Food and Chemical Toxicology 123 (2019) 9–15

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

Complementary feeding may pose a risk of simultaneous exposures to aflatoxin M1 and deoxynivalenol in Indian infants and toddlers: Lessons from a mini-survey of food samples obtained from Kolkata, India

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ARTICLE INFO

Keywords:
Baby foods
Mycotoxin
Aflatoxin M1
Deoxynivalenol

ABSTRACT

A mini-survey of 29 different foods produced by 21 different Indian manufacturers was conducted for the presence of aflatoxins B1, B2, G1 and G2, aflatoxin M1 and deoxynivalenol. The products were purchased from local markets in Kolkata, India and commonly used in the complementary feeding of infants and toddlers in India. Using a previously established direct competitive enzyme-linked immunoassay for this analysis we show that 100% of the samples contained aflatoxin M1 at levels exceeding the recommended European Union levels of 25 ng kg^{-1} by more than an order of magnitude. Also, several (66%) of them contained detectable concentrations of deoxynivalenol with two samples (6.9%) exceeding European Union guidelines for baby food products (200 μg kg^{-1}) and 51.7% samples with DON levels that can lead to dietary intake higher than 1 μg kg^{-1} recommended by the joint FAO/WHO expert committee on food additives. None of the samples contained aflatoxins B1, B2, G1 and G2. The results, therefore, suggest that complementary feeding can put Indian infants and toddlers at risk of simultaneous exposures to deoxynivalenol and aflatoxin M1 and warrant an urgent in-depth research to track, increase surveillance and reduce mycotoxin contamination of baby foods manufactured in India.

1. Introduction

Mycotoxin contamination of crops and food is a global public health issue and is most common in regions with environmental conditions (optimum temperature and humidity) that are conducive to the growth and secondary metabolism of mycotoxin-producing fungi (Marroquin-Cardona et al., 2014; Wambacq et al., 2016). India and the neighboring Southeastern Asian countries, for example, face enormous mycotoxin contamination problems when humidity exceeds 13% and the temperature ranges between 25 °C and 35 °C (Ali et al., 2015; Hesseltine, 1976; Mudili et al., 2014; Priyanka et al., 2014; Reddy et al., 2009; Ruadrew et al., 2013; Vasanthi and Bhat, 1998; Waenlor and Wiwanitkit, 2003). Indian standing crops and food grains are most prone to fungal invasion and mycotoxin contamination during tidal floods coupled with tropical temperature during the monsoon season (Bilgrami and Choudhary, 1998). Some of the most economically valuable food grains of these regions (including rice, maize, wheat, gram, sorghum) are contaminated with the mycotoxins produced by *Aspergillus, Fusarium* and *Penicillium spp.* as a result of pre- and post-harvest conditions. Aflatoxins and deoxynivalenol (DON) are two of the most prevalent foodborne mycotoxins in Indian food products that have significant impacts on the health of humans and animals (Kumar et al., 2016; Payros et al., 2016; Shephard, 2016). It is expected that with the ongoing changes in climate, the rise of temperatures and flash floods,

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https://doi.org/10.1016/j.fct.2018.10.006
Received 18 June 2018; Received in revised form 10 September 2018; Accepted 2 October 2018
Available online 06 October 2018

mycotoxin occurrence in foods will increase as mycotoxin elimination becomes increasingly challenging (Paterson and Lima, 2010).

