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Mini-review

Determination of *Fusarium* mycotoxin exposure in humans based on urine samples, using One Health approach

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ABSTRACT - The role of mycotoxins has been recognized in the etiology of a number of human diseases. Therefore, biomonitoring of human mycotoxin exposure is very important. One of the possible ways to do this is the urinary biomarker-based exposure determination. Over the past few decades, such studies have been conducted in many countries around the world on volunteers of different ages, genders, and eating habits, although these studies do not always use the same measurement, and calculation methods. This review focuses on the most important *fusarium* mycotoxins (deoxynivalenol (DON), zearalenone (ZEA), fumonisins (FUM), T-2, and HT-2 toxins). Because of the presence of mycotoxins in the environment-feed-food chain, One Health strategies should be adopted for the prevention of their exposure.

Keywords: *Fusarium* mycotoxins, human exposure, urinary biomarker

INTRODUCTION

Mycotoxins are secondary metabolites of molds. They cause great economic losses through their frequent occurrence in the food chain and pose a serious health risk to both animals and humans. These toxins are aggressive cytotoxins, resistant to gastric juice, insensitive to high temperatures (100-200 °C), may accumulate in various organs, directly and/or indirectly inhibit the body's specific defence mechanism (*Jávor* and *Szigeti*, 2011 a and b).

Different mycotoxins develop different diseases in different species (liver carcinoma, esophageal cancer, kidney damage, etc.). When different mycotoxins are occurring at the same time, synergistic, additive, or antagonistic interactions can occur (multi-mycotoxic effect) (*Kovács et al.,* 2016).

Fusarium mycotoxins occur worldwide in cereal grains. Mammalian cell cultures were used to show the cytotoxicity of the most common *Fusarium* mycotoxins; deoxynivalenol (DON), zearalenone (ZEA), fumonisin B1 (FB1) and moniliformin (MON). For each *Fusarium* mycotoxin the most sensitive cell line was determined for further toxicological experiments as an alternative of living animal testing. For DON and FB1 Chinese hamster ovary cells (CHO-K1) were found to be the most sensitive, the IC50 values were 0.27 and 85.5 µg/ml,

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respectively, after 48-h exposure. For MON the hepatocellular carcinoma cells (HepG2) showed the highest sensitivity, the IC50 values were 39.5 µg/ml for 48 h and 26.8 µg/ml for 72-h exposure. For ZEA Balb/c mice keratinocyte cell line (C5-O) was found to be the most sensitive, the IC50 value was 24.1 µg/ml after 72-h exposure. In this study DON was found to be the most cytotoxic of all the mycotoxins they tested, MON was the second most cytotoxic, followed by ZEA, and FB1. The results suggests that CHO-K1, C5-O, and HepG2 cells are the appropriately sensitive cell lines for biomonitoring of DON, ZEA and MON contaminated feed and food extracts (*Cetin & Bullerman*, 2005).

Methods for testing mycotoxins and their residues have evolved a lot, and become widely known and available.

Formation of mycotoxins can vary between fungal species as well as within a given species. Numerous physical, chemical, and biological methods have been developed to address the mycotoxin problem, but large-scale, practical, and cost-effective methods for treating mycotoxin-containing feeds are not currently available. For the contaminated foods and feeds detoxification strategies should be used to reduce or eliminate the adverse effects of mycotoxins, improving food and feed safety and prevent economic losses (*Manal et al.,* 2012). Depending on their mode of action, feed additives may act either by binding mycotoxins to their surface (adsorption), or by degrading or transforming them into less toxic metabolites (biotransformation) (*Kolosova & Stroka*, 2011). From the several mycotoxin reduction methods, it should not be stated that a single method is unconditionally effective to eliminate mycotoxin contamination of plants or to prevent the resulting health effects. Prevention strategies, cleaning and sorting methods are widely used as they serve as a first-line barrier to rid the material of various contaminants, including mycotoxins. However, other feed production technologies that also have a mycotoxin reducing effects, such as milling, dehulling and thermal methods can be controversial and limited by different practical conditions. Some physical removal methods results high weight loss, which can be a dilemma in practical manufacturing. Feed additives specifically against mycotoxins are promising but they are still in their infancy, as in the in vitro performance of some products is inconsistent and their in vivo performance requires more evidence (*Peng et al.,* 2018).

