Natural contaminants in infant food:

The case of regulated and emerging mycotoxins

Dominik Braun[a], Maximilian Eiser[a], Hannes Puntscher[a], Doris Marko[a], Benedikt Warth[a]*

[a]University of Vienna, Faculty of Chemistry, Department of Food Chemistry and Toxicology, Währinger Straße 38, 1090 Vienna, Austria

Corresponding Author

*Benedikt Warth, University of Vienna, Faculty of Chemistry, Department of Food Chemistry and Toxicology, Währinger Straße 38, 1090 Vienna, Austria.

Phone: +43 1 4277 70806

E-mail: benedikt.warth@univie.ac.at

Abstract

Breast milk substitutes, e.g. infant formulae, are commonly introduced to the diet within the first months of life. As the infants' detoxifying capability is not fully developed, it is of vital importance to minimize their exposure to food contaminants. Here, we present a comprehensive multi-mycotoxin assessment in infant food. Samples from the Austrian and Czech market (n=59) were screened for 46 mycotoxins and key metabolites using two complementary LC-MS/MS methods. Trace levels of 17 mycotoxins, including aflatoxin B_1 (0.4 µg/kg), zearalenone (<2.3 µg/kg), deoxynivalenol (<131 µg/kg) and fumonisin B_1 (<39 µg/kg) were detected. Infant formulae were contaminated at lower levels compared to cereal-based products. Overall, the concentrations of most toxins were near or below their respective LOQ values. However, two raw flour samples exceeded the regulatory limit of aflatoxin B_1 for infant foods. Interestingly, two toxins not reported previously, namely aflatoxicol and sterigmatocystin, were identified in 3 and 17% of infant foods, respectively.

Keywords

Food safety; infant and public health; environmental contaminants; infant formulae; complementary food

1. Introduction

Mycotoxins, a diverse class of toxic secondary metabolites produced by various fungal species frequently contaminate agricultural crops pre- or postharvest. This leads to serious economic losses and furthermore, the chronic exposure to contaminated foodstuff has been linked to long-term effects on human health (Miller, et al., 2014). Several mycotoxins are strictly regulated by the Commission Regulation (EC) No 1881/2006 (EC, 2006) and monitored in the European Union. This includes aflatoxins (AFB_1, B_2, G_1, G_2 and M_1), deoxynivalenol (DON), fumonisins (FB_1 and B_2), ochratoxin A (OTA), zearalenone (ZEN), T2 and HT-2-toxin (T2; HT-2). In addition, various strategies have been elaborated to minimize the contamination of foodstuffs over the last years (Magan, Aldred, Mylona, & Lambert, 2010). However, insufficiently toxicologically characterized mycotoxins remain. These so-called emerging mycotoxins are neither legislatively regulated, nor routinely analyzed in food. Most of these emerging mycotoxins are produced by *Aspergillus, Penicillium, Fusarium,* and *Claviceps* species. These are microscopic filamentous fungi that are commonly found as plant pathogens infesting a wide variety of food crops ranging from grains and nuts to fruits (Fraeyman, Croubels, Devreese, & Antonissen, 2017). *Alternaria* mycotoxins, considered relevant emerging mycotoxins produced by *Alternaria* fungi, gained more and more interest in the last decade. Extracts of *Alternaria* cultures, especially alternariol (AOH), alternariol monomethyl ether (AME) and the altertoxins (ATX I, II and III) have shown genotoxic and mutagenic effects in different cell lines (Aichinger, et al., 2019; Jarolim, et al., 2017).

Children, especially infants, are generally more sensitive to toxic exposures from the environment. In fact, in the first months of life the human metabolism is not fully developed, leading to a lower capacity for the detoxification of environmental contaminants. In addition, infants have a higher intake of food per kilogram body weight compared to adults (Cohen Hubal, et al., 2000). Moreover, their nutrition is mostly resticted to breast milk, fruits, milk-based infant formulae and cereal products. As a result, the occurrence of toxic contaminants in these foodstuffs may lead to a higher exposure compared to adults. Considering estimates of mycotoxin contamination in up to 80% of the global supply of grains (Eskola, et al., 2019), the accurate determination of mycotoxins in cereal-based infant food is of particular interest. In recent years several reports have been published investigating the mycotoxin contamination of infant foods (Gotthardt, et al., 2019; Lombaert, et al., 2003; Meucci, Razzuoli, Soldani, & Massart, 2010). However, critical data gaps were identified to understand the impact of chronical dietary low background exposures on infant health (LaKind, et al., 2018; Lehmann, et al., 2018). Yet, most of the published studies relied on complex and time-consuming sample preparation procedures, like solid phase extraction (SPE) (Lombaert, et al., 2003), included a very limited number of analytes or investigated only single toxins (Asam & Rychlik, 2013; Mahnine, et al., 2011; Meucci, et al., 2010). However, co-occurrence of multiple mycotoxins is very common in food commodities and may lead to combinatory effects even below the threshold of the single toxin (Vejdovszky, Hahn, Braun, Warth, & Marko, 2017). Single or mycotoxin class based extraction procedures are not feasible for a multi-analyte approach covering a large number of chemically diverse targets. Sample preparation protocols that

enable the simultaneous extraction of analytes covering a wide range of polarities are required. A number of established multi-analyte approaches utilize *'dilute and shoot'* protocols . In these cases, the matrices are extracted in a single step,followed by dilution in neat solvent and direct analysis without any further clean-up steps. Other approaches make use of a *QuEChERS* (Quick, Easy, Cheap, Efficient, Rugged and Safe) method, which is usually followed by a SPE to remove interfering matrix components and to concentrate the analytes of interest (Braun, et al., 2018).

