / 0.003)	0.75 (0.03-19.99)		
			4.2	α-ZEL (0.003 / 0.01)	1.27 (0.52-2.52)		
			5.8	β-ZEL (0.001 / 0.003)	0.88 (0.06-2.74)		
South Africa	Females (19-97 yrs)	54	87	FB1 (0.01 / 0.02)	0.342 (0.007-2.27 ng/mg CRN)	single biomarker LC-MS/MS	Shephard et al. 2013
			100	DON (0.25 / 0.5)	20.4 (0.445-353ng/mg CRN)		
			96	FB1 (0.04 / 0.12)	1.52 (0.026-9.99 ng/mg CRN)	Multiple biomarker (Dilute and shoot) LC-MS/MS	
			87	DON (0.45 / 1.51)	11.3 (0.312-190 ng/mg CRN)		
			92	α-ZEL (0.009 / 0.029)	0.614 (0.006-13.2 ng/mg CRN)		
	Urine		75	β-ZEL (0.016 / 0.054)	0.702 (0.010-21.1 ng/mg CRN)		
			100	ZEN (0.002 / 0.007)	0.529 (0.012-11.2 ng/mg CRN)		
			98	OTA (0.002 / 0.007)	0.041 (0.001-0.629 ng/mg CRN)		
			0	AFM1 (0.01 / 0.02)	n.a.		
Sudan	Maternal breast milk	94	54.25	AFM1 (0.013 / n.s.)	0.401 (0.007-2.561) ng/g	HPLC-FLUO	Elzupir et al. 2012
Tanzania	Children (12-22 mos) Plasma	146	84	AF-alb (3 pg/mg ALB / n.s.)	12.9 (9.9-16.7)* pg/mg	ELISA	Shirima et al. 2013
	Urine	147	96	FB1 (0.02 / n.s.)	167.3 (135.4-206.7)* pg/mg	HPLC/MS	
Tanzania	Children (24 mos) Plasma	60	71	AFB1-lysine (0.4 pg/mg ALB)	5.1 (0.28-25.1) pg/mg ALB	(SPEs) columns UPLC-MS/MS	Chen et al. 2018
	Children (24 & 36 mos) Urine	94	80	FB1 (0.01 / n.s.)	1.3 (<LOD-16.6)	LC/MS	

Tunisia (SouthEast)	Non-NEPH patients serum NEPH patients (wo BT) serum NEPH patients (with BT) serum	62 26 21	100	OTA (n.s. / 0.1)	0.53 (0.12- 8.06) 1.116 (< 0.10- 5.80) 0.339 (< 0.10-0.74)	Immunoaffinity-HPLC	Grosso et al. 2003 (#)
Tunisia	1st study CIN patients[a] Blood 1st study CIN patients[b] Blood 1st study Healthy people Blood 2nd study CIN patients[a] Blood 2nd study CIN patients[b] Blood 2nd study Healthy people Blood	20 40 20 20 20 20	93 83 71 100 78 62	OTA (n.s. / n.s.)	44.4 (17.4-140.5) 8.11 (0-73.19) 2.6 (0-7.5) 50.4(18.4-171.35) 12.36 (1.68–29) 1.22 (0-3.2)	HPLC	Hassen et al. 2004
Tunisia	Adults (20-84 yrs) Serum	107	28	OTA (0.5 / 2)	0.49 (0.12-11.67)	HPLC	Karima et al. 2010 (#)
Tunisia	Adults (17-75 yrs) Serum NEPH patients (18-88 yrs) Serum	138 270	49 76	OTA (0.05 / 0.15)	3.3 (1.7-8.5) 18 (1.8-65)	HPLC	Zaied et al. 2011 (#)

ALB - albumin; BT - bladder tumor; CRN - creatinine; NEPH - Nephropathy; mos – months; yrs – years; n.a. – not applicable; n.s. – not specified

a Chronic interstitial nephropathy patients of unknown etiology

b Chronic interstitial nephropathy patients of known etiology

validated method

3.4. Mycotoxin biomonitoring studies in Europe

A total of 38 studies on biomonitoring of mycotoxin exposure in Europe, conducted since 2001, have been included in this survey (Table 3). Among all countries, the highest number of studies originate from Portugal, followed by Germany, Turkey and Italy. The most commonly investigated human sample type in European studies was urine, while similar number of studies was performed on maternal breast milk and blood and blood components. The cohort of majority studies were healthy adults, while less than 10% of the studies focused on the incidence of mycotoxin biomarkers in children and patients with disease endemic NEPH and urinary disorders.

