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Dietary exposure to mycotoxins and health risk assessment in the second French total diet study

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ABSTRACT

Mycotoxins are produced in plants by micro-fungi species, and naturally contaminated the food chain. In the second French total diet study (TDS), mycotoxins were analyzed in 577 food samples collected in mainland France to be representative of the population diet and prepared ⟨⟨as consumed⟩⟩. Highest mean concentrations were found in wheat and cereal-based products (bread, breakfast cereals, pasta, pastries, pizzas and savoury pastries...). Exposure of adult and child populations was assessed by combining national consumption data with analytical results, using lowerbound (LB) and upperbound (UB) assumptions for left-censorship management. Individual exposures were compared with available health-based guidance values (HBGV). Only the exposure to deoxynivalenol (DON) and its acetylated derivatives was found to significantly exceed the HBGV in LB in adults (0.5% [0.1; 0.8]) and children (5% [4; 6]). HBGV was exceeded in UB only for T-2 and HT-2 toxins by, respectively, 0.2% [0.02; 0.05] and 4% [3; 5] of adults, and 11% [9; 12] and 35% [32; 37] of children. Although the exposures assessed were generally lower than the previous French TDS, the results indicated a health concern for trichothecenes and a need to reduce dietary exposure as well as analytical limits.

1. Introduction

Mycotoxins are secondary metabolites produced by filamentous micro-fungi species belonging to various genera such as *Aspergillus*, *Penicillium*, *Fusarium* and *Byssochlamys*, and that are capable of causing disease and death in humans and animals (Bennett and Klich, 2003; Richard, 2007). Mycotoxins are produced in plants (wheat, maize, rice, beans, oily seeds, dried fruits, nuts, grape, etc.), in the fields or during storage if the conditions are favorable for the growth of fungi (Richard, 2007). The production of mycotoxins depends on the strain of fungus, the substrate, and on the temperature and humidity conditions (Bennett and Klich, 2003). Infestation or growing of the fungi is more likely to occur on damaged plants or with lesions. Mycotoxins are usually thermostable during baking and other thermal processes (Deshpande, 2002; Scudamore et al., 2003; Turner et al., 2009). They persist more or less during the transformation of contaminated plants, depending on the process, and are usually not eliminated during cooking and sterilization. Due to their lipophilic properties and their ability to bind with plasmatic proteins, mycotoxins can persist in organisms when the exposure is chronic or repeated. Animals, which may be fed with contaminated feed of vegetable origin, may be chronically exposed to mycotoxins. Due to transfer and metabolism, animal products such as milk or offal can be contaminated.

The toxicity of mycotoxins differs depending on the kind of toxin, and it was observed in animals that it was related to the species, the dose ingested, the duration of the exposure, and their sex and age. Aflatoxins (AFs), in particular aflatoxin B1 (AFB1), the major form mostly occurring, are considered as the most strong natural genotoxic carcinogen (Squire, 1981). Experimental data showed that AFB1 carcinogenicity could be considered as 10 fold higher than aflatoxin M1 (AFM1) carcinogenicity. Of note, AFM1 is a hydroxylated metabolite of AFB1 found in milk of animals that have been exposed to AFB1, and then in dairy products (Allcroft and Carnaghan, 1963). The International Agency for Research on Cancer (IARC) classified AFB1 in group 1: "carcinogenic to humans"

Abbreviations: 3-Ac-DON, 3-Acetyldeoxynivalenol; 15-Ac-DON, 15-Acetyldeoxynivalenol; α-ZAL, alpha-zearalanol; β-ZAL, beta-zearalanol; α-ZOL, alpha-zearalenol; β-ZOL, beta-zearalenol; AF, aflatoxin; AFB1, aflatoxin B1; AFB2, aflatoxin B2; AFG1, aflatoxin G1; AFG2, aflatoxin G2; AFM1, aflatoxin M1; BW, body weight; DON, deoxynivalenol; EFSA, European Food Safety Authority; FB, fumonisin; HBGV, health-based guidance value; IARC, International Agency for Research on Cancer; INCA, French individual national consumption survey; JECFA, Joint FAO/WHO Expert Committee on Food Additives; LOD, limit of detection; LOQ, limit of quantification; NIV, nivalenol; NOAEL, no-observed-adverse-effect level; OTA, ochratoxin A; OTB, ochratoxin B; PAT, patulin; PTMDI, provisional maximum tolerable daily intake; PTWI, provisional tolerable weekly intake; SCF, Scientific Committee on Food; TCT, trichothecene; TDI, tolerable daily intake; TDS, total diet study; ZEA, zearalenone.