The aim of the present work was to identify and quantify 46 regulated and emerging mycotoxins in processed cereal-based infant foods and infant formulae samples obtained from the Austrian and Czech market. Moreover, raw flour samples of rice and maize provided by a local mill were included in the survey, as these were intended for the production of infant cereals. Thus, a total of 59 samples composed of diverse matrices (rice, maize, wheat, spelt, millet, oatmeal and milk powder) were analyzed by two analytical methods based on LC-MS/MS after a single extraction step. Therefore, we present the first multi-mycotoxin assessment in infant food covering a wide range of different mycotoxin classes. Our findings aim to contribute to more comprehensive exposure data and thus, improve risk assessment of mycotoxins found in complementary infant food.

2. Materials and Methods

2.1. Chemicals and reagents

LC-MS grade solvents (acetonitrile (ACN), methanol (MeOH) and water) were purchased from VWR and Honeywell, respectively. Glacial acetic acid (ACS, ≥99.7%) was purchased from Fisher Chemicals, while acetic acid (LC-MS grade, ≥100%,), ammonia solution (LC-MS grade, 25% in H_2O) and ammonium acetate solution (LC-MS grade, 5 M in H_2O) were purchased from Sigma-Aldrich. For the preparation of calibration standards and spiking solutions, a multi-component mixture of aflatoxin B_1, B_2, G_1, G_2, M_1, M_2, P_1, Q_1 (AFB_1, AFB_2, AFG_1, AFG_2, AFM_1, AFM_2, AFP_1, AFQ_1), aflatoxin B_1-N7-guanine (AFB_1-N7-Gua), aflatoxicol (AFL), sterigmatocystin (STC), ochratoxin A, B and α (OTA, OTB, OTα), citrinin (CIT), dihydrocitrinone (DH-CIT), nivalenol (NIV), DON, T-2, HT-2, FB_1, FB_2, ZEN, α-zearalenol (α-ZEL), β-zearalenol (β-ZEL), beauvericin (BEA) as well as enniatin A, A_1, B and B_1 (EnnA, $EnnA_1$, EnnB, $EnnB_1$) was prepared according to Braun, et al. (2018). The concentration of the individual analytes in this solution were as follows: NIV (8 µg/mL); FB_1 (7.5 µg/mL); DON, HT-2 and FB_2 (4.5 µg/mL); AFL and AFB_1-N7-Gua (1.5 µg/mL); OTα (1 µg/mL); α-ZEL and β-ZEL (800 ng/mL); ZEN, DH-CIT, T-2, OTA and OTB (600 ng/mL); AFB_1, AFB_2, AFG_1, AFG_2, AFM_1, AFM_2, AFP_1, AFQ_1 (300 ng/mL); STC (150 ng/mL); BEA, EnnA, $EnnA_2$, EnnB, $EnnB_1$ (60 ng/mL). A second multi-standard mixture, containing the *Alternaria* toxins AOH, AME, altenuene (ALT), isoaltenuene (isoALT), tenuazonic acid (TeA), tentoxin (TEN), altenuic acid III (AA-III), altenusin (ALS), alterperylenol (ALP), ATX-II, AOH-3-glucoside (AOH-3-Glc), AOH-9-glucoside (AOH-9-Glc), AOH-3-sulfate (AOH-3-S), AME-3-glucoside (AME-3-Glc) and AME-3-sulfate (AME-3-S) was prepared according to (Puntscher, et al., 2018). A solution of ATX-I (10.35 µg/mL) in ACN was additionally prepared.

The concentrations of the individual analytes were as follows: TeA (30 µg/mL); ALT, isoALT, ALS, AA-III (15 µg/mL); ATX-I (10.35 µg/mL); AME-3-Glc (5.0 µg/mL); AOH, AOH-3-Glc, AOH-3-S, AME-3-S, ATX-II, ALP (2.5 µg/mL); TEN (800 ng/mL) and AME (500 ng/mL). All solutions were stored at -20 °C.

A working solution containing all toxins was prepared by diluting 300 µL of the first multi-standard stock, 360 µL of the *Alternaria* toxin multi-standard stock and 90 µl of the stock solution containing ATX-I with 150 µL MeOH in an amber glass vial. This working solution was subsequently vortexed for 30 s and stored at -20 °C.

