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Human exposure to mycotoxins and their masked forms through cereal-based foods in Belgium

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HIGHLIGHTS

- An assessment of exposure of the population to (masked) mycotoxins was performed.
- A deterministic and probabilistic exposure assessment was executed.
- Mean mycotoxin intakes for all cereal-based foods were beneath tolerable daily intake.
- Sub-populations can be exposed to high levels of zearalenone-equivalents.
- Systematic monitoring of all mycotoxins is a prerequisite to reach safety levels.

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ABSTRACT

In the present study, a quantitative dietary exposure assessment of mycotoxins and their masked forms was conducted on a national representative sample of the Belgian population using the contamination data of cereal-based foods. Cereal-based food products ($n = 174$) were analysed for the occurrence of deoxynivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, zearalenone, α-zearalenol, β-zearalenol, T-2-toxin, HT-2-toxin, and their respective masked forms, including, deoxynivalenol-3-glucoside, zearalenone-4-glucoside, α-zearalenol-4-glucoside, β-zearalenol-4-glucoside and zearalenone-4-sulfate. Fibre-enriched bread, bran-enriched bread, breakfast cereals, popcorn and oatmeal were collected in Belgian supermarkets according to a structured sampling plan and analysed during the period from April 2010 to October 2011. The habitual intake of these food groups was estimated from a national representative food intake survey. According to a probabilistic exposure analysis, the mean (and P95) mycotoxin intake for the sum of the deoxynivalenol-equivalents, zearalenone-equivalents, and the sum of HT-2-and T-2-toxin for all cereal-based foods was 0.1162 (0.4047, P95), 0.0447 (0.1568, P95) and 0.0258 (0.0924, P95) $\mu g\,kg^{-1}$ body weight day^{-1}, respectively. These values were below the tolerable daily intake (TDI) levels for deoxynivalenol, zearalenone and the sum of T-2 and HT-2 toxin (1.0, 0.25 and 0.1 $\mu g\,kg^{-1}$ body weight day^{-1}, respectively). The absolute level exceeding the TDI for all cereal-based foods was calculated, and recorded 0.85%, 2.75% and 4.11% of the Belgian population, respectively.

Abbreviations: DON, deoxynivalenol; 3ADON, 3-acetyl-deoxynivalenol; 15ADON, 15-acetyl-deoxynivalenol; DON3G, deoxynivalenol-3-glucoside; β-ZEL, β-zearalenol; α-ZEL, α-zearalenol; ZEN, zearalenone; ZEN4G, zearalenone-4-glucoside; ZEN4S, zearalenone-4-sulfate; β-ZEL4G, β-zearalenol-4-glucoside; α-ZEL4G, α-zearalenol-4-glucoside; HT-2, HT-2 toxin; T-2, T-2 toxin; TDI, tolerable daily intake; DON-equivalents, DON3ADON, 15ADON and DON3G; ZEN-equivalents, ZENZEN4G, ZEN4S, α-ZEL, β-ZEL, α-ZEL4G, β-ZEL4G; LOD, limit of detection; LOQ, limit of quantification.

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1. Introduction

Fusariotoxins are secondary metabolites produced by toxigenic micromycetes of the genus *Fusarium* (*F*) (Conkova et al., 2003). *Fusarium* species might endanger human health through the action of their toxic metabolites, mycotoxins (Bauer et al., 1980). Besides fumonisins, most dominant mycotoxin production includes trichothecenes and myco-estrogens. The clinical outcome is known as mycotoxicosis, which shows a variety of clinical symptoms where synergistic or additive effects between several mycotoxins can enhance the adverse health effects for the exposed organism (Grenier and Oswald, 2011).

Trichothecenes are a family of cyclic sesquiterpenoids and according to their functional groups, they are divided into four groups (A, B, C and D). Type A trichothecenes, T-2-toxin (T-2) and HT-2-toxin (HT-2), produced by *F. poae*, *F. langsethiae* and *F. sporotrichioides*, as well as deoxynivalenol (DON, type B), produced by *F. graminearum* and *F. culmorum*, are the most abundant trichothecenes (Richard et al., 2007). Type C-trichothecenes possess an additional epoxide nevertheless they are not produced by *Fusarium* species. Type D are also non-*Fusarium* mycotoxins and contain a macrocyclic ring; these airborne *Stachybotrys* mycotoxins include satratoxins, roridins and verrucarins and are prevalent in indoor environments. Type A and B are widely distributed in cereals as natural pollutants, whereas the macrocyclic trichothecenes rarely occur in food and feed.

Trichothecenes (type A and B) bind readily to eukaryotic ribosomes and are potent inhibitors of the translation process, inducers of apoptosis in lymphatic and haematopoietic tissues, and cause damage to cellular membranes (Ostry, 1998; Pestka and Smolinski, 2005). Acute exposure induces radiomimetic effects and gastrointestinal manifestations such as diarrhoea, vomiting and melena, while chronic exposure reported effects as anorexia, reduced weight gain, retarded growth, nausea and degeneration of the immune, neural and reproductive systems.