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(IARC, 1993). Based on epidemiological data in human, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) calculated for European adults a risk per unit dose of 0.013×10^{-5} (ng/kg bw/day)$^{-1}$, i.e. an increase in the incidence of cancer in the general population of 0.013 cancers per year per 100,000 subjects per ng AFs per kg body weight per day during life-years, or 13 cancers per 100 million people (JECFA, 1998). AFM1 was classified in group 2B by IARC: "possibly carcinogenic to humans" (IARC, 1993), as well as ochratoxin A (OTA) and fumonisins B1 and B2 (FB1, FB2) (IARC, 2002). Nevertheless, the JECFA proposed a provisional maximum tolerable daily intake (PMTDI) of 2 μg/kg bw/day for fumonisins alone or combined, based on the nephrotoxic effects observed in rats (JECFA, 2001). In human, OTA has also been associated with Balkan endemic nephropathy (Tatu et al., 1998), and could be associated with immunotoxic and neurotoxic effects (EFSA, 2006). In 2007, the JECFA confirmed in 2007 the provisional tolerable weekly intake (PTWI) of 100 ng/kg bw/week proposed in 1995 based on early nephrotoxic effects in pigs (JECFA, 1996, 2007). However, based on the same effects, the European Food Safety Authority (EFSA) proposed in 2006 a PTWI of 120 ng/kg bw/week (EFSA, 2006), and confirmed it in 2010 based on new data (EFSA, 2010b).

Some other mycotoxins were considered by IARC as "not classifiable as to its carcinogenicity to humans" (group 3); it is the case for patulin (PAT) (IARC, 1986), some zearalenone (ZEA) metabolites (IARC, 1993), some trichothecenes (TCTs), in particular T-2 toxin, nivalenol (NIV), and deoxynivalenol (DON) (IARC, 1993). For those mycotoxins, other chronic toxicological effects than carcinogenicity have been reported. PAT has been shown to be cytotoxic and genotoxic. In 1995, the JECFA proposed a PMTDI of 0.4 μg/kg bw/day based on a study on rats (JECFA, 1995). This value was confirmed by the Scientific Committee on Food (SCF) in 1996 and 2000 (SCF, 1996, 2000a).

TCTs from type A, including T-2 and HT-2 toxins, induce hematological modifications in animal, immunotoxic and reprotoxic effects including, notably, inhibition of hormonal production, malformations of newborns, and fetus mortality. In 2001, the JECFA proposed a PMTDI of 60 ng/kg bw/day for T-2 toxin and HT-2 toxin, alone or combined, based on immunotoxic and hematotoxic effects in pigs (JECFA, 2001), and this value was confirmed by the SCF (SCF, 2002). Using a benchmark dose analysis the EFSA established a group TDI of 100 ng/kg bw for the sum T-2 + HT-2 toxins (EFSA, 2011b). In animal, DON could be immunotoxic and hematotoxic. In 2010, the JECFA considered the toxicity of the acetylated derivatives (3-Acetyldeoxynivalenol (3-Ac-DON) and 15-Acetyldeoxynivalenol (15-Ac-DON)) equal to that of DON and extended the previous PTMDI of 1 μg/kg bw/day (EFSA, 2007; JECFA, 2001; SCF, 2002) to a group PTMDI for the three compounds (JECFA, 2010). For NIV, the SCF established a TDI of 0.7 μg/kg bw/day based on immunotoxic and hematotoxic effects observed in mice (SCF, 2002).

ZEA may have an estrogenic activity and induce troubles of the reproduction function: lower fertility, fetal wastage, and lower hormone levels. The JECFA established a group PMTDI of 0.5 μg/kg bw/day for ZEA, based on the hormonal effects on saws (JECFA, 2000) but the SCF proposed a lower provisional tolerable daily intake (TDI) of 0.2 μg ZEA/kg bw/day based on a study on pig (SCF, 2000b). In 2011, the EFSA proposed a new TDI of of 0.25 μg/kg bw/day based on more recent data on pig, but also taking into account comparisons between pigs and humans (EFSA, 2011c).

Performed in several countries using a standardized method recommended by international bodies (EFSA, 2011a; WHO, 2005, 2006), the total diet studies (TDS) aim at providing contamination data on food prepared as consumed by the population and exposure data, in order to help the risk manager in making public health decisions. In 2001–2005, the National Institute of Agricultural Research (INRA) implemented, in collaboration with the French Food Safety Agency (AFSSA), the first French TDS including mycotoxins (Leblanc et al., 2005). In 2006–2010, the French Agency for Food, Environmental and Occupational Health Safety (ANSES, formerly AFSSA) conducted the second French TDS, also covering mycotoxins. This article aims at presenting the results of the exposure to some mycotoxins of the general French population, and evaluating the risk with regards to the international health-based guidance values.

