Food and Chemical Toxicology 118 (2018) 541–548

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Multiple mycotoxin exposure of infants and young children via breastfeeding and complementary/weaning foods consumption in Ecuadorian highlands

Johana Ortiz[a,b,*], Liesbeth Jacxsens[a], Gabriela Astudillo[b], Adriana Ballesteros[b], Silvana Donoso[b], Lieven Huybregts[a,c], Bruno De Meulenaer[a]

[a] Department of Food Safety and Food Quality, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000 Ghent, Belgium
[b] Department of Biosciences, Food Nutrition and Health Research Unit, Faculty of Chemical Sciences, Cuenca University, Av. 12 de Abril s/n Cdla. Universitaria, P.O. Box 01.01.168, Cuenca, Ecuador
[c] Child Health and Nutrition Unit, Department of Public Health, Institute of Tropical Medicine, Nationalestraat 155, 2000 Antwerp, Belgium

ARTICLE INFO

Keywords:
Exposure assessment
Mycotoxins
Breast milk
Staple cereals
Ecuadorian children

ABSTRACT

The dietary exposure to mycotoxins in Ecuadorian children aged 0–23 months (320 rural and 603 urban) was evaluated based on the intake of breast milk and staple cereals used as complementary/weaning foods. A probabilistic distribution approach by first order Monte Carlo simulation was adopted to assess the locally occurring mycotoxins (aflatoxins M_1 and B_1 in breast milk, ochratoxin A and deoxynivalenol in wheat noodles and oat flakes, and HT-2 toxin in polished rice). Overall, exposure was modest but higher for rural children due to their monotonous diet. Aflatoxin exposure by breast milk intake were of health concern in both areas (Margin of Exposure and Combined Margin of Exposure Index < 10,000). Mycotoxin exposure by staple cereals intake was considered tolerable across feeding stages for individual mycotoxin-cereal combination (Hazard Quotient < 1) and combined exposure (Hazard Index < 1). The major exposure was to HT-2 toxin by rice intake at complementary feeding (15% rural and 4% urban above TDI) and at weaning stage (26% rural and 6% urban above TDI). Since the usual Ecuadorian diet is based on the same staple cereals, risk management actions could lead to a better protection of young children and also ensure higher safety of the recommended breastfeeding practices by protecting nursing mothers.

1. Introduction

Mycotoxins contaminate the diet of a large proportion of the world's population. Dietary exposure to mycotoxins might be higher in developing countries because of several conditions such as favorable environment for fungal growth and mycotoxin production; reliance on subsistence farming, and poor quality monitoring and enforcement of regulations in local markets (Shephard, 2008; Wild and Gong, 2010).

Infants and young children are particularly at risk and are about three times more susceptible than adults to the adverse effects of mycotoxins due to their higher intake/body weight ratio, higher metabolic rate and lower detoxification capacity (Hulin et al., 2014; Sherif et al., 2009).

The mycotoxin risk in children depends on the magnitude and frequency of exposure (Blankson and Mill-Robertson, 2016). A high risk is expected when consuming monotonous cereal-based diets that are typically contaminated with several mycotoxins (Cheli et al., 2014). At infancy, another potential dietary source of exposure is breast milk due to the possible lactational transfer of several mycotoxins and their metabolites from maternal diet (El-Tras et al., 2011). From those, the hydroxylated metabolite aflatoxin M_1 (AFM_1) is one of the major occurring mycotoxins in breast milk together with its carcinogenic precursor aflatoxin B_1 (AFB_1) (Gürbay et al., 2010; Turconi et al., 2004).

As in other Latin American (LA) countries, cereals are the most important complementary foods for infants and young children in Ecuador (Leonard et al., 2000). A rather low degree of contamination and co-occurrence of the major mycotoxins of health concern in the main staple cereals in Ecuador (polished rice, wheat noodles and oat

Abbreviations: AFB_1, aflatoxin B_1; AFM_1, aflatoxin M_1; BMDL, lower confidence limit of bench mark dose; DON, deoxynivalenol; HI, Hazard Index; HQ, Hazard Quotient; LOD, limit of detection; MOE, margin of exposure; MOET, combined margin of exposure index; PMTDI, provisional maximum tolerable daily intake; PTDI, provisional tolerable daily intake; OTA, ochratoxin A

* Corresponding author. Department of Biosciences, Food Nutrition and Health Research Unit, Faculty of Chemical Sciences, Cuenca University, Av. 12 de Abril s/n Cdla. Universitaria, P.O. Box 01.01.168, Cuenca, Ecuador.
E-mail address: johana.ortiz@ucuenca.edu.ec (J. Ortiz).

https://doi.org/10.1016/j.fct.2018.06.008
Received 30 December 2017; Received in revised form 3 June 2018; Accepted 4 June 2018
Available online 05 June 2018
0278-6915/

flakes) was previously reported (Ortiz et al., 2013a). Despite this however, a risk of chronic exposure might be expected as well as a hazard from combined exposure to multiple mycotoxins (Assunção et al., 2015).

This study is the first report of dietary exposure to co-occurring mycotoxins in infants and young children aged 0–23 months in Ecuador and LA countries. The exposures to HT-2 toxin through polished rice; deoxynivalenol (DON) and ochratoxin A (OTA) through wheat noodles; DON and OTA through oat flakes, and AFM_1 and AFB_1 through breast milk intake were evaluated according to the child feeding pattern and as combined exposure. To prioritize risk management strategies, risk characterization was assessed comparing the estimated daily exposures to the reported toxicological levels for chronic exposure of individual mycotoxins. For combined exposure to multiple mycotoxins, the component-based approach based on concentration-addition method was applied.

2. Material & methods

2.1. Food consumption data

Data on the consumption of staple cereals, i.e. rice, wheat noodles and oat flakes, were collected from a total of 998 children aged 0–23 months, 348 from a rural canton (Nabon) and 650 from an urban canton (Cuenca), Azuay province, at the southern Ecuadorian highlands. These data were part of a cross-sectional survey conducted from June to September 2008 to evaluate nutritional status in the Ecuadorian highlands. This study was approved by the Ethics Committee of the University Hospital of Ghent, Belgium (Approval code B67020084011), and the Ethics Committee of the Central University of Quito, Ecuador (N° CBM/COBI 001-08). The sample size of the study was computed to detect a difference of 100 kcal d^{-1} in energy intake between the urban and rural setting, with a statistical power of 90%, type I error of 5% and assuming a 20% of non-response.

The selection of the participants was previously described (Ortiz et al., 2013b). Briefly, the rural canton Nabon is located in the country side at 3000 m above sea level. It has a considerable territorial dispersion which complicates the access the different communities (Municipal, 2012). Rural households were randomly selected from the census register of the children under 24 months from all communities of the canton. The urban canton Cuenca is the third largest city in Ecuador and it is the capital of the province. Cuenca is located at approximately 2550 m above sea level and at 70 km from Nabon (Guía-Oficial, 2012). There was no child register available in the urban canton and therefore, a cluster random sampling scheme was adopted using residential blocks as primary sampling unit. All households belonging to a selected block were visited door-to-door and the surveys were conducted without restriction in the number of infants that could be found per block.

In both settings, individual consumption data were obtained from the primary child caregivers at their homes using 24-h dietary recall questionnaires. Duplicate 24-h recalls were carried out in the urban area, while a single 24-h recall was conducted in the rural area due to limited access of the communities. To estimate portion sizes, each respondent was asked to fill a household recipient with the actual amount of food consumed by the child. This amount was determined in volume (mL) by trained interviewers and then converted into grams using recalled data of the consistency of each food consumed. Detailed recipe data were also collected to calculate the actual amount of consumed rice, wheat noodles and oat flakes in each of the composite dish. Breast milk intake was estimated assuming a proxy conversion factor of 13.5 g of milk per minute of breastfeeding (Da Cunha et al., 2013; Mills and Tyler, 1992). Data entry was done using Lucille food intake software (Ghent-University, 2010; Ochoa-Avilés et al., 2014) which allowed estimating of food intake at ingredient level, based on pre-set food composition databases.

The consumption of the cereal-based staples foods and breast milk per day was calculated and expressed as kg per kg^{-1} body weight (bw) using data from individual weight measurements. Children from who body weight data were not available were excluded from the analysis (n = 75). In total, the exposure assessment was carried out for 923 children, 603 children from the urban area and 320 children from the rural area. The dietary exposure assessment on daily bases was performed separately for children of the urban and rural area. In addition, children were grouped in three feeding pattern categories: group 1 (urban n = 61; rural n = 72): children being exclusively or predominantly breastfed (only water and water-based drinks, vitamins, minerals and medicines could be consumed besides breast milk) (PAHO/WHO, 2003); group 2 (urban n = 303; rural n = 163): children at complementary feeding stage (intake of cereals, cereal products and breast milk); and group 3 (urban n = 239; rural n = 85): children at weaning stage (no breast milk intake).

In the urban area, the individual usual dietary intake was determined from the duplicate 24-h recalls using the Multiple Source Method (MSM) program® (Harttig et al., 2011), that considers the intra-individual variability in consumption. The MSM outputs for habitual consumers were used to construct the distribution of consumption data. In the rural area, no MSM computation was applied and the distributions were constructed based on only one 24-h recall of the consumer population.

2.2. Food contamination data

The co-occurrence of ten mycotoxins of health concern in the main staple cereals identified from the food consumption surveys (polished rice, wheat noodles and oat flakes) was previously assessed and described (Ortiz et al., 2013a). Briefly, samples of polished rice (n = 125) were collected from May to July 2010 (rainy season) from the biggest rice mills in Ecuador located at the lowlands of the coastal region, which are the rice suppliers of the whole country. Samples of oat flakes (n = 70, 9 rural and 61 urban) and wheat noodles (n = 128, 15 rural and 113 urban) were collected during February–March 2010 from open markets and supermarkets of the same areas where food consumption surveys were conducted. About half of the urban samples were randomly selected for multimycotoxin analysis, whereas all rural samples were analyzed (polished rice n = 46; wheat noodles n = 80 and oat flakes n = 42). Co-occurrence of aflatoxin B_1, B_2, G_1 and G_2, OTA, DON, fumonisin B_1, zearalenone, and HT-2 and T-2 toxin was analyzed by UHPLC/TOFMS (Ortiz et al., 2013a). No contamination of aflatoxin B_2 and G_2, fumonisin B_1, zearalenone and T-2 toxin were found in none of the samples. In addition, OTA was analyzed in extra batches of oat flakes samples (n = 35) and wheat noodles (n = 59) by HPLC-FLD that was more sensitive for this mycotoxin (Ortiz et al., 2013a). All cereal samples were analyzed as dried raw material.

In the present study, the occurrence of AFB_1 and AFM_1 in breast milk was additionally evaluated. This analysis was carried out as part of a pilot study conducted from November 2012 to January 2013. Breast milk samples (n = 78) were obtained by self-expression of volunteer nursing mothers from the rural canton Nabon. Samples were collected in sterile plastic containers, transported at 4 °C and then frozen within 1 day at −20 °C until mycotoxin analysis. The analytical procedure is described as follows.

2.2.1. Analysis of AFB_1 and AFM_1: chemicals and reagents

LC grade water, acetonitrile, methanol, phosphate-buffered saline (PBS) solution, AFM_1 standard solution in acetonitrile (10 μg mL^{-1}) and standard of solid pure extract of AFB_1 were supplied by Sigma–Aldrich (St. Louis, MO, USA). Acetic acid (glacial) was supplied by Merck KGaA (Darmstadt, Germany). Easi-extract® Aflatoxin immunoaffinity columns were purchased from R-Biopharm Rhône (Glasgow, Scotland). The standard of AFB_1 was reconstituted using acetonitrile. Aliquots of standard stock solutions in acetonitrile (0.1 μg mL^{-1}) were dried under a gentle stream of nitrogen and stored