2.2. Infant food samples

Commercially available infant food products (cereal-based infant foods and infant formulae) were collected from retail outlets in Vienna, Austria (n=41) and Prague, Czech Republic (n=3). Infant food was manufactured in four different European countries (Supplementary Table S2). All products were labeled and packaged for retail markets and intended for end consumers. Most products were harvested from organic agriculture (67%), while the others were grown conventionally (33%). Incremental sample aliquots were transferred to 15 mL tubes and stored at -20 °C until extraction. In addition, a local mill provided raw flour samples of rice (n=11) and maize (n=4) in vacuum sealed plastic bags containing 20 to 50 g. These were stored at -20 °C until extraction.

2.3. Sample preparation

For sample extraction the protocol according to Puntscher, et al. (2018) was applied. In brief, 1.00 ± 0.01 g of the respective samples were transferred to 15 mL tubes, suspended with 5 mL of an extraction solvent (MeOH:H_2O:HOAc; 79:20:1; v:v:v) and homogenized on an overhead shaker (50 rpm; 22 °C) for one hour. Subsequently, samples were centrifuged at 4500 rpm for 10 min, followed by the dilution of the resulting supernatant (900 µL) with a solvent mixture (900 µL; MeOH:H_2O; 10:90; v:v) and vortexing for 30 s. After a second centrifugation step (14000 rpm, 12 min, 4 °C), samples were filtered (Chromafil®, PTFE, 0.20µm, Macherey-Nagel, Germany), transferred into amber LC vials and stored at -20 °C until analysis.

2.4. Preparation of calibration standards

Calibration standards were prepared covering three orders of magnitude on seven levels (Table 1 and Supplementary Table S1). Due to the lack of certified reference material, and the wide variety of commodities tested in this pilot survey, no matrix-matched calibration was performed. Instead, calibration standards were prepared by diluting the working solution in solvent (MeOH:H_2O, 10:90, v:v). After homogenization, these solutions were transferred to amber vials and stored at -20 °C until analysis. Calibration curves were evaluated using linear regression analysis with a weighting factor of 1/x.

2.5. Spiking experiments

To evaluate the extraction efficiency and matrix effects, spiking experiments in triplicate were conducted in five matrices (rice, maize, wheat, oatmeal and infant formulae; Table 1 and Supplementary Table S1). Since no certified reference materials were available, a sample of each matrix was randomly chosen and spiked in three independent experiments. For spiking experiments 30 µL working solution were used to spike sample aliquots which were left at ambient temperature for 5 min to allow solvent evaporation. After homogenization, these samples were extracted as described above. Spiking levels, as well as apparent recoveries are reported in Table 1. Apparent recoveries were determined by the ratio of sample concentration calculated using the solvent calibration curves and the known spiking level, multiplied by 100.

2.6. LC-MS/MS measurements

An UltiMate 3000 UHPLC system (Thermo Scientific), coupled to a TSQ Vantage triple-quadrupole mass spectrometer via a heated electrospray-ionization source (Thermo Scientific) was utilized. To screen for all mycotoxins of interest two established methods were used.

Method 1: Multi-mycotoxin method

Mycotoxins regulated in infant cereal and infant formulae products as well as several emerging mycotoxins from *Aspergillus, Penicillium* and *Fusarium* were determined using an LC-MS method adapted from Braun, et al. (2018). Briefly, an Acquity HSS T3 column (C18, 1.8 µm, 2.1 × 100 mm, Waters, Vienna, Austria) was used to chromatographically separate the analytes of interest. The autosampler was set to 10 °C while the column was maintained at 40 °C. The mobile phase consisted of eluent A1 (water/ ammonium actetate (5mM)/ acetic acid (0.1%)) and eluent B (MeOH) with a flow rate of 250 µL/min. The injection volume was 5 µL. All instrumental LC-MS parameters such as gradient and MS/MS settings were reported in detail by Braun, et al. (2018).

Method 2: Multi-*Alternaria* toxin method

For the determination of *Alternaria* toxins., the LC-MS/MS method described by Puntscher, et al. (2018) was utilized. Briefly, chromatographic separation was achieved using a Ascentis Express column (C18, 2.7 µm, 2.1 × 100 mm, Supelco, Vienna, Austria). The autosampler was kept at 10 °C and the column maintained at 30 °C. The mobile phase consisted of eluent A2 (aqueous solution of NH_4Ac, 5 mM, pH adjusted to 8.7 with 25% ammonia solution), while MeOH was used as eluent B. The injection volume was 5 µL.

2.7. Quality control and data evaluation

To monitor the instrument's performance and ensure reproducible measurements, quality control samples containing reference standards in solvent were injected in triplicate at the beginning and in the end of each measurement. Peak shapes, peak areas as well as retention times were evaluated to ensure system integrity. Limit of detection (LOD) and limit of quantification (LOQ) was estimated as three and ten times the signal-to-

noise ratio for each analyte using spiked blank samples, respectively. Data acquisition and data evaluation was performed using Xcalibur (version 3.1) and TraceFinder (version 3.3 and 4.1), respectively.