A recent study from Belgium reported the presence of DON biomarkers in all children urine samples, while OTA was present in half of the samples (Heyndrickx et al. 2015). The BIOMYCO study aimed to detect 33 potential biomarkers and metabolites of multiple mycotoxins including aflatoxins, OTA, fumonisins, TCs, zearalenone and DON (Heyndrickx et al. 2015). In this study, urine samples of 155 children and 239 adults were analyzed. Only 9 out of 33 biomarkers were detected in the analyzed samples as shown in Table 3. OTA was detected in most of the samples from the Belgian population. Furthermore, 100% of samples contained DON15GlcA with the highest mean concentration of 58.4 ng/ml and 53.8 ng/ml for children and adults respectively. It is worth mentioning that OTA levels were almost 3 times higher in children compared to the adults (Heyndrickx et al. 2015). High incidence of OTA was also observed in 93% and 100% of children samples from Poland (Postupolski et al. 2006) and from the south of Italy (Solfrizzo et al. 2014) respectively. Interestingly, the study from Poland showed that although OTA was detected in all serum samples from nursing mothers less than quarter of maternal breast milk samples were positive (Postupolski et al. 2006). A significant incidence (above 60%) of OTA and its biomarkers in biological fluids such as milk, plasma, serum and urine was reported in most of the studies originating from Bulgaria (Petkova-Bocharova et al. 2003), Czech Republic (Dohnal et al. 2013; Ostry et al. 2005), Italy (Biasucci et al. 2011; Galvano et al. 2008), Germany (Ali et al. 2017; Munoz et al. 2010), Hungary (Fazekas et al. 2005), Portugal (Duarte et al. 2010; Duarte et al. 2012; Duarte et al. 2009; Lino et al. 2008; Pena et al. 2006), Spain (Coronel et al. 2011; Manique et al. 2008) ,Turkey (Akdemir et al. 2010; Erkekoglu et al. 2010) and UK (Gilbert et al. 2001). Contrary, lower incidence of the same mycotoxin was reported for Croatia (Domijan et al. 2009; Sarkanj et al. 2013), Norway (Skaug et al. 2001), Slovakia (Dostal et al. 2008) and Sweden (Wallin et al. 2015).

The study of Lino *et al.* (Lino et al. 2008) examined OTA level in serum from urban and rural population in three regions of Portugal and correlated the results of OTA levels in serum with those in whole blood samples. The ratio of serum/blood was 2.0±0.7 which is in agreement with previous study(Lino et al. 2008). However, OTA were detected in all serum samples within the range of 0.14 -2.49 ng/ml. The EDI levels in this study were 0.19-3.35 ng/kg bw, which do not exceeded the estimated TDI of 5 ng/kg bw set by the Scientific Committee on Foods (SFC) of the European Union in 2002. Interestingly, the OTA level in rural adult male serum samples was significantly higher than in adult females. A study from southern Italy, found mycotoxin biomarkers in the samples from all the participants (Solfrizzo et al. 2014). OTA, ZEA and α-ZEL were present in all urine samples, while the incidence rates for β-ZEL, DON, FB1 and AFM1 were present in 98%, 96%, 56% and 6% of samples respectively (Solfrizzo 2014). The mean concentration of DON was the highest compared to other mycotoxins (11.89 ng/ml) and detected in 96% of the samples with 40% of the samples exceeding TDI for DON. PDI levels exceeded TDI level for DON. OTA was considerably greater than TDI in 94% of the urine samples, while FB1 and ZEA levels were much less than TDI in all the samples. The exposure to aflatoxin in this region was limited to measurement of AFM1, which was detected in only 3 urine samples (Solfrizzo et al. 2014). Similarly, AFM1 was present in 5% of maternal breast milk samples of Italian residents (Galvano et al. 2008). Controversial findings were reported for the nursing mothers in Turkey, where one study reported the presence of AFB1 and AFM1 in all breast milk samples (Gurbay et al. 2010) while a more recent one indentified AFM1 in 25% breast milk samples (Atasever et al. 2014). This discrepancy could be most likely attributed to the different mycotoxin detection method used, i.e. the first study used HPLC with fluorescence detection and the later one used ELISA assay. The 60% and above incidence of AFM1 in human biological fluids was also reported for the populations of Czech Republic (Ostry et al. 2005) and Serbia (Kos et al. 2014) while in Portugal the AFM1 was detected in 32.8% of maternal breast samples (Bogalho et al. 2018).

Another study in southern Italy assessed OTA level in 327 plasma samples from adult population and OTA was detected in 99.1% of the samples. The level of OTA in 17 plasma samples exceeded 0.5 ng/ml which indicate an increased risk for cancer and kidney toxicity (di Giuseppe et al. 2012). The results of this study confirmed the report of the "European Scientific Committee on Food (SCF)" (SCF 2002) which mentioned that Italian food products such as coffee, cereals, pork meat and olive oil are contaminated with OTA. This study concluded that the wine in Italy is seemingly the most mycotoxin contaminated food product (di Giuseppe et al. 2012).

In Central Europe, DONGlcA and DON were detected in 82% and 29% of the analyzed samples, respectively, from a sample set that is composed of 101 urine samples from a German population (Gerding et al. 2014). A total of 12% of the samples exceeded TDI for DON (1 µg/kg bw according to SCF 2002) with the highest provisional daily intake of 5.67 µg/kg bw. Furthermore, the same study correlated the results with BMEL, the annual German harvest report of 2013 which mentioned that 99% of raw grains in Germany are contaminated with DON. The study analyzed 23 mycotoxin biomarkers and found either single or multiple mycotoxin biomarkers in 87% of the analyzed urine samples. Interestingly, the same study did not detect any aflatoxin metabolite (AFB1, AFB2, AFG2 and AFM1) in any sample. Gerding and coworkers later reported that the German population have in general low exposure to mycotoxins except DON. This is in agreement with the more recent study from Germany (Ali et al. 2016) that detected DON in 100% of samples and DOM-1 in 40% of samples. High incidence of DON and its metabolites was also reported in samples for Croatia (Sarkanj et al. 2013), Sweden (Wallin et al. 2015) and UK (Turner et al. 2011a; Turner et al. 2010).