Aflatoxins are synthesized as secondary metabolites by some species within the genus *Aspergillus* (such as *A. flavus, A. parasiticus*, *A. nomius*) and are responsible for 25,000–150,000 cases of hepatocellular carcinoma worldwide each year (Liu and Wu, 2010). Aflatoxin B1 (AFB1) is an established liver carcinogen (IARC, 1987; WHO and IARC, 1993). Aflatoxin M1 is a hydroxylated metabolite of aflatoxin B1, which contaminates milk obtained from livestock that is exposed to feed containing aflatoxin B1. It is an active cytotoxin and a genotoxin. Given a long line of studies that have demonstrated toxic and carcinogenic effects of AFM1 (Cullen et al., 1987; Kowalska et al., 2017; Milita et al., 2010; Neal et al., 1998; Theumer et al., 2018), IARC now considers Aflatoxin M1 as a Group 1 carcinogen as well (IARC, 2002). A recent study in the low-income areas of Nairobi, Kenya has also shown an association of aflatoxin M1 exposure with stunted child growth and development (Kiarie et al., 2016). Several previous reports on aflatoxin M1 monitoring in Indian baby foods (Kanungo and Bhand, 2014; Rastogi et al., 2004; Siddappa et al., 2012) indicated presence of aflatoxin M1 exceeding the recommended EU level of 0.025 $\mu g\,kg^{-1}$ (European Commision, 2006a), suggesting that children in India are prone to risk of high dietary intake aflatoxin M1.

DON, a trichothlatoecene, also known as vomitoxin, is synthesized by some species within the genus *Fusarium* such as *F. graminearum* and *F. culmorum* (Drochner, 1989; Magan et al., 2010; Wegulo, 2012). Health effects of DON include dysregulation in immune function (immune suppression) and gastrointestinal epithelial atrophy and inflammation (Pestka, 2010; Rotter et al., 1996). Based on the toxicity of DON, EU's recommended maximum permissible limit for DON in baby foods is 200 μg/kg (European Commision, 2006a). Additionally based on toxicokinetic data on DON, the joint FAO/WHO expert committee on Food Additives (JECFA) has recommended a provisional maximum tolerable daily intake (PMTDI) of 1 $\mu g\,kg^{-1}$ body weight (JECFA, 2002). India is the second largest producer of wheat in the world, and frequent occurrence of DON in wheat-based food has been implied through multiple studies (Bhat et al., 1989; Mishra et al., 2013). Although studies showing the presence of DON in Indian baby foods are rare, the baby foods comprising a combination of milk and cereal products are likely to contain DON (as well as other Fusarium toxins) in addition to aflatoxin M1. The Food Safety and Standard Authority of India (FSSAI) has a recommended maximum level of 1 mg/kg of DON in wheat (FSSAI, 2015). There are currently no recommendations for AFM1 in baby foods sold in India. Since AFM1 is a carcinogen it is expected based on the European food safety authority (EFSA) and the scientific committee on food (SCF) recommendations that AFM1 daily intake value should be 'as low as reasonable achievable' (ALARA) (for ALARA principle adopted for food carcinogens see (Crebelli, 2006; EFSA, 2005)).

In this report, we adhere to the WHO's description of complementary feeding (Brown et al., 1998), which comprises any food served to babies as a part of complementary feeding outside breast milk within ages 0–2 years (Brown et al., 1998). Our goal for this study was to evaluate whether complementary feeding can put Indian infants and toddlers (children within ages 0–2 years) at risk of combined dietary intake of aflatoxins and DON. We analyzed food samples produced by 21 different Indian manufacturers (a total of 29 different products). The samples ranged from commercially available ready-to-eat formula foods to food ingredients (such as wheat and oat bran) from which, several homemade baby foods are prepared. Currently, while available evidence supporting single mycotoxin contaminations exist in Indian baby foods (Kanungo and Bhand, 2014; Mishra et al., 2013), the literature provides no evidence suggesting the co-presence of multiple mycotoxins in commercially available baby foods manufactured in India. Based on available information from the literature, the null hypothesis (H_0) of this study was that the likelihood of multiple mycotoxin contaminations in the manufactured food products intended for complementary feeding of Indian infants and toddlers is 0%. The alternative hypothesis (H_1) therefore, was that the likelihood of multiple mycotoxin contaminations in the manufactured food products intended for complementary feeding of Indian infants and toddlers is significantly higher than 0%.