Applying the One Health approach helps to protect the population from the direct (on health) and indirect (economic, on trade and livelihood) effects of mycotoxins. The practical application of this approach is useful for the development of a functioning risk management system. Development initiatives for management systems for the early prevention of toxic exposure are important

(*Ladeira et al.,* 2017). A basic and effective measure to reduce fungal contamination in facilities for the storage of susceptible plants is to regulate the environment by manipulating ecological factors. This minimizes the entry of mycotoxins from fungi into the feed and food chain and ultimately reduces their adverse effects on animal and human health (*Imran et al.,* 2020).

It is a worldwide problem, that mycotoxins contaminate food and feedstuffs. Acute mycotoxicosis caused by high doses is currently rare. But ingestion of low and medium doses of *Fusarium* mycotoxins is quite common. These low amounts may weaken immune function and intestinal health, has effect on pathogen fitness and host-pathogen interactions, thus it can cause a different outcome of the exposure. The exposure of DON and other *Fusarium* mycotoxins generally makes worse the infection with viruses, bacteria and parasites in wide range of host species. For example: coccidiosis in poultry (*Girgis et al.,* 2008; *Girgis et al.,* 2010), enteric septicaemia of catfish (*Manning et al.,* 2005; *Manning et al.,* 2013), salmonellosis in pigs (*Vandenbroucke et al.,* 2011; *Verbrugghe et al.,* 2012) and mice (*Tai & Pestka*, 1988) and necrotic enteritis in poultry (*Antonissen et al.,* 2014 b). On the other hand, the T-2 toxin decreases the colonization capacity of Salmonella in the pig intestine. Although the effect of *Fusarium* mycotoxin exposure on infectious disease in human is less known, the animal model based extrapolations suggest possible aggravation of e.g. salmonellosis and colibacillosis in human, as well (*Antonissen*, 2014 a).

Mycotoxin-producing fungi mainly infect cereals, partly already in the production area (field molds) and partly during storage (storage molds). These toxins can be found in larger quantities mainly in whole-grain bakery products, bread, pasta, cereals, muesli. These compounds are resistant to various food production operations and can accumulate in the body when consumed. Many mycotoxins are renal and/or hepatotoxic and carcinogenic compounds, neurotoxics or endocrine disruptors. At-risk groups include those aged 0-5, those over 70, expectant mothers, and those with chronic diseases that weaken the various immune responses. Consuming cereals from controlled sources is particularly important in these groups. Furthermore, in the case of developing schoolchildren and young people, care should be taken not to overdo the consumption of whole grain-cereals (*NNK,* 2020).

In different type of foods, Regulation (EC) No 1126/2007 also regulates the maximum levels for DON and ZEA (*Table 1*). Commission Recommendation 2013/2007 / EU sets maximum recommended concentrations of T-2 and HT-2 toxins in different type of foods (*Table 2*).

Table 1
Maximum levels for DON and ZEA in foods

Mycotoxin	Product	Maximum levels (ppm)
DON	Cereals intended for direct human consumption, cereal flour, bran and germ as end-product marketed for direct human consumption	0,75
	Pasta (dry)	0,75
	Bread (including small bakery wares), pastries, biscuits, cereal snacks and breakfast cereals	0,5
	Processed cereal-based foods and baby foods for infants and young children	0,2
ZEA	Cereals intended for direct human consumption, cereal flour, bran and germ as end-product marketed for direct human consumption	0,075
	Refined maize oil	0,4
	Bread (including small bakery wares), pastries, biscuits, cereal snacks and breakfast cereals, excluding maize-snacks and maize-based breakfast cereals	0,05
	Maize intended for direct human consumption, maize-based snacks and maize-based breakfast cereals	0,1
	Processed cereal-based foods (excluding processed maize-based foods) and baby foods for infants and young children	0,02

(Commission of the European Communities Regulation (EC) No 1126/2007)

Table 2
Maximum recommended levels for T-2 and HT-2 toxins in food

Mycotoxin	Product		Maximum recommended levels (ppm)
T-2+HT-2	Cereal products for human consumption	Oat bran and flaked oats	0,2
		Breakfast cereals including formed cereal flakes	0,075
		Bread (including small bakery wares), pastries, biscuits, cereal snacks, pasta	0,025
		Cereal-based foods for infants and young children	0,015

(European Commission Recommendation 2013/165 / EU)

Commission Regulation (EC) No 1126/2007 regulates, inter alia, maximum levels for fumonisins (FUM) in different type of foods. The concentrations specified in the Regulation are given for FB1 + fumonisin B2 (FB2) (*Table 3*).