Zearalenone (ZEN) is mainly produced by *F. graminearum*, *F. crookwellense*, *F. sporotrichioides* and *F. culmorum*, consequently co-occurrence with DON, T-2 and HT-2 was described (Pittet, 1998). ZEN, acting similarly to 17β-estradiol, causes strong estrogenic outcomes, alters consequently the reproductive tract and is associated with hyperestrogenism, although haematotoxic and genotoxic properties were also described (Minervini et al., 2005; Ostry, 1998; Turcotte et al., 2005). In humans, the occurrence of ZEN in plasma was associated with precocious puberty, endometrial adenocarcinomas and hyperplasia (Saenz de Rodriguez et al., 1985; Tomaszewski et al., 1998). The most abundant derivatives of ZEN are α-zearalenol (α-ZEL) and β-zearalenol (β-ZEL). Metabolization of mycotoxins by plants can partly occur, and gives rise to the production of so called "masked mycotoxins" (Berthiller et al., 2009a). Three phases of chemical modifications of these xenobiotic compounds can be distinguished during the plant metabolism. The phase I process includes the reduction, oxidation or acetylation of the parent mycotoxin resulting in an activation of the derived molecule and a higher toxicity level (*e.g.* α-ZEL). Phase II consists of the enzymatic transformation of these reactive groups such as conjugation, glucosidation and sulfatation (*e.g.* deoxynivalenol-3-glucoside (DON3G)) leading to the formation of more hydrophilic compounds, facilitating the elimination of the masked mycotoxins and thus a decreased toxicity (Plasencia and Mirocha, 1991; Poppenberger et al., 2003; Vendl et al., 2009). Phase III comprises the compartmentalisation of the mycotoxins into the vacuole of the plant or binding to the cell wall (Berthiller et al., 2009a; Coleman et al., 1997; He et al., 2010; Zinedine et al., 2007).

A potential risk for consumers is the possible hydrolysis of masked mycotoxins into their toxic parent forms during mammalian digestion (Grabley et al., 1992; Berthiller et al., 2011).

The European Safety (EFSA) requested the evaluation of mycotoxins such as Alternaria toxins and zearalenone, however no explicit plan for masked mycotoxins has been considered for masked forms (EFSA, 2011a). The evaluation of masked mycotoxins is not (yet) available due to the lack of occurrence, bioavailability and toxicological data, however the Joint European Commission FAO/WHO Expert Committee (JECFA) considered DON3G and the acetylated forms 3ADON and 15ADON as an additional contributing factor of the total dietary exposure to DON (Codex, 2011; JECFA, 2010). Masked forms of zearalenone were not considered. Making these statements it encouraged industry and research to further investigate masked mycotoxins. Poppenberger et al. (2003) already proved that DON3G dramatically reduces the ability to inhibit protein synthesis of wheat ribosomes *in vitro*. Recently, Berthiller et al. (2011) showed the toxicological relevance of DON3G by demonstrating that several lactic acid bacteria can hydrolyse DON3G *in vitro* (Berthiller et al., 2011). However, Nagl et al. (2012) demonstrated that DON3G is partially bioavailable in rats. The majority of the administered DON3G was cleaved during digestion and subsequently excreted *via* faeces. Thus, DON3G present in food and feed seems to have a significantly lower toxic equivalency compared to DON. However, due to differences regarding anatomy and gut microbiota, bioavailability and metabolization may be species dependent and should be experimentally determined.

Concerning masked ZEN-forms Ayed et al. (2011) argued that ZEN and α-ZEL exhibited the same range of cytotoxicity and genotoxicity, and both were more cyto- and genotoxic than β-ZEL. Recently, a study was executed on the amount of these forms in rats (Versilovskis et al., 2012). After administration of ZEN4G, ZEN was found in the stomach suggesting that hydrolysis is possible as was already shown for the acetylated DON-forms. Small amounts of ZEN4G were detectable in the small and large intestines suggesting that they were not fully hydrolysed. But, the large occurrence of α-ZEL (Videmann et al., 2012) and a sharp decrease of ZEN4G in the smal intestine proved hydrolysis (Versilovskis et al., 2012), as a consequence, the total human exposure and risk assessment to mycotoxins might be underestimated.

To be sure of the statements made for the masked forms of DON and ZEN, a full metabolism study should be carried out, preferably by incorporation of mycotoxins in the feed of reliable species.

The European Union (EU) has set maximum levels for certain mycotoxins as a risk management strategy, and to achieve a high level of public health protection (EC, 2006a). The Scientific Committee on Food has adopted opinions laying down a tolerable daily intake (TDI) for several toxins. It has established a TDI for DON of 1.00 $\mu g\,kg^{-1}$ body weight (bw) day^{-1}, a provisional TDI of 0.25 $\mu g\,kg^{-1}$ bw day^{-1} for ZEN and a combined provisional TDI of 0.06 $\mu g\,kg^{-1}$ bw day^{-1} for the sum of T-2 and HT-2. However, the European Food Safety Authority has recently established a TDI of 0.10 $\mu g\,kg^{-1}$ bw day^{-1} for the sum of T-2 and HT-2 (EFSA, 2011b, 2011c).

Current legal limits and control strategies only focus on the parent mycotoxins. Cereal-based products are very important in the human diet and their quality and safety should be controlled during processing throughout the entire food chain (Yazar and Omurtag, 2008). Foodstuffs which are susceptible to trichothecene and ZEN contamination include wheat, maize, barley and cereal-based products such as breakfast cereals, bread and beer. Furthermore, in these matrices co-occurrence of masked and parent mycotoxins has been previously described (De Boevre et al., 2012a; Berthiller et al., 2009b; Desmarchelier and Seefelder, 2011; He et al., 2010; Kostelanska et al., 2009; Lancova et al., 2008; Vendl et al., 2010). To date however, no risk assessments were performed for masked mycotoxins. The objective of this study was to determine the occurrence of mycotoxins and their masked forms in cereal-based foodstuffs and to their estimate exposure for the Belgian