Since complementary feeding is prevalent in the urban areas in India, we purchased the analyzed samples from the local markets in the city of Kolkata, which is one of the three most populated cities in India. We reasoned that, given the requirement of batch-to-batch consistency as a component of the manufacturers' good manufacturing practice, every product samples randomly picked from this city appropriately represented the quality of all the batches of that product manufactured in India. Reported here are the results of this mini-survey.

2. Materials and methods

2.1. Samples

A total of 29 commercially available food samples manufactured by 21 different manufacturers were obtained randomly from different retail stores in Kolkata. Sampling was conducted by the principles outlined by European Commision (European Commision, 2006b). The samples were transported to the Integrative Mycology Laboratory at Columbia, SC, in dry ice and mycotoxin extraction proceeded on the same day the sample reached Columbia. Samples used for mycotoxin detection (method described below) were stored at 4 °C. The entire procedure from purchase to analysis was conducted within six days. At all times before extraction, we ensured that the food samples remained refrigerated to avoid any temperature fluctuations that could initiate biological activity within the samples and impact the mycotoxin readings.

The primary ingredients of the food samples analyzed sample are listed in Table 1. These food samples were of various types that included infant food (5 products), nutritional drinks (5 products), snacks (6 products), food products used in homemade recipes (6 products), and breakfast foods (7 products). To prevent the identification of the manufacturer, we have not disclosed the manufacturer name or the form (such as biscuits or cereals) in which these are sold. Instead, we provide sample IDs in Table 1 and the individual ingredients that are described by the manufacturer on the package of their food products.

2.2. Mycotoxin extraction and analysis

A total of 50 g from each sample were used for quantitative analysis of aflatoxins B1, B2, G1 and G2 and aflatoxin M1. For each sample, triplicate extracts were analyzed to account for variation in extraction and measurements of aflatoxin M1 and DON Mycotoxins in the samples were extracted using a chloroform: methanol extraction in which samples were initially vigorously mixed with 15 mL of chloroform in a 50 mL conical tube. The chloroform extract was transferred to a glass vial and air-dried. Finally, five mL of 70% methanol was added to the dry extract and shaken vigorously. The methanol extract was finally centrifuged at 10,000 g for 5 min to remove any food particles and debris before analysis. A total of 10 g of each sample was used for DON analysis. The samples were mixed with 100 mL of sterile distilled water. The mixture was allowed to settle and then filtered using Whatman filter paper (grade 1). The filtrate was used for analysis of DON. Quantitative analysis of DON and aflatoxins B_1, B_2, G_1, G_2, and M1 were performed using direct competitive enzyme-linked immunoassay (ELISA) method using three separate high sensitive veratox kits (Neogen, Lansing, MI) for measuring: (a) total aflatoxins, B_1, B_2, G_1, and G_2 (cat. 8035), (b) DON (cat. 8332), and (c) aflatoxin M1 (cat. 8019). The assays were performed using the manufacturer's instructions.

Table 1
Primary ingredient information of the food samples analyzed in this study.