Table 3
Maximum levels for fumonisins in food

Mycotoxin	Product	Maximum levels (ppm)
FB1+FB2	Unprocessed maize, excepted for unprocessed maize intended to be processed by wet milling	4
	Maize intended for direct human consumption, maize-based foods for direct human consumption, excepted for foodstuffs listed in * and **	1
	* Maize-based breakfast cereals and maize-based snacks	0,8
	** Processed maize-based foods and baby foods for infants and young children	0,2

(Commission of the European Communities Regulation (EC) No 1126/2007.)

Exposure can be determined by two different approaches, one indirect by combining food consumption and contamination data, and another, a direct approach based on biomarkers. In both approaches, exposure is expressed as probable daily intake (PDI).

In risk assessment of mycotoxins, food consumption data and occurrence data from the corresponding foods are normally used to estimate population exposure. However, this method cannot estimate the individual intake, usually does not consider all kinds of sources of contamination, so biomarker-based methods are hence more and more used to assess dietary exposure from blood or urine concentrations (*Turner et al.,* 2012).

The determination of maximum tolerable contamination levels for mycotoxins is commonly based on estimations of tolerable daily intakes (TDIs) regarding comprehensive food consumption databases in single countries or regions. The European Food Safety Authority (EFSA) defined TDIs (*Table 4*).

Table 4
Tolerable Daily Intake values (TDIs) defined by European Food Safety Authority (EFSA)

Mycotoxin	TDI (µg/kg body weight/ day)	Source of information
DON	1	EFSA, 2017
ZEA	0,25	EFSA, 2011 a)
T-2+HT-2	0,1	EFSA, 2011 b)
FB1	1	EFSA, 2018

(EFSA, 2017; EFSA, 2011 **a**; EFSA, 2011 **b**; EFSA, 2018)

BIOMARKERS USED FOR EACH *FUSARIUM* TOXIN

FB1 levels in human urine show huge variability even under controlled conditions, which suggests that regulating the urinary excretion of fumonisin is a complex process. Nevertheless, the results confirm that urinary FB1 content is

a useful biomarker to assess exposure in ongoing population-based studies. If the level of exposure is relatively constant, there shouldn't be significant difference between morning and afternoon urine samples. However, it is complicated to link urinary FB1 content to dietary fumonisins because there are significant differences between individuals and the rate of excretion may also vary. Nevertheless, monitoring urinary FB1 levels - combined with the use of multiple-mechanism biomarkers - is an important tool in the investigation of fumonisins (as contributing factor of the development of human diseases such as esophageal cancer), especially in areas where the population consumes large amounts of maize and thus high exposure is probable. Eight volunteers from Guatemala consumed foods contaminated with FB1 (mean 2.94 ± 0.55 μg/kg). The urinary recovery of FB1 in these cases averaged 0.5 ± 0.24% of the dose (*Riley et al.,* 2012).

FB1 is a structural analogue of sphinganine, that is why FB1 is a specific inhibitor of the ceramide synthetase enzyme, thus interfering with the formation of complex sphingolipids (*Wang et al.,* 1992). In animal experiments blood and urine sphinganine/sphingosine (SA/SO) concentrations predicted fumonisin toxicity early, specifically and sensitively. However, it is not a sensitive indicator of the extent of FUM uptake in humans and is not a good biomarker for estimating human exposure (*Van der Westhuizen et al.,* 2008). In the case of fumonisins, urinary FB1 level is probably the most appropriate exposure biomarker in humans. Furthermore, DON, which was detected in urine, was also found to be strongly correlated with the amount of DON consumed (*Turner et al.,* 2011 a).

Shephard et al. (2013) found that urinary biomarker-based mycotoxin measurement is a valuable and efficient method for the detection of various mycotoxins (including DON, ZEA, α-zearalenol (α-ZOL), β-zearalenol (β-ZOL) and FB1). This is especially true in areas where it is difficult to collect food samples and it is hard to study food consumption data. This was the first publication on urinary DON, ZEA, α- and β-ZOL.