2. Material and method

2.1. Consumption data and food sampling

First, foods representative of the diet of the French population were selected from the second national individual dietary consumption survey (INCA2), carried out in 2006–2007 and implemented through a period of 11 months. The sample of 1918 adults aged 18–79 years and 1444 children aged 3–17 years was representative of the French population through stratification (Dubuisson et al., 2010; Dufour et al., 2008). Subjects completed a 7-day food record diary (consecutive days) as well as other questionnaires on anthropometrical and socio-economical factors. Portion sizes were estimate through photographs compiled in a manual adapted from the SuViMax picture booklet (Hercberg et al., 1994). Food records were subsequently coded into 1280 food items.

Core foods were selected on the basis of two criteria: (i) the most consumed foods in terms of quantity by adults and/or children with at least 5% of consumer rate, and (ii) the main known or supposed contributors to the exposure, if they were not selected by the first criterion. The methodology was already described in the literature (Sirot et al., 2009). Two hundred and twelve core foods were selected from the consumption survey, covering 88–89% of the adult and child diet. The whole list of foods included in the TDS can be found in Sirot et al. (2009).

The food sample collection (n = 1319) was performed in eight great metropolitan regions corresponding to the INCA2 regions. In order to be as representative as possible of the French food consumption habits, each food sample was composed of up to 15 subsamples of equal weight of the same food, taking account of the market shares, origin or species, conditioning and packaging, flavoring if any, etc. (Sirot et al., 2009). Samples were prepared as consumed by the population (i.e. peeled, cooked, etc.) according to the cooking habits recorded in the INCA2 survey.

2.2. Analysis of food samples

Samples of foods which were known or supposed to contribute to the exposure to mycotoxins (n = 577) were analyzed for 25 mycotoxins: AFB1, AFB2, AFG1, AFG2 and AFM1, OTA, OTB, PAT and some *Fusarium* toxins: TCTs from type A (T-2 toxin, HT-2 toxin) and type B (NIV, DON, 3-Ac-DON, 15-Ac-DON), zearalenone and its metabolites (α-ZAL, β-ZAL α-ZOL and β-ZOL), and fumonisins B1 and B2. Based on available data from literature, on the expertise of analysts specialized in the routine analysis of raw and processed food products and on monitoring program reports, the above listed mycotoxins and their metabolites can occur only in certain types of food products. For this reason, it was not necessary to consider all types of collected food products on the list for all listed mycotoxin determination. The occurrence of some mycotoxin metabolites such as AFM1 was also considered in food from animal origin. The analyses were carried out by the Laboratory of Development and Analysis located in Ploufragan, F-22440 (LDA22), under accreditation and quality assurance conditions according to the standard NF EN ISO/CEI 17025 v2005. Series of tests were performed during each analysis: concentrations of stock solutions were checked using spectrophotometry, calibration curves were drawn for each series of analyses, spiked samples were used to calculate the performance parameters for each group of food products corresponding to the specific method (Table 1). Recovery rates were calculated for each type of food products and each assay series. Every analytical result was corrected according to the recovery rate. However results with a recovery rate below 60 or above 120% were considered as being outside of the acceptable analytical performance parameter range and consequently were not used for exposure assessment calculation. LDA22 is accredited by the French Accreditation Committee COFRAC for the program 99.1 'Mycotoxins'.

The standard NF EN 12 955 (AFNOR, 1999) was used for the detection and determination of AFB and G. Briefly this analytical technique consists of an extraction by using water + methanol solvent mixture (or methanol for fatty products), a clean-up step by using an immuno-affinity column containing anti-AF antibodies). The purified extract is injected to a reversed phase Liquid Chromatography followed by a derivatization system and fluorescence detector (IAC–LC–FD). The standard NF EN ISO 14 501 (AFNOR, 2007) was used for the detection and determination of AFM1 in milk. Briefly this analytical technique consists of a combined extraction and clean-up step of defatted liquid milk sample by using an immuno-affinity column (containing anti-AF antibodies). The purified extract is injected to a reversed phase Liquid Chromatography followed by a fluorescence detector (IAC–LC–FD). An in-house validated method was performed for the detection and determination of AFM1 in cheeses, butter, pork and poultry products. Briefly this analytical technique consists of an extraction by using methyl chloride and clean-up step by using an immuno-affinity column (containing anti-AF antibodies). The purified extract is injected to a reversed phase Liquid Chromatography followed by a fluorescence detector (IAC–LC–FD).