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Cumulative health risk assessment of co-occurring mycotoxins of deoxynivalenol and its acetyl derivatives in wheat and maize: Case study, Shanghai, China

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ABSTRACT

Humans are naturally and frequently exposed to a multitude of mycotoxins, but health risk assessments are usually performed on individual mycotoxins, which may underestimate the total risks. In this study, we assessed for the first time the cumulative health risks of concomitant exposure via dietary intake (DI) to multiple mycotoxins, namely deoxynivalenol (DON) and its acetyl derivatives of 3-acetyldeoxynivalenol (3-ADON) and 15-acetyldeoxynivalenol (15-ADON), based on the concentration addition (CA) concept. A cross-sectional study was conducted in seven districts in Shanghai, China with 1269 participants and 330 wheat and maize samples analyzed. After probabilistic analysis using Monte Carlo simulation, the results showed no health risks to the population in Shanghai considering individual mycotoxins. However, if the cumulative health risks were calculated based on the combined consideration of DON with either 3-ADON or 15-ADON or both, the DI values in 95th percentile were up to 1087 ng/kg body weight/day, exceeding the Provisional Maximum Tolerable Daily Intake (PMTDI) of 1000 ng/kg body weight/day and hence representing potential health risks to the population in Shanghai. The integrated study proposed here could be a model strategy for cumulative health risk assessment on the co-occurring hazards in the fields of food safety combined with environmental contaminants.

1. Introduction

Mycotoxins are an extremely diverse group of environmentally persistent compounds produced by fungi, which when ingested, inhaled or absorbed from environmental sources, can cause adverse health effects or even death in humans and animals (Khan et al., 2008; McLaughlin et al., 2009; Stoev, 2013). They can contaminate various agricultural commodities, especially maize and wheat, either before harvest or under post-harvest conditions (Jin et al., 2010; Kana et al., 2013; Rodriguez-Carrasco et al., 2013).

Deoxynivalenol (DON), mainly produced by *Fusarium graminearum* and *Fusarium culmorum*, is one of the most abundant contaminants in maize and wheat worldwide, and the most frequently occurring mycotoxin in China (Jin et al., 2010; Li et al., 2011; Ren et al., 2007; Ward et al., 2002; Wei et al., 2012). Acute and chronic ingestion of DON by humans and animals can elicit a variety of toxic effects, including feed refusal, weight loss and vomiting (Marin et al., 2013). Multiple outbreaks of vomiting illness during 1961–1985 in China were linked to consumption of cereal grains contaminated with DON (Luo, 1989). Recently, some mycotoxin derivatives, especially the acetylation products of the parent mycotoxins, are observed to be excreted directly by fungi. Consumer health risks may result from hydrolysis of these conjugates into their toxic parent forms during mammalian digestion (De Boevre et al., 2013). The best known substances in this respect are 3-acetyldeoxynivalenol (3-ADON) and 15-acetyldeoxynivalenol (15-ADON), arising from a common precursor of 3,15-diacetyldeoxynivalenol and both are biosynthetic precursors of DON (Pinton et al., 2012).

Maize and wheat can be infected by the toxigenic molds leading to the co-occurrence of DON, 3-ADON and 15-ADON, and reportedly, more than 30% of the DON-contaminated samples contained one or two derivatives (Ediage et al., 2011; Juan et al., 2013; Spanjer et al., 2008). The issue of combined toxicity is complex, but generally it can be concluded that co-exposure to several different

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mycotoxins may result in an additive or synergistic effect, particularly when the mycotoxins exert their toxicity through a common mode of action. Consequently, the acetylated forms of DON have received increasing attention and are regarded as a critical contributing factor toward total dietary exposure to DON. In 2010, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) reconsidered the toxicity of the acetylated derivatives (3-ADON and 15-ADON) to be similar to that of DON and extended the previous Provisional Maximum Tolerable Daily Intake (PMTDI) of 1 μg/kg body weight/day (JECFA, 2001) to a group PTMDI for the three compounds (JECFA, 2010). Due to the high toxicity and co-occurrence of the targeted mycotoxins, assessments of cumulative health risks associated with dietary intake (DI) of multiple mycotoxins are required (Calow and Forbes, 2013; Sexton and Linder, 2014).

Several studies have been performed to evaluate the health risks of DON, 3-ADON and 15-ADON, as single compounds in isolation (Sirot et al., 2013; Soubra et al., 2009). However, compounds in a mixture can work synergistically and cause effects greater than the individual components. Hence, assessing mycotoxins singly may underestimate the total risks.

A number of methods for the hazard and risk assessment of chemical mixtures of environmental contaminants have been developed to predict the combined toxicity of mixtures and their risks. The most straightforward way is to test the mixture of interest in its totality, which closely follows single substance assessments and does not require any special methodology. However, this approach has several bottle-necks, the most important one of which is to obtain the mixture itself making this approach largely unfeasible (Backhaus et al., 2010). Recently, cumulative risk assessments of environmental contaminants, i.e., perfluoroalkylated and polyfluoroalkylated substances (PFASs), have employed the methods based on the concepts of independent action (IA) and concentration addition (CA) (Borg et al., 2013; Sarigiannis and Hansen, 2012; SCHER, 2011). IA assumes that the individual components act independently of each other, whereas CA considers that the mixture components act in the same way only differing in the concentrations for eliciting their toxic effects. The latter approach seems much more suitable for the cumulative risk assessment of the concomitant mycotoxins such as DON, 3-ADON and 15-ADON because of their similar actions and toxicities.