In the case of DON, urinary metabolites and in vitro results indicate that the major detoxification pathway is glucuronidation (*Maul et al.,* 2012). The epoxidation pathway is likely to be less significant in humans (*Piekkola et al.,* 2012). T-2 and HT-2 toxins and their metabolites are rare in human urine (*Fan et al.,* 2019).

ANALYTICAL DETERMINATION OF URINE BIOMARKERS

Numerous studies have been published worldwide on risk assessment of urine-based biomarkers. The urine sample on which the test is based is collected as follows: after the volunteer has accurately recorded the food which they consumed for 3 days the volunteer collects urine for 24 hours on the fourth day. Participants complete a questionnaire about their health status before conducting the studies. Samples from individuals with liver and /or kidney disease are generally not considered due to potential disturbances in mycotoxin and creatinine metabolism. Samples are stored frozen before transport to the site of analysis. Frozen samples are assayed for multi-mycotoxins (for example DON, DOM-1, FB1, FB2, ZEA, α-ZOL, β-ZOL, ochratoxin A (OTA), aflatoxin M1 (AFM1), T-2 toxin, HT-2 toxin, nivalenol (NIV), etc.). Urine samples are thawed and centrifuged. The samples are then treated with β-glucuronidase / sulfatase enzyme. The hydrolyzed urine is then diluted with water and usually purified using an immuno-affinity column. (The column is specific for the measured mycotoxins. For example when DON is measured, they use an immuno-affinity column specific for DON. In case of a multi mycotoxin measurement, they can use multi-mycotoxin immuno-affinity columns, which are specific for several mycotoxins.) The analyses are carried out by high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS). Toxin content is usually expressed in "µg/L urine" (*Solfrizzo et al.,* 2014; *Gerding et al.,* 2014; *Heyndrickx et al.,* 2015; *Gambacorta et al.,* 2018; *Mitropoulou et al.,* 2018; *Franco et al.,* 2019; *Lemming et al.,* 2020).

EVALUATION OF RESULTS (CALCULATION OF PDI)

Using the urine biomarker concentrations measured in different urine tests, the PDI of each mycotoxin can be calculated according to the following formula (*Solfrizzo et al.,* 2014; *Gerding et al.,* 2014):

$$PDI = (C \times V \times 100) / (W \times E)$$

where: PDI =probable daily intake of mycotoxin (µg/kg body weight/day)
C - human urinary biomarker concentration normalized for creatinine (µg/L)*
V - 24 h human urine volume measured for each volunteer (L)
W- human body weight measured for each volunteer (kg)
E - mean urinary excretion rate of mycotoxin*

*Urinary creatinine levels provide important data on excretion rate and renal function. Urine creatinine content is usually determined by a kinetic colorimetric assay based on the Jaffe method (Toora & Rajagopal, 2002). The measured toxin concentrations are normalized to the urinary creatinine level (mycotoxin concentration/creatinine concentration).

The results obtained are finally usually compared with TDIs.

*A wide variety of excretion rates are used, which greatly influences the PDI value and whether it will exceed the TDI value. For example, *Gambacorta et al.* (2013) found strong correlations between the amount of relevant biomarkers excreted in 24 h post-dose urine and the amount of mycotoxins ingested in piglets. Many studies used these excretion rates in the past years. They found that the mean percentages of dietary mycotoxins excreted as biomarkers in 24 h post-dose urine was 36.8% for ZEA, 28.5% for DON and 2.6% FB1. On the other hand *Shephard et al.* (2013) found, that the excretion rate for FB1 varies only 0,5-0,8%. *Riley et al.* (2012) also found a 0.5% excretion rate in the case of FB1 and they calculated with a 50% excretion rate for DON in human. In contrast, *Warth et al.* (2013) found, that the excretion rate for DON is 68%, but for ZEA is only 9,4% in humans.

ESTIMATES OF EXPOSURE IN TROPICAL AND SUBTROPICAL AREAS

In sub-Saharan Africa (Cameroon) measurements from human urine samples have shown that some Cameroonians are highly exposed to various mycotoxins. For example, for fumonisins, DON, and NIV the PDI has been found to exceed the TDI in some cases. Concentrations measured for FB1 are of concern, as both the average probable daily intake (APDI) and the maximum probable daily intake (MPDI) exceed the TDI (*Abia et al.,* 2013).