Table 3. Summary of studies measuring mycotoxin levels in human samples from Europe.

Country	Sample type	No. of samples	Positive samples (%)	Mycotoxin type (LOD / LOQ) ng/mL	Mean (Range) ng/mL	Method of detection	Reference
Belgium	Children (3-12 yrs) Urine	155	70	DON (0.2 / n.s.)	5.2 (0.5-32.5)	LC-MS/MS	Heyndrickx et al. 2015 (#)
			91	DON3GlcA (0.2 / n.s.)	10.6 (0.7-43)		
			100	DON15GlcA (0.2 / n.s.)	58.4 (4.3-343)		
			17	DOMGlcA (0.2 / n.s.)	91.7 (1.1-526.1)		
			51	OTA (0.001 / n.s.)	0.0795 (0.0038-3.683)		
			n.d.	α-ZEL (0.061 / n.s.)	n.d (n.a.)		
			n.d.	β-ZEL14GlcA (0.117 / n.s.)	n.d (n.a.)		
	Adults (19-65 yrs) Urine	239	37	DON (0.2 / n.s.)	3.9 (0.5-129.8)		
			77	DON3GlcA (0.2 / n.s.)	7.5 (0.5-126.2)		
			100	DON15GlcA (0.2 / n.s.)	53.8 (1.1-460.8)		
			22	DOMGlcA (0.2 / n.s.)	16.9 (0.6-172)		
			35	OTA (0.001 / n.s.)	0.0278 (0.0027-0.3681)		
			0.4	α-ZEL (0.061 / n.s.)	5 (5-5)		
			0.8	β-ZEL14GlcA (0.117 / n.s.)	0.8 (0.6-1)		
Bulgaria	Adults (20-30yrs) Serum	16	100	OTA serum (n.s. / 0.1)	0.00159 (0.0001-0.0109)	HPLC (IAC)	Petkova-Bocharova et al. 2003 (#)
	Urine		95	OTA urine (n.s. / 4)	0.0508 to 0.16864 (0.01- 1.91)		
Croatia	Area of residence			OTA (0.005 / n.s.)		HPLC	Domijan et al. 2009 (#)
	Endemic NEPH	45 (2000*)	43		0.007 (0.005-0.086)		
	Urine	45 (2005*)	18		0.001 (0.005-0.015)		
	Control	18 (2000*)	28		0.003 (0.005-0.02)		
	Urine	18 (2005*)	6		0.005 (0.01)		
Croatia	Pregnant women (26-33 yrs) Urine	40	76	DON (4 / 13)	18.3 (4-275)	LC–ESI–MS/MS	Sarkanj et al. 2013
			98	DON-15-GlcA (3 / 11)	120.4 (3-1237.7)		
			83	DON-3-GlcA (6 / 20)	28.8 (6-298.1)		
			10	OTA (0.05 / 0.17)	<0.17 (0.05-<0.17)		
Czech Republic	Healthy volunteers Urine	100 (1997*)	72	AFM1 (n.s. / 0.000125)	367 (19-6064) pg/g CRN	ELISA (IAC)	Ostry et al. 2005 (#)
		105 (1998*)	46		414 (21-19219) pg/g CRN		
	Serum	2206	94.2	OTA (n.s. / 0.1)	0.28 (0.1-13.7)	HPLC with FLUO	

Czech Republic	Females (19-40yrs) Serum	115	100	OTA (n.s. / 0.05)	0.135 (0 -1.073) 0.140 (0.050 -1.130)	ELISA HPLC with FLUO	Dohnal et al. 2013 (#)
Italy	Maternal breast milk	82	5 74	AFM1 (0.003 / 0.007) OTA (0.0052 / 0.005)	0.05535 (<0.007-0.14) 0.03043 (<0.005- 0.405)	HPLC (IAC)	Galvano et al. 2008 (#)
Italy	Nursing mothers Maternal breast milk Serum	130	78.8 99	OTA milk (0.0005 / 0.001) OTA serum (0.025 / 0.05)	0.01 (0.0011- >0.0751) 0.4998 (0.084-4.835)	HPLC	Biasucci et al. 2010 (#)
Italy (South)	Children and Adults (3-85 yrs) Urine	52	6 100 96 56 98 100 100	AFM1 (n.s. / 0.02) OTA (n.s. / 0.006) DON (n.s. / 1.5) FB1 (n.s. / 0.01) β-ZEL (n.s. / 0.054) α-ZEL (n.s. / 0.03) ZEN (n.s. / 0.007)	0.068 (Max 0.146) 0.144 (Max 2.129) 11.89 (Max 67.36) 0.055 (Max 0.352) 0.09 (Max 0.135) 0.077 (Max 0.176) 0.057 (Max 0.120)	UPLC-MS/MS	Solfrizzo et al. 2014 (#)
Germany	Adults (20-57 yrs) Urine Plasma	13	100	OTA (0.02 / 0.05) OT α (0.02 / 0.05) OTA (0.07 / 0.5) OT α (0.07 / 0.5)	0.07 (0.02–0-14) 2.88 (0.49–7.12) 0.25 (0.19–0.29) 0.95 (0.07–1.64)	HPLC with FLUO (IAC)	Munoz et al. 2010 (#)
Germany	Adults (19-65 yrs) Urine	101	29.41 82.35 3.96	DON (0.5 / 2) DON3GlcA (2 / 4) ZEN-14-GlcA (0.75 / 1.5)	3.38 (2.48-17.34) 12.21 (4.37-92.95) <LOQ	LC-MS/MS	Gerding et al. 2014 (#)
Germany	Male 30 yrs, plasma Male 30 yrs, urine Male 60 yrs, plasma Male 60 yrs, urine	7 7		OTA (0.05 / 0.1) OT α (0.05 / 0.1) OTA (0.01 / 0.02) OT α (0.01 / 0.02) OTA (0.05 / 0.1) OT α (0.05 / 0.1) OTA (0.01 / 0.02) OT α (0.01 / 0.02)	0.42 (0.34-0.58) 0.45 (0.39-0.52) 0.06 (0.04-0.16) 0.06 (0.02-0.11) 1.64 (1.14-1.97) 0.20 (0.08-0.31) 0.24 (0.06-0.62) 2.22 (0.21-3.78)	HPLC-FD	Ali et al. 2017 (#)
	Adults, urine	50	100 78	OTA (0.01 / 0.02) OT α (0.01 / 0.02)	0.21 (0.02-1.82) 1.33 (n.d.-14.25)		