2.8. Preliminary exposure assessment

The estimated daily intake of mycotoxins via complementary infant food products was calculated utilizing a deterministic approach. Here, we assumed an upper bound worst case scenario by considering the LOD values as maximum concentrations for analytes, which are regulated but were not detected. LOQ values or the quantified maximum concentrations were used for analytes, which were detected in the samples (Table 2). The hypothetical daily intake was calculated on the basis of the upper bound concentration in food, daily food consumption and infant body weight (bw). The following equation was used to calculate the hypothetical upper bound mycotoxin intake (hUBI) on a daily base:

$$hUBI = \frac{C_{mycotoxin\ (upper\ bound)} \times IR}{bw}$$

While hUBI is given in micrograms per kilogram body weight per day, C represents the upper bound concentration of the respective mycotoxin (microgram per kilogram) and IR is the intake rate of food by the infant (g/day). The IR was based on exclusive intake of infant food products and the recommended daily dose at the age of six months (100 g/day) was used as stated on the package leaflet. In addition, an median infant weight (8 kg) was selected by using published weight-for-age standards (WHO, 2006).

3. Results and discussion

3.1. Method performance and limitations

The parameters describing the method's performance of the detected toxins (n = 17) in the analyzed samples are reported in Table 1. The absence of reference material to evaluate the extraction efficiency and matrix effects was compensated by the determination of the apparent recovery using the calibration slope of solvent standards. Here, most mycotoxins (n = 30) in the spiked matrices were recovered in the range of 50 to 150%. For five analytes, apparent recoveries below 50% were observed in the evaluated matrices. This could be the results of limited analyte extraction efficiency, high signal suppression or the combination of both. Interestingly, for seven analytes apparent recoveries above 150% were determined, which most likely was the result of significant signal enhancement. Matrix-matched standard calibration, however, was not feasible, as neither certified reference material, nor appropriate blank samples were available for the infant food products. The apparent recovery of four analytes, BEA, $EnnA_1$, EnnB and $EnnB_1$, was not successfully evaluated for oatmeal, as the sample used for spiking was naturally contaminated. This was also the case for EnnB and $EnnB_1$ in wheat samples. The results of these analytes were not corrected for recovery in these matrices. Spelt and millet samples were deemed to be sufficiently similar to wheat, thus the respective recovery of wheat was utilized to correct for matrix effects and extraction efficiency. FB_2 was not successfully

evaluated, as this toxin exhibited significant carry-over. Mycotoxins detected in naturally contaminated samples were arranged by product type and the respective data are reported in Table 2.

3.2. Mycotoxin contamination in infant food products

Infant food products and raw flour samples (n=59) were screened for 46 different mycotoxins using two LC-MS methods. Overall, 17 different mycotoxins were detected, while the quantification was possible for 59% of contaminated samples. In addition, co-occurrence of mycotoxins was found in 59% of samples (n = 35). Interestingly, an organic spelt cereal sample was contaminated with nine mycotoxins, while in ten samples (17%) no mycotoxin was detected.

3.2.1. Regulated mycotoxins

Commercially available cereal-based infant foods (n = 35) were grouped considering their main ingredients: rice (n=8), maize (n=1), wheat (n=6), spelt (n=5), millet (n=5), oatmeal (n=6) and products containing skim milk powder and cereal flour (milk cereal; n=4). Additionally, 15 raw flour samples intended for the production of infant foods, were classified as rice flour (n=11) and maize flour (n=4).

The mycotoxins AFB_1, ZEN, DON, T-2 and FB_1, regulated by the Commission Regulation (EC) No 1881/2006 (EC, 2006) for cereal-based food intended for the consumption by infants, were determined in 30 samples. In contrast, the regulated mycotoxins OTA and HT-2 were not detected in any sample. The highest prevalence in cereal-based food was observed for T-2 and FB_1 with 26% and 20%, respectively. Concentration levels of up to 3.0 µg/kg T-2 were detected in commercial samples, while only one raw maize flour sample contained 0.9 µg/kg T-2. The concentration of FB_1 did not exceed 8.3 µg/kg in commercial samples. Comparably higher FB_1 concentrations ranging from 13 to 39 µg/kg were only found in raw flour samples. Here, processing practices may reduce the mycotoxin concentration in the finished products. However, all samples in which T-2 and FB_1 was detected were below their maximum residue limit (MRL) of 15 µg/kg and 200 µg/kg, respectively. No contamination trend towards any specific commodity was observed, although T-2 was not found in spelt and FB_1 was not detected in wheat and oatmeal samples. The high incidence of T-2 was not expected, as only few studies have reported this toxin in infant foods in very low concentrations. The highest incidence of T-2 was found by Tanaka, et al. (2010) in Japan, reporting a concentration range from 0.1 to 0.6 µg/kg in 12% of biscuits intended for infant consumption. Zhang, Flannery, Oles, and Adeuya (2018) detected T-2 in only 3 of 147 tested infant foods in the US below the LOQ of 1.6 µg/kg. Furthermore, Lu, Ruiz Leal, Míguez, and Fernández-Franzón (2013) reported T-2 in a single sample of cereal-based infant food at 0.039 µg/kg. A probable cause for the high incidence may be the large share of cereal samples from organic agriculture, as 7 of the 9 samples positive for T-2 were labeled organic. Similarly, the majority of FB_1 positive samples in the present study (10 out of 12) were organic samples. However, label of organic or conventional production does not address influencing factors for mycotoxin contamination such as storage, transport or processing practices. Moreover the sample size in this study was rather limited. The reported concentration levels for FB_1 were below those detected by previous studies, e.g. D'Arco, Fernandez-Franzon, Font, Damiani,