Sample ID	Primary ingredient information as described on the product package (Country of origin of all these products is India)
1	Formula milk consisting of milk fat, milk Protein, carbohydrates, vitamins and minerals.
2	Sugar, Corn Maltodextrin, Milk Protein Concentrate, Safflower Oil, Canola Oil, Soy Protein Isolate
3[a]	White Rice Flour, Dried Skimmed Milk, Sucrose, Palm Olein, Rapeseed Oil, Coconut Oil, Sunflower Oil, Maltodextrin, Flavor, Minerals & Vitamins.
4[a]	Rice flour, maize maltodextrin, vitamins, minerals and traces of milk
5[a]	Wheat flour, Rice, milk solids, sucrose, soybean oil, corn, legumes, vegetables (tomatoes, carrot, spinach), malt extract, vitamins and minerals
6	Wheat Samples (Flour,bran, fibre), Maize Starch, Milk Solids, Corn flakes, flavoring agents and emulsifiers
7	Wheat Flour, Vegetable Oil, Potato Starch, Maltodextrin, Edible Vegetable Fat, Tomato Paste, salt, sugar, condiments, coloring and flavoring agents
8	Wheat flour, milk, honey, nuts
9	Wheat, rice, green gram, nuts
10	Wheat Flour, Vegetable Oil, Yeast, Salt, Sugar, Milk Solids, Malt Extracts, Emulsifier, and baking powder.
11	Rice meal, corn meal, gram meal, vegetable oil, spices, condiments, salt, sugar, citric acid, tartaric acid and milk solids.
12	Whole wheat powder
13	Cashew and oils
14	Powdered gram
15	Oat bran
16	Milk powder (with traces of sugar and soy)
17	Oat Bran
18	Skimmed cow's milk, maltodextrin, vegetable oils, sucrose, flavouring agents, coloring agents, salts, vitamins and minerals.
19	Wheat Flour, malted barley, dried whey (milk), dried skimmed milk, sugar, salts, oild, vitamins and minerals
20[b]	Skimmed milk, maltodextrin, lactose, vegetable oil, sugar, glucose, fat reduced cocoa powder, dextrose, flavourings, magnesium sulphate, thickener, vitamins and minerals
21[b]	Brown rice syrup, milk protein concentrate, canola oil, cane sugar, flavoring agent, vitamins and minerals
22	Malt extract, milk solids, sugar, liquid glucose, cocoa solids, caramel, emulsifiers, minerals, vitamins, liquid vanilla flavor and salt.
23[c]	Corn grits, malt extract, sugar beetroot extract, strawberry puree, vitamins and minerals
24[c]	Corn, soluble corn fiber, wheat starch, coconut and soybean oil, sugar, salt and coloring agent
25[c]	Corn, barley, malt extract, sugar, salt, vitamins and minerals
26[c]	Wheat bran, wheat, soy grits, rice, gluten, soy protein isolate, whole grain wheat, salt, malt flavoring, sucralose, natural and artificial flavor
27[c]	Whole grain oat flour, whole wheat flour, rice flour, oat fiber, corn bran sugar, skim milk, soybean oil, palm oil, cocoa, chocolate, soy lecithin, vanilla extract, salt, and artificial flavor
28[c]	Whole grain wheat, wheat bran, malt flavoring and sugar
29[c]	Rice meal, vegetable oil, corn meal, gram meal, condiments, salt, sugar, and milk solids.

([a] [b] [c] Superscripts indicate the products from the same brand).

2.3. Validation experiments

Validation experiments for the aflatoxin M1 ELISA kit were conducted using both milk based and non-milk-based validation matrices including dried skim milk powder, wheat powder, and cornmeal. Samples were spiked with 0.01, 0.02 and 0.05 μg kg^{-1} (target concentration) of aflatoxin M1. Validation of DON ELISA kit was conducted using wheat flour, cornmeal and dried skim milk powder spiked with 0.5, 2 and 5 mg kg^{-1} (target concentration) of DON. Validation of the total aflatoxin ELISA kit was performed using wheat flour, cornmeal, and dried skim powder with the 5 and 20 μg kg^{-1} (target concentrations) of aflatoxin B1.

2.4. Estimation of the dietary intake of aflatoxin M1 and DON through complementary feeding

Exposure to mycotoxins DON and aflatoxin M1 were calculated based on per capita consumption data of grains, grain-based snacks, ready-to-eat cereals, and milk-based baby foods available from multiple sources that include reports from Food and Agricultural Organization (FAO, 2013) and the National Sample Survey Organization of the ministry of statistics and program implementation of the Government of India (NSSO, 2001, 2014). The estimated daily intake (EDI) of the mycotoxins were calculated according to the equation below:

EDI = mean mycotoxin concentration in the samples × average food consumption per body weight

2.5. Statistical power analysis

To determine if the sample size was enough to test the hypotheses H_0 and H_1, we conducted a posthoc statistical analysis as described below:

If p denotes the true proportion of samples containing more than one mycotoxin, and if the 0% likelihood of samples containing multiple mycotoxin contamination is denoted by p_0, then the null hypothesis H_0: $p = p_0$ *versus* the alternate hypothesis, H_1: $p > p_0$ can be tested using an asymptotic Z-test with test statistic (McClave and Sincich, 2012)

$$Z = \frac{\hat{p} - p_0}{\sqrt{\frac{p_0(1-p_0)}{n}}} > z_\alpha,$$

where, $\hat{p}$ is the observed sample proportion out of n samples that shows multiple mycotoxin contaminations. The p-value for the test is given by $P(Z > T)$, where Z follows a standard Normal distribution.

Suppose α is the level of significance or probability of type I error used for the test and z_α is the $(1-\alpha)^{th}$ percentile of the Normal distribution. Then the power of this test at the non-null value p_1 ($> p_0$) of p observed in our analysis can be derived using the following expression:

$$Power = P\left(Z > \frac{(p_0 - p_1) + z_\alpha\sqrt{\frac{\hat{p}(1-\hat{p})}{n}}}{\sqrt{\frac{p_1(1-p_1)}{n}}}\right)$$

3. Results

3.1. Levels of aflatoxin M1 in food samples

At a detection limit of 0.005 μg kg^{-1} for milk-based samples and 0.01 μg kg^{-1} for non-milk based samples, our analyses revealed that aflatoxin M1 dominated in all the food samples and ranged between 0.80 and 3.2 μg kg^{-1}, which were an order of magnitude higher than the EU recommended levels of 0.025 μg kg^{-1}. The aflatoxin M1 range detected for the different foods are shown in Table 2. Alongside

Table 2
Aflatoxin M1 and DON levels in the analyzed samples of baby foods available in Kolkata, India for ages 0–2 years. The table indicates the types of food that were analyzed.

Table 2A: Aflatoxin M1 levels

Food Type	Number of samples	Mean concentration range (μg Kg^{-1}) from triplicate readings (± mean SE in the readings)	Percent samples exceeding EU recommended levels (0.025 μg Kg^{-1})
Infant foods	5	1.0–3.2 (± 0.01)	100
Snacks	6	0.8–2.6 (± 0.01)	100
Food ingredients	6	1.0–3.3 (± 0.02)	100
Nutritional drinks	5	1.7–2.5 (± 0.02)	100
Breakfast foods	7	1.1–2.4 (± 0.01)	100

Table 2B: DON levels

Food Type	Number of samples	Concentration range (μg Kg^{-1}) from triplicate readings (± mean SE in the readings)	Percent samples exceeding EU recommended levels (200 μg Kg^{-1})
Infant foods	5	0 -14 (± 0.01)	0
Snacks	6	1- 228 (± 0.03)	33
Food ingredients	6	0–77 (± 0.02)	0
Nutritional drinks	5	3–76 (± 0.02)	0
Breakfast foods	7	0–49 (± 0.02)	0

Table 3
Results from validation experiments on the commercial ELISA kits used for the study.

A. Aflatoxin M1 ELISA Kit validation results

Matrices	Spike level ($\mu g\ Kg^{-1}$)	Average Recovery (from 5 replicates)	Standard deviation
Dried Skim Milk	0	0.002	0.001
Dried Skim Milk	0.01	0.01	0.001
Dried Skim Milk	0.02	0.02	0.001
Dried Skim Milk	0.05	0.06	0.002
Wheat powder	0	0.002	0.001
Wheat powder	0.01	0.01	0.002
Wheat powder	0.02	0.02	0.001
Wheat powder	0.05	0.06	0.003
Corn meal	0	0.002	0.001
Corn meal	0.01	0.01	0.002
Corn meal	0.02	0.02	0.001
Corn meal	0.05	0.06	0.003

B. DON ELISA Kit validation results

Matrices	Spike level ($mg\ Kg^{-1}$)	Average Recovery (from 5 replicates)	Standard deviation
Wheat flour	0	0	0.0
Wheat flour	0.5	0.5	0.0
Wheat flour	2	2.0	0.07
Wheat flour	5	5.2	0.15
Corn meal	0	0	0
Corn meal	0.5	0.6	0.06
Corn meal	2	2.1	0.05
Corn meal	5	5.1	0.22
Dried Skim Milk	0	0	0
Dried Skim Milk	0.5	0.4	0.09
Dried Skim Milk	2	1.8	0.17
Dried Skim Milk	5	4.6	0.15

C. Total aflatoxin ELISA Kit validation results

Matrices	Spike level ($\mu g\ Kg^{-1}$)	Average Recovery (from 5 replicates)	Standard deviation
Wheat flour	0	0	0.0
Wheat flour	5	5.8	0.61
Wheat flour	20	23.1	2.07
Corn meal	0	0	0
Corn meal	5	6	1.31
Corn meal	20	18.9	1.05
Dried Skim Milk	0	0	0
Dried Skim Milk	5	4.1	0.69
Dried Skim Milk	20	17.8	1.72

aflatoxin M1, we conducted a quantitative analysis of aflatoxins B_1, B_2, G_1 and G_2, which indicated non-detectable levels of these mycotoxins in the baby foods analyzed (data not shown). An unexpected finding from these measurements was the detection of aflatoxin M1 even in non-milk based food samples. To ensure that the aflatoxin M1 results in these samples were not false-positives due to the interaction of aflatoxin M1 antibodies with blank matrices a detailed validation analysis was conducted (validation results are indicated below and shown in Table 3).

3.2. DON levels detected in food samples

At a detection limit of 5 μg kg^{-1}, DON was detected in 19 out of 29 samples (66%). The concentration levels of DON detected in the samples ranged from levels below the limit of detection (LOD) to 228 μg kg^{-1} (Table 2). In two of these samples (6.9%), the concentrations exceeded the EU recommended 200 mg kg^{-1} maximum limit for baby foods. Wheat-based food samples had higher DON contamination than milk-based foods.

3.3. Validation of ELISA readings

We also conducted a series of validation experiments using non-spiked and spiked samples of the milk-based as well as non-milk-based matrices to eliminate any possibility of false-positive readings through cross-reactivity of the blank matrices with the ELISA antibodies against aflatoxin M1, DON, and total aflatoxin. Results of these validation experiments are provided in Table 3 A-C. The results from this validation suggested that the commercial kit could satisfactorily detect the spiked mycotoxins with recovery values close to the spiked concentrations. Negligible cross-reactivity of aflatoxin M1 antibodies were detected in some samples. However, the levels of aflatoxin M1 detected as false positives due to interactions with blank matrices were 1000-fold lower than the readings obtained from the food samples. Results in Table 3, therefore, confirmed the validity of our aflatoxin M1 and DON readings.

3.4. Calculations of estimated dietary intake of DON

Since aflatoxin M1 is an IARC classified carcinogen (IARC, 2002) European food safety authority (EFSA) and the scientific committee on food (SCF) recommends the adoption of the ALARA principle (Crebelli, 2006; EFSA, 2005) and hence no recommended PMTDI value exists for aflatoxin M1 in baby food. Hence we only estimated a possible daily dietary intake DON in infants and toddlers in Kolkata (Table 4) using the DON concentration levels in our analyzed food samples.

The EDI of DON ranged between 0 and 22.8 μg kg^{-1} with 51.7% samples containing DON that would lead to EDI values exceeding the PMTDI recommended level of 1 μg kg^{-1} (JECFA, 2010). These calculations suggest that 51.7% food samples used in India for complementary feeding can put Indian infants and toddlers at risk of simultaneous dietary exposure to DON and aflatoxin M1 at levels, which potentially may negatively affect their growth and development.

Table 4
Estimated intake pattern of DON in infants and toddlers in Kolkata, India. Estimated Daily Intake (EDI) was calculated based on an average per capita baby food consumption value of 0.1 Kg per Kg body weight (NSSO, 2014).

	Estimated Daily Intake (μg Kg-1)	Percent samples leading EDI greater than recommended levels (**)
DON	Undetected - 22.8	51.7

**The provisional maximum tolerable daily intake (PMTDI) recommended by joint FAO/WHO expert committee on Food Additives (JECFA) is 1 μg/Kg body weight.

3.5. Posthoc statistical analysis of the study

The proportion of samples co-contaminated with aflatoxin M1 and DON was 51.7% in this survey. Using the observed proportion $\hat{p} = 0.517$, the p-value of the test was 1.26×10^{-8}, which suggests that the co-contamination proportion is significantly larger than 0% with very strong statistical evidence.

Finally, we determined the statistical power of the study. Since 51.7% of the samples analyzed had at risk simultaneous exposure level of aflatoxin M1 and DON, we verified if the sample size of 29 had enough power to detect a 51.7% co-contamination proportion. Using the statistical description with $p_1 = 0.517$, using $p_0 = 0$ and level of significance, $\alpha = 0.05$, our calculations showed that the statistical power of this study was > 95%. This suggested that our sample size of 29 had sufficient power of discerning a significantly correct detection at the level observed in the survey.

These findings suggest that without rigorous monitoring and prevention of mycotoxins, baby food products manufactured in India may pose a potential health hazard through simultaneous aflatoxin M1 and DON exposures.

4. Discussion

In this survey, we highlight a significant public health issue that complementary feeding may put children of ages 0–2 years at risk of dietary exposure to multiple mycotoxins. Although none of the samples analyzed indicated the presence of aflatoxins B_1, B_2, G_1, and G_2, all of the food samples representing 21 different brands contained aflatoxin M1 at levels ~30–120 fold higher than the EU recommended levels and ~15–60 fold higher than the recommended levels (0.00005 μg kg^{-1}) in India (FSSAI, 2011). Interestingly, aflatoxin M1 levels detected in our samples were also higher than previous reports on aflatoxin occurrence in baby foods in India (Kanungo and Bhand, 2014; Rastogi et al., 2004; Siddappa et al., 2012). The presence of aflatoxin M1 in milk-based foods suggest high contamination of cattle feeds with aflatoxin B1. It may also suggest the use of raw ingredients purchased from other countries with different aflatoxin regulations. Our results therefore prompt the need to impose stricter mycotoxin surveillance and enforce regulations on aflatoxin M1 levels in food that are used for complementary feeding of children. In addition to aflatoxin M1, 51.7% of the analyzed food samples also contained DON that has the potential to result in an EDI significantly higher than PMTDI recommended by JECFA. Collectively our study demonstrates the feasibility of simultaneous exposure to aflatoxin M1 and DON from the commercially available baby food in Indian markets.

Two unexpected, alarming and unaccounted for observations in this study were the findings of aflatoxin M1 in non-milk based samples and the presence of detectable DON in milk-based samples. These data may be indicative of mycotoxin contamination in raw materials from unexpected sources, which manufacturers are not aware of and hence can be prevented by careful mycotoxin monitoring during the manufacturing process. Whether changing climates, insecticide and pesticide applications may influence mycotoxin profiles of the *Aspergillus spp*. that contaminate Indian crops needs to be investigated. Also, our observation of DON occurrence in milk-based food products contradicts the current understanding that unmetabolized DON cannot be present in milk products as established earlier (Keese et al., 2008). That study showed that oral ingestion of DON gets quickly biotransformed into diepoxy-deoxynivalenol (DOM-1). Hence only low percentage (0.22%) of total DON ingested gets transferred into cow milk and therefore should be below the LOD of ELISA kits that are typically 0.5 ng mL^{-1} for liquid milk and 0.5 ng kg^{-1} for milk powder. However, we emphasize here that this is true under the condition that liver function and metabolism in the animal is NOT impaired upon chronic oral ingestion of extremely high amounts of DON contaminates animal feed. This issue of high contamination of animal feed with Fusarium toxins (such as

DON) is becoming substantial with the ongoing climate changes as reflected in a recent study in milk samples of Croatia (Pleadin et al., 2017), which demonstrated that cow milk samples could be DON positive and readily detectable by ELISA. The presence of unmetabolized DON in cow milk in that study was correlated with extremely high DON concentrations in cattle feed and maize silage. Our results are in line with these findings and therefore indicate the need to investigate DON levels in milk-based products in India as well.