Germany	Adults, urine	50	100 40	DON (0.16 / 0.3) DOM-1 (0.1 / 0.2)	9.02 (0.16–38.44) 0.21 (0.10–0.73)	LC-MS/MS	Ali et al. 2016 (#)
Hungary	Healthy volunteers (8-80 yrs) Urine	88	61	OTA (0.004 / 0.006)	0.013 (0.006–0.065)	HPLC (IAC)	Fazekas et al. 2005 (#)
Norway	Maternal breast milk	80	21	OTA (0.01 / 0.01)	0.03 (0.01- 0.182)	HPLC	Skaug et al. 2001
Poland	Lactating women Serum Maternal breast milk Fetal serum	30	100 16.6 93	OTA serum (0.005 / 0.015) OTA milk (0.02 / 0.06)	1.14 (0.14-3.41) 0.0056 (0.0053-0.017) 1.96 (0.6-4)	HPLC with FLUO (IAC)	Postupolski et al. 2006
Portugal	Adults (19-82 yrs) Urine	60	70	OTA (n.s. / 0.02)	0.038 (0.021 -0.105)	HPLC-FD	Pena et al. 2006 (#)
Portugal	NEPH patients Serum	95	n.s.	OTA (n.s. / 0.05)	0.49 to 0.50 (0.12- 1.52)	HPLC with FLUO	Dinis et al. 2007 (#)
Portugal	Healthy volunteers (15-67 yrs) Urine	30	43.3	OTA (n.s. / 0.007)	0.019 (0.011 - 0.208)	HPLC with FLUO (IAC)	Manique et al. 2008 (#)
Portugal	Adults (19-92 yrs) Serum	104	100	OTA (n.s. / 0.1)	n.s. (0.14 -2.49)	HPLC-FD (IAC)	Lino et al. 2008 (#)
Portugal	Adults (18-75 yrs) Urine	43	100	OTA (n.s. / 0.008)	0.026 (n.d–0.071)	HPLC with FLUO (IAC)	Duarte et al. 2009 (#)
Portugal	Adults (18-96 yrs) Urine	155	92.2	OTA (n.s. / 0.008)	0.018 (n.d.–0.069)	HPLC	Duarte et al. 2010 (#)
Portugal	Adults (20-83 yrs) Urine	95	81.1 (summer) 87.4 (winter)	OTA (0.0024 / 0.008)	0.016 (n.d.-0.040) 0.022 (n.d.-0.071)	HPLC–FD	Duarte et al. 2012 (#)
Portugal	Maternal breast milk	67	32.8	AFM1 (0.005 / n.s.)	7.4 (5.1-10.6)	ELISA	Bogalho et al. 2018
Serbia	Maternal breast milk	10	60	AFM1 (0.0015 / 0.005 ng/g)	0.01 (0.006-0.022) ng/g	ELISA	Kos et al. 2014 (#)
Slovakia	Maternal breast milk	76	30.2	OTA (0.0048 / 0.0144)	n.s. (0.0023-0.0603)	HPLC	Dostal et al. 2008 (#)
Spain	Adults (18-53 yrs) Urine	31	80.6	OTA (n.s. / 0.007)	0.032 (0.007–0.124)	HPLC with FLUO (IAC)	Manique et al. 2008 (#)
Spain	Adults (18-45 yrs)	72				HPLC with FLUO	