and Manes (2009) recorded mean levels of 159 µg/kg in organic and 3 µg/kg in conventional maize samples. In conformity with a survey of cereal-based infant food in Canada conducted by Lombaert, et al. (2003), no FB_1 was detected in oat-based samples. Others reported that fumonisins were below their detection limit in infant food products (Cirillo, Ritieni, Galvano, & Amodio Cocchieri, 2003).

In processed cereal samples, DON was detected only in one sample of spelt and oatmeal (25 and 62 µg/kg), which is clearly below the MRL of 200 µg/kg. Equally to T-2 and FB_1, the DON concentration was higher in two raw flour samples with 126 and 131 µg/kg. Even higher frequencies and concentrations in baby foods were reported before. Juan, Raiola, Manes, and Ritieni (2014) found DON in 76% of cereal-based baby food samples and Lombaert, et al. (2003) in 63%. Cirillo, et al. (2003) reported DON in 60% of infant foods. The rare occurrence of DON was not expected and may be a result of more rigorous controls during the food production process. ZEN was detected in a single commercial wheat-based sample (1.2 µg/kg), which did not exceed the MRL of 20 µg/kg for grain-based infant foods. Moreover, ZEN concentrations of 2.2 and 2.3 µg/kg were determined in raw maize flour samples, co-occurring with DON.

Interestingly, the highly carcinogenic AFB_1 was detected in two raw rice flour samples, one at 0.4 µg/kg and one below the respective LOQ value of 0.3 µg/kg. These samples exceeded the MRL of AFB_1 in processed cereal-based infant foods (0.1 µg/kg). However, due to the fact that AFB_1 was only detected in raw flour samples, the concentration in the processed product will likely be below the MRL. Recently, AFB_1 was detected in infant foods, e.g. Hernandez-Martinez and Navarro-Blasco (2010) reported several samples (n=7) even above the MRL. Others reported either only a single occurrence (Alvito, Sizoo, Almeida, & van Egmond, 2010) or no contamination of AFB_1 (Beltrán, et al., 2011; Juan, et al., 2014; Zhang, et al., 2013). OTA was not found in any of the analyzed samples, which is most certainly due to the high limit of detection (0.4 µg/kg). In fact, this toxin is frequently detected in cereal-based infant food products across Europe and North America at concentrations lower than the LOD value of the present study and thus not likely a concern for infant health (Alvito, et al., 2010; Juan, et al., 2014; Lombaert, et al., 2003).

3.2.2. Non-regulated aflatoxins and derivatives

Besides regulated aflatoxins, AFL and STC, two non-regulated aflatoxin derivatives were detected (Figure 1), although not co-occurring with AFB_1. A quarter of all processed cereal foods (n=8) exhibited a contamination with STC, a precursor of AFB_1. STC was detected in all commodities except for the single maize-based sample in concentration levels up to 0.5 µg/kg. The occurrence of STC in infant foods has previously been investigated by Sartori, de Moraes, Santos, Souza, and da Nobrega (2017) (LOD 0.04 µg/kg) and Liu, Fan, Huang, Jin, and Zhu (2016) (LOD 0.11 µg/kg), although both studies did not identify any positive sample. In addition, we report AFL in one oatmeal sample below the respective LOQ value in the present study. Moreover, in one milk cereal sample a concentration of 1.1 µg/kg AFL was detected. To the best of our knowledge, this is the first study which reports both AFL and STC in infant food products.