In a survey from Egypt: only 2% (n = 69) of urine samples from pregnant women were positive for DOM-1 with values between 0.1 and 0.12 ng/ml. It was concluded that deepoxy-deoxynivalenol (DOM-1) is not the main route of detoxification (*Piekkola et al.,* 2012).

During experiments in Brazil, mycotoxins were detected in 53% of food samples and 93% of urine samples. Based on the results, high exposure of the studied population to DON and fumonisins was established. Although the incidence of aflatoxins was low, the measured concentrations reached potentially hazardous values for health. Incidence and exposure levels showed an inverse pattern in food and urine samples: measurement based on food samples showed smaller, while measurement based on urine samples showed higher exposure (*Franco et al.,* 2019).

Investigations in South Africa showed that urinary FB1 levels are adequate to indicate FB1 exposure. The use of this biomarker improves the evaluability of exposure data, thereby contributing to the mapping of FUM contamination, the development of contamination reduction strategies, and the mapping of

health effects (*Van der Westhuizen et al.,* 2011). In South Africa, scientists detected FB1 in nearly 96% of urine samples in a group of home-grown corn-consuming people (*Shephard et al.,* 2013). Urine studies in Mexico have found the LC / MS-MS method to be sufficiently sensitive for the detection of FB1 (*Gong et al.,* 2008). The One Health approach is particularly justified in rural areas of Africa and can prevent population from direct (on health) and indirect (e.g. economies) effects of mycotoxins, which represent a serious health problem as well considerable economic losses. In these poor regions usually no regulations are available, the infrastructure for prevention and controlling food contamination is less developed and they do not allow the rejection of contaminated food. A complex risk management system is needed (*Ladeira et al.,* 2017).

In urine samples from Haiti, and Bangladesh (n = 42 and 95, respectively), FB1 was not detectable in samples from Bangladesh and only 1% of Haitian samples contained FB1 (*Gerding et al.,* 2015).

T-2 and HT-2 toxins were also not detected from urine samples from India (n=60) (*Warth et al.,* 2014). Neither T-2, HT-2 and nor HT-2-4 glucuronic acid (HT-2-4-GlcA) were detected in samples from Bangladesh and Haiti (n = 42 and 95, respectively) (*Gerding et al.,* 2015). Furthermore, HT-2 was not detected in the urine samples collected in Nanjing (China) (n=260) and only 2.0% of the samples contained T-2 (mean concentration, 2.45 µg/L; range, 0.742–3.61 µg/L) (*Fan et al.,* 2019).

ESTIMATES OF EXPOSURE IN EUROPE

Based on the first approach interpretation of urinary ZEA concentrations, PDIs do not exceed TDI (0.25 µg/kg body weight) for European samples (however, for urine samples from Haiti and African countries, PDIs exceed TDI). There is no difference between men and women in the urinary α-ZoL/ZEA ratio. This ratio ranges from 0.83 to 10. Furthermore, the data support that estimation based on urine biomarkers is a suitable method to biomonitoring the ZEA exposure (*Mally et al.,* 2016).

In the UK adult urine survey for DON and DON glucuronides (n = 34), only two samples from the same volunteer were positive at very low DOM-1 concentrations (0.5-0.8 ng/ml), that was about 1% of the detected DON + DON glucuronides (57.9 and 61.8 ng/ml). It was concluded that deepoxy-deoxynivalenol (DOM-1) is not the main route of detoxification. In contrast, deoxynivalenol conjugated with glucuronic acid (DON-GlcA) by uridine diphosphate

glucuronyltransferase (UDP-GT) appears to be the main metabolite. In addition, unconjugated DON also persisted in the body and was excreted in the urine in 68% of the studied group (*Turner et al.,* 2011 b).

In urine and blood serum studies of Swedish adolescents, and it was found that the concentration of DON in the urine is generally low, however, in 2% of cases, the PDI exceeds the TDI. In the case of DON, a significant correlation was found between cereal consumption and exposure (*Lemming et al.,* 2020).

Extended urine multi-biomarker analysis of Swedish adults and children found that biomonitoring of mycotoxins is a useful tool to confirm mycotoxin exposure and in trend analyses. Furthermore, this test method is also an important tool to support the association of exposure with the consumption of certain food groups, at least when there is a major source of mycotoxin intake. In addition, the method has a role in exploring the influence of other factors (such as the socio-economic situation). The development of these studies is highly dependent on the validation of sampling procedures and analytical methods, as well as the development of reference materials and toxicokinetic studies in humans (*Mitropoulou et al.,* 2018).