	Urine		12.5 61.1	OTA (0.034 / 0.112) Otα (0.023 / 0.076)	0.237 (0.057–0.562) 0.441 (0.056–2.894)		Coronel et al. 2011 (#)
Sweden	Adults Urine	252	63 8 37 21 18 6 23 51	DON (n.s. / 0.2) DOM-1 (n.s. / 0.89) ZEA (n.s. / 0.01) α-ZEL (n.s. / 0.04) β-ZEL (n.s. / 0.04) FB1 (n.s. / 0.01) FB2 (n.s. / n.s.) OTA (n.s. / 0.006)	3.37 (n.s.) 0.18 (n.s.) 0.03 (n.s.) 0.03 (n.s.) 0.02 (n.s.) 0.004 (n.s.) 0.01 (n.s.) 0.46 (n.s.)	LC-MS/MS	Wallin et al. 2015
Turkey	Healthy volunteers Serum Urinary disorders patients Serum	40 93	n.s.	OTA (0.2 / n.s.)	0.4 (0.19-1.43) n.s(0.3-5.5)	Fluorescence	Ozcelik et al. 2001
Turkey	Adults (18-65 yrs) Urine	233	83	OTA (0.006 / 0.018)	14.34 (Max 75.60 ng/g CRN)	HPLC	Akdemir et al. 2010 (#)
Turkey	Adults (6-80yrs) Serum	239	98 (summer) 81 (winter)	OTA (0.025 / 0.025)	0.312 (0.028 - 1.496) 0.137 (0.0306 - 0.887)	ELISA	Erkekoglu et al. 2010
Turkey	Maternal breast milk	75	100 100	AFB1 (0.005 / 0.005) AFM1 (0.005 / 0.005)	n.s. (0.0945–4.1238) n.s. (0.0609–0.29999)	HPLC with FLUO	Gurbay et al. 2010
Turkey	Maternal breast milk	73	24.6	AFM1 (0.01 / n.s.)	0.83 (1.3–6.0) ng/l	ELISA	Atasever et al. 2014 (#)
UK	Plasma Urine	50	100 92	OTA plasma (0.1 / n.s.) OTA urine (n.s. / 0.01)	n.s. (0.15- 2.17) n.s. (< 0.01 - 0.058	HPLC (IAC)	Gilbert et al. 2001
UK	Adults (21-59 yrs) Urine	210	94.2	DON (n.s. / 2)	11.6 (n.d.-78.2)	LC–MS	Turner et al. 2010 (PMID: 20572795)
UK	Adults (21-59 yrs) Urine	34	68 3	DON (n.s. / 0.5) DOM-1 (n.s. / 0.06)	2.4 (0.5–9.3) n.s.	LC–MS	Turner et al. 2011

ALB - albumin; CRN - creatinine; NEPH - Nephropathy; mos – months; yrs – years; n.a. – not applicable; n.s. – not specified

* year of sample collection; # validated method

3.5. Mycotoxin biomonitoring studies in Asia

Biomonitoring studies in Asia covered by this survey include 13 studies, with majority originating from Iran and Malaysia (Table 4). The cohort of all studies included healthy adults while only one study from South Asia also included children. More than 70% of studies from Asia focused on the exposure to the class I carcinogen aflatoxin by measuring the AFB1, AFM1 and AFB1-lysine biomarkers of exposure. In majority of the countries the incidence of aflatoxin biomarkers was above 93% regardless of the analyzed sample type (Azarikia et al. 2018; Groopman et al. 2014; Leong et al. 2012; Maleki et al. 2015; Mohd Redzwan et al. 2014; Omar 2012; Sabran et al. 2012). Leong and colleagues (Leong et al. 2012) have evaluated the presence of AFB1-lysine in blood for the Penang population in Malaysia where people consume large quantities of nuts products. Only 2 samples exceeded the concentration 20 AFB1-lysine pg/mg albumin. Furthermore, based on the amount of adducts found (0.20 to 23.16 pg/mg albumin) the study calculated the dietary intake EDI to be in the range of 0.01 to 0.60 µg AFB1 per day. According to this study, 97% of serum samples contained AFB1-lysine adducts which, despite the low amounts detected, indicates that the population of Penang is exposed to AFB1(Leong et al. 2012). Lower exposure to aflatoxins has been reported for the population of Bangladesh, where the positive samples ranged between 26% in winter to 40% in summer with the maximum mean value of 27.7 pg/mL AFM1 (Ali et al. 2017).

Several studies also investigated human exposure to OTA and DON (Afshar et al. 2013; Ali et al. 2016; Assaf et al. 2004; Dehghan et al. 2014; Turner et al. 2011b). A study conducted in Lebanon (Assaf et al. 2004) evaluated OTA in the most popular foodstuffs by analyzing plasma samples from 250 volunteers from different regions of the country such as north, south, capital city and village. The analysis of foodstuff showed that 82% of beer samples, 61% of burghul samples, 12% of wheat samples and 7.6% of maize samples were contaminated with OTA. In addition, 33% of the total plasma samples showed traces of OTA. The consumption of large amounts of cereals and burghul has been correlated with higher incidence of OTA in plasma samples. However, the mean concentration of plasma samples did not vary considerably (0.16-0.18 ng/ml) between the analyzed regions. EDI have been calculated in plasma samples based on the eq. 4. (i.e. EDI of OTA (ng/kg bw per day) = concentration of OTA in plasma (ng/mL) · 1.34). The value of EDI for the mean concentration of OTA in all plasma samples was 0.23 ng/kg bw/day, which is considerably lower than the maximum allowed level set by JECFA (see table 3 for TDI levels). Even the highest value detected in plasma (0.87 ng/ml) was lower than EDI limit (1.16 ng/kg bw per day). A study by Assaf and co-workers (Assaf et al. 2004) showed that the most

commonly consumed food in Lebanon (i.e. wheat, burghul, beans, lentil and beer) contained considerable amounts of OTA. In this case, OTA concentration found in the beer samples (0.42 and 1.12 ng/ml) was higher than the level advised by the European Union (i.e. 0.2 ng/ml).

The DON Biomonitoring studies reported the incidence of positives in more than 96% of the analyzed sample from China (Turner et al. 2011b) and 33% or 44% of samples collected in summer or winter, respectively from Bangladesh (Ali 2016). Mean DON values were significantly higher in China compared to the reported value for urine samples from Bangladesh population. In a study by Turner and colleagues (Turner et al. 2011b) the free and conjugated forms of DON were determined in 70 urine samples obtained from female individuals originating from Shanghai. In the Shanghai region, the population consume large quantities of rice and wheat daily. However, DON biomarkers have been detected in 96.7% of the samples with mean concentration of 4.8 ng/ml. In contrast, DON-1 was not detected in any of the samples. The results of this study were compared to the results of a study conducted on the female cohort from the UK. DON mean concentration in urine samples from UK females was twice higher than the concentration measured in the samples from Chinese females. Although based on the survey studies, the amount of daily intake of wheat in UK is 4 times higher than the amount of wheat consumed by the Chinese population. This probably means that wheat in China is more contaminated with DON than in UK but due to dietary habits it represent lower risk than for population in UK (Turner et al. 2008a; Turner et al. 2008b; Turner et al. 2009; Turner et al. 2010).

Table 4. Summary of studies measuring mycotoxin levels in human samples from Asia.

Country	Sample type	No. of samples	Positive samples (%)	Mycotoxin type (LOD / LOQ) ng/mL	Mean (Range) ng/mL	Method of detection	Reference
Bangladesh	Adults Urine	62 (summer) 95 (winter)	27 31	DON (0.16 / 0.3) DON (0.16 / 0.3) DOM-1 (0.1 / n.s.)	0.17 (0.16–1.78) 0.16 (0.16–1.21) n.d.	LC–MS/MS	Ali et al. 2016 (#)
Bangladesh	Adults Urine Pregnant women Urine	69 (summer) 95 (winter) 54	>40 26 31	AFM1 (0.0017 / 0.005)	13.5 (1.7–104) pg/mL 27.7 (1.8–190) pg/mL 13.9 (1.7–141) pg/mL	HPLC-FD (IAC) ELISA	Ali et al. 2017 (#)
China	Adults (40-70 yrs) Urine	60	96.7	DON (0.5 / n.s.)	5.9 (nd - 30.5) ng/mg CRN	HPLC	Turner et al. 2011b (#)
Iran	Maternal breast milk	136	1.47 0.73	OTA (n.s. / 0.5) AFM1 (n.s. / 0.1)	0.09 and 0.14 (2 positive) 0.02 (1 positive)	ELISA HPLC	Afshar et al. 2013
Iran	Maternal breast milk	87	96.6	OTA (n.s. / n.s.)	0.02457 (0.0016-0.06)	ELISA	Dehghan et al. 2014
Iran	Maternal breast milk	85	100	AFM1 (n.s. / n.s.)	0.00591 (0.002-0.01)	ELISA	Maleki et al. 2015
Iran	Maternal breast milk	88	93.2 100	AFB1 (0.01 / 0.0157) AFM1 (0.00004 / 0.00625)	0.02418 (0.01- 0.08) 0.00316 (0.0001-0.0136)	ELISA	Azarikia et al. 2018 (#)
Jordan	Maternal breast milk	80	100	AFM1 (n.s. / 0.01)	67.78 (9.71–137.18) ng/kg	ELISA	Omar et al. 2012
Lebanon	Adults (16- ≥60 yrs) Plasma	250	33	OTA (n.s. / 0.6)	0.17 (0.1 - 0.87)	HPLC	Assaf et al. 2004
Malaysia	Adults (18-85 yrs) Serum	170	97	AFB1-lysine (0.4 pg/mg ALB)	7.67 (0.20 - 23.16) pg/mg ALB	HPLC-FLUO	Leong et al. 2012
Malaysia	Adults (25-55 yrs) Urine	22	100	AFM1 (0.0247 / n.s.)	0.0421 (0.0289 - 0.1547)	ELISA	Sabran et al. 2012 (#)
Malaysia	Adults (23-75 yrs) Serum	71	100	AFB1-lysine (0.05 / 0.17)	6.85 (1.13–18.85) pg/mg ALB	HPLC	Redzwan et al. 2014 (#)
	Pregnant women and		94	AFB1-lysine (n.s. / 0.2 pg/mg ALB)	25.28 (0.45-2939.30) pg/mg ALB	UPLC-MS	

South Asia Nepal and Bangladesh	children (6-7yrs) serum Nepal Bangladesh	 4926 44567						Groopman et al. 2014

ALB - albumin; yrs – years; n.a. – not applicable; n.s. – not specified

* year of sample collection; # validated method

3.6. Mycotoxin biomonitoring studies in America

This survey covers 12 studies reported for both South and North America (table5). Majority of the studies monitored the exposure to aflatoxin and ochratoxin A in breast milk, blood and urine. Most of the biomonitoring studies targeted healthy adults and lactating woman, except (Brewer et al. 2013) study, which targeted patients with chronic fatigue syndrome.