3.2.3. Fusarium toxins

Besides DON and T2, only NIV was detected among the trichothecenes in two cereal-based samples. NIV was found in one rice sample at a concentration of 20 µg/kg and in one wheat sample below the LOQ of 16 µg/kg. In contrast to commercial samples, raw flour samples were not contaminated. In addition, no co-occurrence with any of the other trichothecenes (DON, T-2) was observed. Hexadepsipeptides like BEA and the enniatins, especially EnnB, were frequently found in all cereal-based commodities analyzed in concentration levels up to 40 µg/kg. The highest incidence of EnnB was found in 60% of processed cereal-based samples. In contrast, only two raw maize flour samples were contaminated by EnnB. Other hexadepsipeptides monitored, namely BEA, $EnnB_1$, $EnnA_1$ and EnnA, were detected in 26, 17, 11 and 3% of the processed cereal-based samples, respectively. These results are in agreement with results published by Juan, Manes, Raiola, and Ritieni (2013). Analyzing cereal-based infant foods from Italy, these authors reported that EnnB, $EnnB_1$, BEA, EnnA and $EnnA_1$ were detected in 70, 26, 17, 13 and 9% of all samples, respectively. However, concentration levels in the present study were significantly lower. Others did not detect this mycotoxin class in wheat-based products from Morocco (Mahnine, et al., 2011). This is in contrast to the present study, where all six wheat-based samples tested contained at least one hexadepsipeptide. However, the sensitivity of the applied analytical method was higher, resulting in significantly lower LOD values than those of Mahnine, et al. (2011). In addition, two out of six rice-based samples in the study of Mahnine, et al. (2011) were contaminated with either BEA or enniatins. In the present study, rice products contained the least variety of *Fusarium* toxins of all commodities tested. In raw flour samples, the highest occurrence was observed for BEA. Here, all maize-based samples and one rice-based sample were contaminated with this mycotoxin.

3.2.4. Emerging *Alternaria* toxins

Infant food samples were screened for the occurrence of 16 mycotoxins and mycotoxin-conjugates produced by the fungal species *Alternaria*. Four of these, namely AME, TeA, TEN and ALP were found in both processed cereal-based infant foods and raw flour samples. AME was detectable in rice, millet, oatmeal and milk cereal samples. While two raw samples of rice flour were contaminated below the LOQ value, the maximum AME concentration of 1.1 µg/kg was observed in a commercial oatmeal sample. In contrast, AOH was not detected in any of the analyzed samples. Previously, Scott, Zhao, Feng, and Lau (2012) analyzed AOH and AME contamination of Canadian cereal products including infant foods. They frequently detected both toxins in concentration levels up to 4.4 and 9.0 µg/kg, respectively. TEN was the most common *Alternaria* toxin, found in 34% of processed cereal-based samples at concentrations up to 1.5 µg/kg, followed by TeA in 31% of the samples at concentrations up to 124 µg/kg. The highest concentrations of TeA were detected in millet-based samples, similar to previous published results: For example, Asam and Rychlik (2013) reported that on average, sorghum-based infant foods contained higher amounts of TeA than infant foods based on other cereals (550 µg/kg versus 20 µg/kg respectively). Except one spelt sample (99 µg/kg) all other TeA-positive samples in the present study contained levels below the LOQ value of 24 µg/kg. One raw rice flour sample was contaminated with TEN lower than the LOQ, while six out of eleven raw rice flour samples contained TeA

297 in concentrations up to 54 µg/kg. Other *Alternaria* toxins or key metabolites thereof were not detected in
298 any of the analyzed samples.

299 **3.2.5. Contamination patterns**

300 Most cereal-based commodities analyzed (wheat, spelt, millet, oatmeal) showed traces of mycotoxins
301 deriving from both *Fusarium* and *Alternaria* fungi, without a notable trend towards any specific type of cereal
302 (Figure 2). Interestingly, rice-based samples, both commercial and raw samples, predominantly contained
303 toxins from *Alternaria* spp. and almost no *Fusarium* toxins. This contamination pattern is unusual considering
304 the widespread occurrence of *Fusarium* toxins in other commodities. *Fusarium* fungi have been shown to
305 readily produce mycotoxins using rice as a substrate (Mateo, Mateo, & Jimenez, 2002). On the other hand,
306 all five maize-based samples contained no *Alternaria* toxins on detectable levels, while all four raw maize
307 flour samples were contaminated with a variety of *Fusarium* mycotoxins.

308 **3.2.6. Infant formulae**

309 Compared to cereal-based infant foods, the infant formulae (n=9) analyzed in the present study were
310 contaminated only by a small number of mycotoxins. Moreover, mycotoxins found in these samples were
311 mostly below the respective LOQ values or not detectable at all. In infant formulae only AFM_1 is regulated
312 with a MRL of 0.025 µg/kg. This toxin was not detected in any of the nine infant formulae samples. However,
313 the applied screening method was not suitable to detect levels of AFM_1 below 0.3 µg/kg. Thus, it cannot be
314 excluded that AFM_1 might exceed the MRL level. Results from previous studies indicate that AFM_1 does not
315 frequently occur in infant formulae (Beltrán, et al., 2011; Meucci, et al., 2010; Zhang, et al., 2013). Hence,
316 early-life exposure towards AFM_1 resulting from this commodity may not imply a relevant health risk. ZEN
317 and FB_1, regulated for cereal-based foods, were detected in two and three infant formulae samples,
318 respectively. ZEN was quantified in one sample with a concentration of 0.7 µg/kg. All detections of FB_1 were
319 below the LOQ of 7.0 µg/kg. Similar to cereal-based food, OTA was not detected in a single sample, while
320 Meucci, et al. (2010) reported OTA in 63% of powdered infant formulae in Italy. However, the sensitivity of
321 Meucci, et al. (2010) was higher compared to the present study (1.25 ng/L compared to 0.4 µg/kg) as a result
322 of an immuno-affinity clean-up. Following the fact that no OTA concentrations above 0.7 µg/L were detected
323 by Meucci, et al. (2010) the absence of OTA in the present study can be reasonably explained. Similarly to
324 cereal-based food STC was found in two samples, while emerging mycotoxins such as $EnnB_1$ and AME were
325 detected only below their respective LOQ value in this matrix.