The objectives of the present study were (i) to determine the occurrence of DON and its derivatives in maize and wheat originating from Shanghai, China; (ii) to make the first attempt using CA concepts to quantitatively assess the potential risks to Shanghai residents associated with the intake of co-occurring mycotoxins, individually and in combination; (iii) to clarify the mechanism of the interaction and cumulative effects of different mycotoxins; (iv) to investigate the relationship between the mycotoxins exposure and the dietary habits of Shanghai residents as well as the other elements, i.e., age and gender, in order to better understand the causal factors of the health risks to Shanghai populations and to be able to set-up effective prevention and control actions.

2. Materials and methods

2.1. Reagents and materials

The chemical standards of DON, 3-ADON and 15-ADON were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile and methanol, purchased from Merck (Darmstadt, Germany), were both HPLC grade. Milli-Q quality water (Millipore, Billerica, MA, USA) was used throughout the analyses. All other reagents were of HPLC or analytical grade.

2.2. Apparatus

The quantification of DON, 3-ADON and 15-ADON was performed by LC-MS/MS (SHIMADZU, Kyoto, Japan). Inertsil ODS column (100 mm × 2.1 mm, 3 μm) (GL Sciences B.V., Eindhoven, Netherlands) at 35 °C was utilized for separation with a linear gradient elution using (A) water and (B) methanol as the mobile phase under the negative electrospray ionization mode (ESI^-). The elution program was set as follows: 20% B (initial), 20%–80% B (0–6 min), 80–20% B (6–6.1 min) and hold on for a further 5.9 min for re-equilibration, giving a total run time of 12 min. The flow rate was 0.3 mL/min and the injection volume was 5 μL (partial loop with needle overfill). MS/MS detection was performed with the following parameters: nebulizing gas flow of 3 L/min, drying gas flow of 15 L/min, interface voltage of 4.5 kV, DL temperature of 250 °C, heat block temperature of 400 °C. Quantitation was performed in multiple reaction monitoring (MRM) mode and the parameters were optimized for each mycotoxin during infusion (Supplementary Table S1).

2.3. Dietary consumption data

Three different zones were targeted according to the population distribution and comprised of the city center, countryside and island. The four districts representing the central part of Shanghai included Xuhui, Hongkou, Changning and Yangpu, while Pudong and Fengxian districts represented the suburbs. Congming district was the only island in Shanghai. A schematic view of the map of Shanghai showing the geographical location of the studied areas is represented in Fig. 1.

Representative food consumption data were obtained from the Shanghai Food Consumption Survey of 2012–2013. The recruitment of participants took gender into consideration and comprised of a total of 1269 participants, including 608 males and 661 females, with the age older than 7, using a balanced design as recommended by Schiebinger (2014). A 24-hour dietary recall questionnaire was administrated, in which the wheat and maize products used in preparing home meals were clearly listed. A carefully structured questionnaire was designed and administered by trained interviewers and included all basic information such as demographic factors (age, sex and education), socioeconomic and general health status.

2.4. Samples for analysis

From 2009 to 2012, a total of 330 samples including wheat (n = 198) and maize (n = 132) were randomly collected from supermarkets located in the same three studied zones of Shanghai. The distributions of the samples across the different years were as follows: 18 wheat and 30 maize samples were collected in 2012, 20 wheat and 20 maize samples in 2011, 40 wheat and 37 maize samples in 2010, 120 wheat and 45 maize samples in 2009. All samples were ground into powders and maintained in zipper top paper bags to prevent humidity changes stored at −20 °C prior to analysis. The concentrations of DON were determined in all samples collected during the 4-year period, while 3-ADON and 15-ADON were only analyzed in the samples collected in 2011 and 2012 after the regulations for these toxins were issued by JECFA (JECFA, 2010).

2.5. Sample preparation

The wheat and maize samples were pretreated according to the standard operating procedures regulated in China (Yang et al., 2012). Portions of 5 g of tested samples were accurately weighed and put into 50 mL polypropylene centrifuge tubes. Then, 10 mL of water, 5 g of sodium chloride and 16 mL of acetonitrile were added. The mixture was shaken for 30 minutes, and then centrifuged at 5000 *g* for 5 minutes. An aliquot of 8 mL of the supernatant was collected and mixed with 4 mL of n-hexane saturated with acetonitrile. After mixing for 3 minutes and centrifugation at 5000 *g* for 2 min, the acetonitrile layer was selected and dried by nitrogen gas at 45 °C. The residues were dissolved in 5 mL of water and passed through preconditioned Oasis HLB SPE cartridges at a rate of about 2 mL/min, and then 5 mL of water was passed through the cartridges at a rate of about 2 mL/min. All targets were eluted with 5 mL methanol and then dried by nitrogen gas at 40 °C. The residues were reconstituted in 1 mL methanol/water (20/80, v/v) solution, passed through a 0.22 μm nylon filter (Millipore) and ready for injection.

2.6. Risk assessment

The health risks of DON were first assessed using all samples collected in the period 2009–2012. In order to compare the health risks related to single and multiple mycotoxins, the risks related to DON, 3-ADON, 15-ADON and the combinations of these mycotoxins (DON + 3-ADON, DON + 15-ADON, 3-ADON + 15-ADON and DON + 3-ADON + 15-ADON) were further evaluated using only the samples collected in the period 2011–2012.

Two mathematical approaches, point evaluation (deterministic approach) and Monte Carlo simulation (probabilistic analysis) were used for the computation of the risk assessments for DON, 3-ADON and 15-ADON. The point evaluation multiplied the mean/highest mycotoxin concentration data by the mean/highest food-consumption data. The Monte Carlo approach simulated both the food-consumption data and the concentration levels of mycotoxins as distributions. Best fit distributions were formed for DON and its derivatives individually and in combination in maize and wheat and also to the respective consumption data. First order Monte Carlo simulations were performed considering 5000 iterations. By means of Monte Carlo simulation, the inherent uncertainty and variability associated with the mycotoxin contents of different foodstuffs, the food consumption pattern and in the