As presented on table 5, nursing mothers are exposed to aflatoxin at various level. AFM1 was detected in 90% and 89% of maternal breast milk samples collected in Columbia and Mexico, respectively (Cantú-Cornelio et al. 2016; Diaz and Sanchez 2015), while the incidence of AFM1 was found in less than 15% of maternal breast milk samples from Brazil (Iha et al. 2014; Ishikawa et al. 2016; Navas et al. 2005) and Ecuador (Ortiz et al. 2018). Another study of aflatoxin exposure in Brazil found AFM1 in 65% of Brazilian samples within lower mean (range) concentration 0.37 (0.25 – 12.68) pg/mg CRN (Jager 2016) while a study in USA found AFM1 positive in 11% of the analyzed samples with high mean (range) concentration of 223.85 (1.89–935.49) pg/mg CRN (Johnson 2010). Furthermore, AFB1-lysine was detected in American healthy adults within mean (range) concentration of 3.84 (1.01–16.57) pg/mg ALB, but not detected in Brazilian healthy adults. Furthermore, Aflatoxins biomarkers have been detected in CFS american patients with elevated mean (range) concentration of 4.67 (1.1-9.4) ng/ml (Brewer et al. 2013).

Exposure to ochratoxin A was reported in six studies covered by this survey. Based on the collected data it is evident that more than half of the both North and South American healthy population are exposed to OTA. It seems that population in Chile is the most exposed to OTA (Munoz et al. 2014; Munoz et al. 2006), followed by population in Argentina and Brazil (Iha et al. 2014; Navas et al. 2005; Pacin et al. 2008). Interestingly, the highest concentration of OTA was reported for CFS American patients with the incidence of 83%, where the mean (range) concentration was 6.2 (2-14.6) ng/ml (Brewer et al. 2013).

Table 5. Summary of studies measuring mycotoxin levels in human samples from South and North America.

Country	Sample type	No. of samples	Positive samples (%)	Mycotoxin type (LOD / LOQ) ng/mL	Mean (Range) ng/mL	Method of detection	Reference
Argentina	Adults Mar del Plata, plasma General Rodrigues, plasma	 199 236	 63.8 62.3	OTA (0.012 / 0.019)	 0.15 (0.019-50) 0.43 (0.019-80)	HPLC	Pacin et al. 2008
Brazil	Maternal breast milk	50	2 4	AFM1 (n.s. / 0.01) OTA (n.s. / 0.01)	n.a. (0.024) n.a. (0.011 and 0.024)	HPLC-FLUO	Navas et al. 2005 (#)
Brazil	Maternal breast milk	100	2 66	AFM1 (0.0003 / 0.0008) OTA (0.0003 / 0.0008)	n.a. (0.0003 and 0.0008) n.s. (<0.0003 -0.021)	LC-FLUO	Iha et al. 2014 (#)
Brazil	Maternal breast milk	94	5.3	AFM1 (0.003 / 0.004)	0.018 (0.013 -0.025) ng/g	HPLC	Ishikawa et al. 2016 (#)
Brazil	Adults (18-60 yrs) Serum	 113	 0	 AFB1-lysine (6 / 20 pg/mg ALB)	 n.d.	UPLC	Jager et al. 2016 (#)
	Urine	113	65 0	AFM1 (0.075 / 0.25 pg/mg CRN) AFB1-N7-guanine (0.003 / 0.01)	0.37-1.7 (0.25-12.68) pg/mg CRN n.d.		
Chile	Adults (18-83 yrs) Colbu´n zone, plasma San Vicente de zone, plasma	 88	 54 91	OTA (0.1 / 0.4)	 0.44 (0.07–2.75) 0.77 (0.22–2.12)	HPLC	Munoz et al. 2006 (#)
Chile	Lactating women Maternal breast milk Maternal plasma Infant urine	 50 45 39	 79 95.5 n.s.	OTA milk (0.01 / 0.03) OTA plasma (0.07 / 0.1) OTA urine (0.03 / 0.05)	 0.052 (0.01–0.639) 0.200 (0.072-0.639) 0.0652 (0.03–0.433)	HPLC-FLUO	Munoz et al. 2014 (#)
Columbia	Maternal breast milk	50	90	AFM1 (0.0006 / 0.0018)	0.052 (0.0009- 0.0185)	HPLC-FLUO	Diaz et al. 2015 (#)
Ecuador	Maternal breast milk	78	13 9	AFM1 (0.33 / 0.66) AFB1 (0.023 / 0.046)	0.045 (0.053- 0.458) 0.024 (0.056–0.291)	HPLC	Ortiz et al. 2018 (#)
Mexico	Maternal breast milk	112	89	AFM1 (0.00092 / 0.00279)	0.01278 (0.00301–0.03424)	ELISA	Cantu-Cornelio et al. 2016
USA	Adults (18-83 yrs) Serum Urine	 184	 20.6 11.7	 AFB1-lysine (10 pg/mL / n.s.) AFM1 (0.5 pg/mL / n.s.)	 3.84 (1.01–16.57) pg/mg ALB 223.85 (1.89–935.49) pg/mg CRN	HPLC-FLUO	Johnson et al. 2010

USA	CFS patients Urine	112	12 44 83	Aflatoxins (1 / n.s.) TCs (0.2 / n.s.) OTA (2 / n.s.)	4.67 (1.1-9.4) 0.85(0.21–5.72) 6.2 (2-14.6)	ELISA	Brewer et al. 2013 (#)

ALB - albumin; CFS - Patients with Chronic Fatigue Syndrome; CRN - creatinine; FLUO - fluorescence; yrs – years; n.a. – not applicable; n.d. - not detected; n.s. – not specified

validated method

3. Conclusion

Current review summarizes studies of the population exposure in four continents i.e. Asia, Africa, Europe and America, to major mycotoxins which include AFB1, OTA, FB1, ZEN and DON. The search of the current literature does not identified any biomonitoring study in the last 18 years for Australia. This review also outlines the metabolism of mycotoxins in the human organism and the use of these metabolites as biomarkers to assess mycotoxin exposure. Vast majority of the human mycotoxin exposure studies have assessed the main mycotoxins which have been classified by IARC either as carcinogenic group 1, 2 and 3. Human exposure to mycotoxins contamination is one of the major food borne health concerns especially in developing countries. Since the major source of contamination is food, the most efficient risk assessment approach to assess population exposure is to evaluate the mycotoxin intake from food. JECFA, FAO/WHO and European Commission established hazardous limits for the tolerated daily and weekly intakes as well as regulations specifying analytical approaches to measure the level of mycotoxins in animal feed and human food in order to prevent the consumption of contaminated feed/food. Yet, several countries still lack monitoring efforts and regulatory enforcement of mycotoxin in foodstuff such as Pakistan (FAO 2004).

Information on mycotoxin biomarkers and biotransformation of mycotoxin at the molecular level is important to assess the actual exposure to mycotoxins and to evaluate the current biomonitoring approach to measure all forms of mycotoxins in food and in human body. This has particular importance, since exposure analysis should include measurement of all metabolites and bioconjugated forms of mycotoxin to accurately assess the toxicity and risk for disease upon exposure. Biomonitoring of all metabolites and bioconjugated forms of mycotoxins could lead to a better causality determination between the elevated level of certain mycotoxin forms and various chronic diseases. The maximal permissible levels in food taking into account all mycotoxin forms should be revised to minimize human exposure. Evaluating all possible forms of mycotoxins in easy accessible body fluids such as blood and urine allow to select the best set of compounds and the best sample type to assess mycotoxin exposure. However, to this require to setup a complex study where all mycotoxin forms are measured in these body fluids over a period of time following exposure to allow to determine long, medium and short term exposure. Since intentional human exposure cannot be performed, this requires to either select individuals based on population screening or obtaining samples from individuals who become known for expose for an accidental event, which makes challenging planning and execution of such studies. Strict control and enforced legislation should be followed by development of more sensitive analytical methods and constant biomonitoring worldwide

to control mycotoxins exposure of human population. The first method for biomarker detection was developed for AFB1 almost 30 years ago. However, only from 2010 researchers started to develop and adopt multianalyte protocols to detect a large number of mycotoxins and their metabolites as well as the bioconjugated forms (Turner et al. 2012). Despite these analytical efforts the biological activity of each compound remains to be elucidated.

As evident from this review the most of the cited papers analyzed mycotoxins by liquid chromatography coupled to mass spectrometry and the most commonly investigated human sample was urine. In Asia the exposure to AFB1 and DON was proven to be common, while the most significant exposure results were found in African children who have been exposed to the most toxic mycotoxins such as AFB1 and FB1. In other parts of Africa such as South Africa, exposure to DON and ZEN are prevalent. Even in Europe, DON, ZEN and OTA have been detected in a majority of the population involved in mycotoxin exposure studies. There is a lack of knowledge on the DON and ZEN exposure in both North and South America in the last 18 years. Recent studies proved that there is a significant underestimation of the amount of mycotoxin actually ingested through food and accumulation effects in different body parts should be addressed in future human population exposure studies. Moreover, mycotoxin biotransformation and deposition in organs has not been fully studied by the scientific community, which information is required to fully understand the impact of mycotoxin on human health. To improve the risk assessment, future studies should monitor different forms of mycotoxins such as glucuronide conjugates as well as mycotoxins that have been rarely addressed such as T-2/HT-2 toxin, nivalenol, citrinin (Warth et al. 2013). In addition, some of the mycotoxin biomarkers are difficult to analyze due to the lack of analytical standards and reference materials. Therefore, synthetic laboratories should put more efforts in synthesizing mycotoxin bioconjugates and metabolite standards (Pierron 2016) and in developing novel analytical techniques that will overcome the obstacles such as the extraction and analysis of chemically diverse mycotoxins and their derivatives with large differences in their physico-chemical properties such as polarity and solubility properties (Murugesan et al. 2015). We are persuaded that multi-analyte biomonitoring approaches will contribute to improve the risk assessment of mycotoxins exposure and will contribute to identify population subgroup, which are at higher risk towards mycotoxin exposure such as patients with renal dysfunction.

4. Conflict of interest:

The authors declare that they have no conflict of interest

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