326 **3.3. Estimation of the daily intake of mycotoxins from infant food products**

327 The estimated daily intake of mycotoxins was based on an upper bound worst case scenario assuming
328 contamination of infant food products at the detected maximum concentration. For the three regulated
329 mycotoxins AFM_1, CIT and OTA, which have not been detected in any sample, hypothetical contamination at
330 the respective LOD values were assumed. The exposure to food contaminants in the critical time window of
331 infancy should be as minimal as possible, due to the less developed immune system. This is reflected by the

established MRL for mycotoxins in the Commission Regulation (EC) No 1881/2006 (EC, 2006). Here, maximum concentrations set for mycotoxins in infant food are generally lower. In addition, the European Food Safety Authority (EFSA) stated that the tolerable daily intake (TDI) should be adjusted for infants for the first months of life (EFSA, 2017). The calculated worst case scenario was thus compared to an infant age-corrected TDI (Table 3). Based on the results, we conclude that the intake of DON via infant food may exceed the guidance value by a factor of five, although the respective maximum concentration used for the exposure estimate did not exceed the MRL. However, the exposure was assumed as an upper bound worst case scenario and the maximum concentration was found in a raw flour sample. As already discussed, processing practices clearly influence mycotoxin levels and thus it is likely that the final infant food product contains less and thus potentially negligible amounts of DON. The rather lipophilic toxins BEA and EnnB were recently detected in breast milk from Austrian and Nigerian mothers (Braun et al., 2020a; Braun et al., 2020b). Exposure estimates based on infant food products were 10x and 500x higher for BEA and EnnB, respectively, compared to the estimates for breast milk consumption. For the xenoestrogen ZEN, which has recently been shown to cross the placental barrier (Warth, et al., 2019) a hUBI of 0.029 µg/kg bw/d was calculated. Based on the worst case scenario in infant food the intake of ZEN is twice as high compared to the breast milk estimates we calculated earlier (Braun, et al., 2018).

Moreover, cow milk is frequently used for the preparation of infant food which may not be mycotoxin free and thus extend the contamination pattern. These results clearly highlight that breastfeeding should not be avoided based on the potential presence of mycotoxins, as alternatives are more likely to be contaminated and lack the tailored nutritional composition for an developing infant.

4. Conclusions

Low concentration levels of 17 mycotoxins were detected in 83% of infant food and infant food raw flour samples. All detected mycotoxins were present in cereal-based foods or raw flour samples, whereas only six mycotoxins were found in infant formulae products. In addition, the toxin levels of infant formulae products were near or below the respective LOQ values, confirming that the exposure to mycotoxins from these foods is low. Even though the mycotoxin levels were low in cereal-based products too, co-occurrence of toxins was frequently observed and in a single sample nine different mycotoxins were detected. Within this study we could confirm that regulated mycotoxins did not exceed the MRL, however, sample size and geographical distribution limit the overall conclusions and have to be verified in subsequent studies. In contrast to ready-to-use products, in two raw flour samples AFB_1 was detected above the MRL. To the best of our knowledge, we describe the first detection of STC and AFL in cereal-based infant foods. Samples from organic agriculture, which represented the majority of the samples in this preliminary study, were more likely to be contaminated than those from conventional agriculture. However, the levels of toxins detected in both groups did not differ significantly. In conclusion, infant food products included in this study (cereal-based infant foods and milk-based infant formulae) can be considered as safe regarding this class of natural food contaminants. Certainly,

breast feeding is still considered to be the most advantageous nourishment for infants and should be favored over complementary foods.

Acknowledgements

The authors want to gratefully thank the Mass Spectrometry Centre of the University of Vienna for technical support. This research was financed by the University of Vienna, Austria and the Austrian Science Fund (FWF): P 33188-B.

Author contributions

D.B. conceived, designed and planned the experiments, supported sample analysis, LC-MS/MS measurement, data evaluation, interpretation and drafted the paper. M.E. collected and analyzed samples, evaluated data. H.P. conceived the experimental design and evaluated results. D.M. was involved in the experimental design and data interpretation. B.W. designed and supervised the study and supported analyses and data evaluation/interpretation. All authors contributed to manuscript writing.

Competing interests

The authors declare no competing interests.

Materials & Correspondence

Correspondence and requests for materials should be addressed to B.W.

Data availability

The authors can confirm that all relevant data are included in the paper and/or its Supplementary Information files.

References

Aichinger, G., Kruger, F., Puntscher, H., Preindl, K., Warth, B., & Marko, D. (2019). Naturally occurring mixtures of Alternaria toxins: anti-estrogenic and genotoxic effects in vitro. *Archives of Toxicology, 93*(10), 3021-3031.