The EDI values have been calculated based on per capita consumption values obtained from food consumption report from Food and Agricultural Organization (FAO, 2013), and the National Sample Survey Reports from Government of India (NSSO, 2001, 2014). However we emphasize here that to conduct a detailed risk assessment study similar to a recent study on aflatoxin M1 contamination in different milk types sold in the Greek market (Tsakiris et al., 2013), more rigorous baby food consumption data in Indian infants and toddlers is necessary. Two commodities (samples 8 and 19) were not covered in the report and were approximated as a grain-based snack and milk-based drink (respectively) based on the relatively higher percentage of grain and milk component present in those commodities.

We emphasize here that recommended PMTDI levels for mycotoxins are based on toxicity studies conducted using single mycotoxins. However, it is possible that multiple mycotoxin exposures may occur which may cause additive and/or synergistic effects resulting in significantly higher toxicity than single mycotoxin exposures as suggested in a recent *in vitro* study that showed a significantly higher cytotoxicity of aflatoxin M1 in Caco-2 cells in presence of, ochratoxin A, zearalenone and α-zearalenone (Gao et al., 2016). Studies to investigate the effects of co-exposure to multiple mycotoxins are therefore essential at this point and are a key focus of our ongoing research.

In this study, we have used samples made by 21 different brands. Given that manufacturers ensure batch-to-batch consistency of product constituents as a component of good manufacturing practice (GMP), we reason that our results are a close representation of the food samples that are manufactured in India. Similar mini-surveys of foods and agricultural products focused on highlighting hazards that need urgent attention have been reported previously and made significant impacts in ensuring food and feed safety. Examples include a 28 sample size study highlighting the presence of aflatoxins in herbal medicinal products of Thailand (Tassaneeyakul et al., 2004), a 32 sample size study highlighting the issue of mycotoxin contamination in commercial pet foods in China (Shao et al., 2018), a 28 sample size study highlighting the issue of multiple mycotoxin contamination in dried date palm fruits in Egypt (Abdallah et al., 2018) and a study using 6 brand samples to demonstrate the problems of mycotoxin contamination of premium and grocery brands of pelleted cat food in South Africa (Singh et al., 2017).

In conclusion, our study provides the first evidence to the best of our knowledge, that complementary feeding may pose a risk of exposure to multiple mycotoxins to infants and toddlers in India. Similar contaminations have been reported in other countries. For example, the presence of multiple mycotoxins was observed in baby foods commercially obtained in Italy (Juan et al., 2014), Canada (Lombaert et al., 2003) and Turkey (Baydar et al., 2007). Chronic high daily intake of aflatoxin M1 and DON may have severe impacts on growth, metabolic pathways, development of tissues and organs and immune function of children (Gong et al., 2003; Ishikawa et al., 2016; Magoha et al., 2016) which may result in increasing immune compromised populations, that may be more prone to several diseases.

Future directions of this work will focus on tracking the sources of mycotoxin contaminations, the results of which can provide a guiding framework for the Indian food manufacturers and regulatory agencies to combat mycotoxin contamination issues in raw materials. Such guiding regulatory framework will very useful in establishing standards for premixed and packaged complementary foods. These foods contain an adequate nutrient density and are commonly manufactured and sold in developing countries to fight malnutrition (Masters et al., 2017). Such food monitoring initiatives are essential for regulation and prevention of mycotoxins in baby foods and are therefore critical to prevent the detrimental impacts of the foodborne mycotoxins on the health of children worldwide.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

This work was funded by the National Institute of Food and Agriculture (Grant No. 2017-67017-26167) and the incentive funds (to A.C.) from the Department of Environmental Health Sciences, University of South Carolina.

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.fct.2018.10.006.

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