In two Italian volunteers, DOM-1 was not detected in the urine even after treatment with β-glucuronidase enzyme. In parallel, a 1.7-fold increase in DON concentration was observed using β-glucuronidase enzyme (*Lattanzio et al.,* 2011). In contrast, when analyzing the urine samples of 32 Belgian volunteers, 25% of the samples were positive for deepoxy-deoxynivalenol glucuronic acid (DOM-1-GlcA) (*Huybrechts et al.,* 2015).

In southern Italy, mycotoxins (including DON, FB1, ZEA, α-ZOL and, β-ZOL) were determined by urinary biomarkers. Several mycotoxins were found in all urine samples of volunteers in the study. The PDI estimated by the urinary biomarker approach for DON, FB1, and ZEA was found to fit well with the intake calculated from the dietary approach reported in the literature (*Solfrizzo et al.,* 2014).

In urine samples from Germany (n=50), FB1 was not detectable in the samples (*Gerding et al.,* 2015). In general, low FB1 concentrations can be found in human urine samples (*Vidal Corominas et al.,* 2018). That is because of low oral bioavailability of FB1, which is 5% or less (*Schelstraete et al.,* 2020). Neither T-2, HT-2 and nor HT-2-4 glucuronic acid (HT-2-4-GlcA) were detected in samples from Germany (*Gerding et al.,* 2015).

In Central Europe, studies based on urinary biomarkers have found that mycotoxin exposure in the German population is low, except DON and DON-GlcA, which had a higher incidence and, PDIs calculated from the measured concentrations were close to the bid values and exceeded the bid values in

12% of cases. No significant correlation could be found between the dietary habits of the participants and the mycotoxin exposure. This is presumably due to the relatively low number of samples and low exposure values (*Gerding et al.,* 2014). However, a strong quantitative correlation was found between dietary DON and urinary DON content. Furthermore, urinary monitoring of DON was found to be essential for experiments investigating DON exposure and health effects (*Turner et al.,* 2010).

In a study of 27 Austrian volunteers, the mean measured DON+DON-GlcA concentration was found to be 20.4 ng/ml (the LOD was 4 ng/ml for DON and 6 ng/ml for DON-GlcA). 96% of the samples were positive for DON-GlcA, and in 22% was free of DON. However, DOM-1 was not detectable. On average, 86% (79–95%) of total DON (DON and DON metabolites) was DON-GlcA (*Warth et al.,* 2012). Conjugation is probably the main route of detoxification and de-epoxidation is less important (*Schelstraete et al.,* 2020).

In Hungary, fumonisin exposure was estimated based on population consumption data of the Hungarian National Food Chain Safety Office (NÉBIH) and Hungarian Central Statistical Office (KSH), and FB1 & FB2 contamination of edible maize-based foods. The results showed that the average toxin intake of the population was well below the reference values set by *JECFA* (2016): 2 µg/kg bw/day FB1+FB2+fumonisin B3 (FB3) and EFSA (2018): 1 µg/kg bw/day FB1. However, in 1% of the subjects (n = 60), the PDI (in one case it was 1,81 µg/kg body weight (bw)/day) exceeded TDI (1 µg/kg bw/day). Children's involvement was 2.5 times bigger than the mean (*Zentai et al.,* 2019). On the other hand, when exposure assessment was carried out based on urine multi-mycotoxin analysis, the ratio of volunteers with PDI exceeding TDI was approx. 12% (calculated by the excretion rate in pigs, according to *Gambacorta et al.,* 2013) (unpublished data).

CONCLUSIONS

Urine biomarker-based research are widespread worldwide and are widely used. The method has a significant scientific background. In different countries, volunteers of different ages, genders, and diets were studied using this method. Urine biomarker-based research is considered a very good method to determine *Fusarium* mycotoxin exposure and assess the human health risk they pose. However, it is important to mention that in some cases huge differences can be found between the excretion rates, and in addition, the data and calculation methods found in the literature are not always uniform. For this reason, the different results can only be compared by taking these into account.

Furthermore, the holistic approach reported by One Health is typically not taken into account when evaluating results.

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