Alvito, P. C., Sizoo, E. A., Almeida, C. M. M., & van Egmond, H. P. (2010). Occurrence of Aflatoxins and Ochratoxin A in Baby Foods in Portugal. *Food Analytical Methods, 3*(1), 22-30.

Asam, S., & Rychlik, M. (2013). Potential health hazards due to the occurrence of the mycotoxin tenuazonic acid in infant food. *European Food Research and Technology, 236*(3), 491-497.

Beltrán, E., Ibánez, M., Sancho, J. V., Cortés, M. A., Yusà, V., & Hernández, F. (2011). UHPLC–MS/MS highly sensitive determination of aflatoxins, the aflatoxin metabolite M1 and ochratoxin A in baby food and milk. *Food Chemistry, 126*, 737-744.

Braun, D., Ezekiel, C. N., Abia, W. A., Wisgrill, L., Degen, G. H., Turner, P. C., Marko, D., & Warth, B. (2018). Monitoring Early Life Mycotoxin Exposures via LC-MS/MS Breast Milk Analysis. *Analytical Chemistry, 90*(24), 14569-14577.

Braun, D., Ezekiel, C. N., Marko, D., & Warth, B. (2020a). Exposure to Mycotoxin-Mixtures via Breast Milk: An Ultra-Sensitive LC-MS/MS Biomonitoring Approach. *Frontiers in Chemistry, 8*, 423.

Braun, D., Schernhammer, E., Marko, D., & Warth, B. (2020b). Longitudinal assessment of mycotoxin co-exposures in exclusively breastfed infants. *Environment International, 142*, 105845.

Cirillo, T., Ritieni, A., Galvano, F., & Amodio Cocchieri, R. (2003). Natural co-occurrence of deoxynivalenol and fumonisins B1 and B2 in Italian marketed foodstuffs. *Food Addit Contam, 20*(6), 566-571.

Cohen Hubal, E. A., Sheldon, L. S., Burke, J. M., McCurdy, T. R., Berry, M. R., Rigas, M. L., Zartarian, V. G., & Freeman, N. C. (2000). Children's exposure assessment: a review of factors influencing Children's exposure, and the data available to characterize and assess that exposure. *Environmental Health Perspectives, 108*(6), 475-486.

D'Arco, G., Fernandez-Franzon, M., Font, G., Damiani, P., & Manes, J. (2009). Survey of fumonisins B1, B2 and B3 in conventional and organic retail corn products in Spain and Italy and estimated dietary exposure. *Food Additives & Contaminants, Part B: Surveillance, 2*(2), 146-153.

EC. (2006). Commission Regulation (EC) No 1881/2006 Setting Maximum Levels for Certain Contaminants in Foodstuffs. In *European Union Commission, Off. J. Eur. Communities L*.

EFSA. (2006). Opinion of the Scientific Panel on contaminants in the food chain [CONTAM] related to ochratoxin A in food. In *EFSA Journal* (Vol. 4, pp. 365.): Wiley Online Library.

EFSA. (2011). Scientific Opinion on the risks for public health related to the presence of zearalenone in food. In *EFSA Journal* (Vol. 9, pp. 2197.).

EFSA. (2012). Scientific Opinion on the risks for public and animal health related to the presence of citrinin in food and feed. In *EFSA Journal* (Vol. 10, pp. 2605.): Wiley Online Library.

EFSA. (2013a). Deoxynivalenol in food and feed: occurrence and exposure. In *EFSA Journal* (Vol. 11, pp. 3379).

EFSA. (2013b). Scientific Opinion on risks for animal and public health related to the presence of nivalenol in food and feed. In *EFSA Journal* (Vol. 11, pp. 3262).

EFSA. (2017). Guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age. In *EFSA Journal* (Vol. 15).

Eskola, M., Kos, G., Elliott, C. T., Hajslova, J., Mayar, S., & Krska, R. (2019). Worldwide contamination of food-crops with mycotoxins: Validity of the widely cited 'FAO estimate' of 25. *Critical Reviews in Food Science and Nutrition*, 1-17.

Fraeyman, S., Croubels, S., Devreese, M., & Antonissen, G. (2017). Emerging Fusarium and Alternaria Mycotoxins: Occurrence, Toxicity and Toxicokinetics. *Toxins (Basel), 9*, 228.

Gotthardt, M., Asam, S., Gunkel, K., Moghaddam, A. F., Baumann, E., Kietz, R., & Rychlik, M. (2019). Quantitation of Six Alternaria Toxins in Infant Foods Applying Stable Isotope Labeled Standards. *Front Microbiol, 10*, 109.

Hernandez-Martinez, R., & Navarro-Blasco, I. (2010). Aflatoxin levels and exposure assessment of Spanish infant cereals. *Food Additives & Contaminants, Part B: Surveillance, 3*(4), 275-288.

Jarolim, K., Del Favero, G., Ellmer, D., Stark, T. D., Hofmann, T., Sulyok, M., Humpf, H. U., & