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Single-compound and cumulative risk assessment of mycotoxins present in breakfast cereals consumed by children from Lisbon region, Portugal

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ABSTRACT

Humans can be exposed to multiple chemicals, but current risk assessment is usually carried out on one chemical at a time. Mycotoxins are commonly found in a variety of foods including those intended to consumption by children namely breakfast cereals. The present study aims to perform, the risk assessment of single and multiple mycotoxins present in breakfast cereals consumed by children (1–3 years old) from Lisbon region, Portugal. Daily exposure of children to ochratoxin A, fumonisins and trichothecenes showed no health risks to the children population considering individual mycotoxins, while exposure to aflatoxin B_1 (AFB_1) suggested a potential health concern for the high percentiles of intake (P90, P95 and P99). The combined exposure to fumonisins and trichothecenes are not expected to be of health concern. The combined margin of exposure (MoET) for the aflatoxins group could constitute a potential health concern and AFB_1 was the main contributor for MoET. Legal limits and control strategies regarding the presence of multiple mycotoxins in foodstuffs is an urgent need. To the best of our knowledge, this is the first time a cumulative risk assessment was performed on multiple mycotoxins present in breakfast cereals consumed by children.

1. Introduction

Mycotoxins are toxic and carcinogenic metabolites produced by fungi that colonize food crops and they can occur in cereal based products as breakfast cereals. Cereals are among the first solid foods eaten by children and thus constitute an important food group of their diet (Schwartz et al., 2008). Several commercial brands provide breakfast cereals primarily marketed for this particular population group. Co-contamination of foodstuffs with known or unknown mycotoxins is being reported at an increasing high rate (Stoev, 2015) and there is a rising concern due to the hazard of exposure of combined mycotoxins to humans, which could be expected to exert greater toxicity and carcinogenicity than exposure to single mycotoxins (Bouaziz et al., 2008). Toxicological studies led the International Agency for Research on Cancer (IARC) to consider aflatoxins (AFB_1, AFB_2, AFG_1, and AFG_2) as human carcinogens (Group 1), aflatoxin M_1 (AFM_1), ochratoxin A (OTA) and fumonisins (FB_1 and FB_2) as possibly carcinogenic to humans (Group 2B) and the trichothecenes (T-2/HT-2, nivalenol or NIV and deoxynivalenol or DON) as not classifiable as to its human carcinogenicity (Group 3) (IARC, 2002). 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 risk characterization of food chemicals is based on the comparison between the dietary exposure and the relevant health-based guidance value. Dietary exposure assessment consists of combining deterministically or probabilistically food consumption

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figures with occurrence of a given chemical substance in a number of food categories. Within the general framework of chemical risk assessment, a difficult step in dietary exposure assessment is the handling of concentration data reported to be below the limit of detection (LOD) of the analytical method. These data are known as non-detects and the resulting distribution of occurrence values is left-censored. EFSA has so far mainly used substitution methods (EFSA, 2010).

Humans are naturally and frequently exposed to multiple mycotoxins, but health risk assessments are usually performed on individual mycotoxins, which may underestimate the total risks. A number of methods have been developed to predict the toxicity and risk of mixtures based on their chemical composition and knowledge of the toxicities of the mixture components. Most of these methods are based on the concepts of Concentration Addition (CA) and Independent Action (IA). CA assumes that the individual components act via a similar mode of action, only differing in their relative potency to elicit a toxic effect, whereas IA, assumes that the individual components act independently of each other (Backhaus et al., 2010; EFSA, 2013). Examples of cumulative risk assessment methods include the Hazard Index (HI) and the Combined Margin of Exposure Index (MoET) (Borg et al., 2013). The HI does not predict the overall health effect of the mixture, but provide a measure of the total risk based on the individual risk of each component. The MoE (margin of exposure) is proposed for the risk assessment of substances that have both genotoxic and carcinogenic properties and the MoET is usually used for the cumulative risk assessment (EFSA, 2013).

The few reports available in the Europe on the children dietary exposure to mycotoxins were mainly conducted to estimate the individual exposure to these toxins. In Spain, Catalonian infants (0–3 years) were identified as the most exposed population group to fumonisins through baby foods (Cano-Sancho et al., 2012). In The Netherlands, a risk assessment of the dietary exposure of young children (2–6 years) to contaminants suggested that the health risk for FB_1, patulin (PAT) and DON was negligible (Boon et al., 2009).

Considering the scarce information on the risk assessment of children to multiple mycotoxins in breakfast cereals and the fact that they are the main food source of whole grains in children diet, the present study aims to perform, for the first time, the risk assessment of mycotoxins present in breakfast cereals consumed by children from Lisbon region, Portugal.

2. Materials and methods

2.1. Mycotoxins occurrence data

Twenty six breakfast cereals primarily marketed for children were purchased from supermarkets in Lisbon region, in 2014, including in their composition maize, wheat, rice and multigrain. Samples were homogenized in a food homogenizer, saved in plastic bags and stored in fridge at 4 °C until further analysis.

2.1.1. Aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2 and AFM_1) and ochratoxin A (OTA)

Aflatoxins and ochratoxin A determination was performed according to the method described in EN15851, with few modifications (EN ISO, 2010). Mycotoxins were quantified by RP-HPLC with post column derivatization involving bromination followed by fluorescence detection. HPLC analysis was performed using a Waters® Alliance 2695 equipped with fluorescence detector Waters® 2475 (Milford, MA, USA) with Empower® Chromatography Software. Mycotoxin standard solutions were from Biopure® (Austria).

2.1.2. Trichothecenes (DON, NIV, T2-Toxin and HT-2 Toxin)

Trichothecenes extraction was performed according a procedure based on QuEChERS methodology previously developed by Pereira et al. (2015). GC–MS analysis was performed on an Agilent® (Little Falls, DE, USA) gas chromatograph 6890 equipped with an electronically controlled split/splitless injection port and an inert 5973N mass selective detector with electron impact (EI) ionization chamber. Standards of DON and NIV were purchased from Fluka® (West Chester, PA, USA), while T2-Toxin and HT-2 Toxin were purchased from Sigma® (St. Louis, MO, USA). The internal standards (IS) used were α-chloralose (IS1) and $^{13}C_{15}$-DON solution (IS2), purchased from Sigma® and Fluka®, respectively. Dispersive-SPE sorbents for experiments including C_{18}-bonded silica were purchased from Waters® (Milford, MA, USA) and primary secondary amine (PSA; particle size 50 mm) from Supelco® (Bellefonte, PA, USA).

2.1.3. Fumonisins (FB_1 and FB_2)

Fumonisins extraction was described by Ndube et al. (2011) with minor modifications. Extracts were analyzed on a Waters® Acquity I-Class UPLC system (Milford, MA, USA) equipped with a BEH C_{18} column (2.1 × 50 mm, 1.7 μm) and coupled to a Waters® Xevo TQ-S mass spectrometer (Milford, MA, USA). The column was kept at 40 °C during analysis, and samples were maintained at 15 °C. The fumonisin B_1 and B_2 standards were purchased from Sigma® (St. Louis, MO, USA).

2.1.4. Performance of the analytical methods

Method performance was evaluated and limits of detection (LOD) and quantification (LOQ) (μg kg^{-1}), linearity range (μg kg^{-1}), coefficient of determination (R^2) and recoveries (%) were determined for all the studied mycotoxins.

2.2. Consumption data

The food consumption data were obtained from a pilot study performed between February and June 2014, in a Primary Health Care Unit in Lisbon region (Cidadela, Cascais, Portugal). This survey included a sample of 103 children, aged between 0 and 3 years old and selected from all children who were enrolled and attended the Primary Health Care Unit in the period of the survey. Information to the participant, with a standard explanation of why the survey was being carried out, was given to each parent before the interview. An informed consent was signed. The interview, in two parts, consisted of a brief personal history, followed by the explanation about how to fill the food diary. The brief personal history included among other gender, age, body weight (bw), height, birth date, food intolerances, physical activity and family data. The food diary was filled from three consecutive days for each child participant. This survey was conducted according to the guidelines laid down in the declaration of Helsinki and was approved by the Ethical Committee of the National Institute of Health Doutor Ricardo Jorge and by the Portuguese Data Protection Authority. Considering that children may begin to eat breakfast cereals after one year old, a subsample of 75 children aged between 1 and 3 years old was considered in the present study.

2.3. Exposure assessment

Two mathematical approaches, point evaluation (deterministic approach) and Monte Carlo simulation (probabilistic approach) were used for the computation of the exposure assessment of mycotoxins.

Four different scenarios were included for the mycotoxin dietary exposure assessment in relation to the data treatment of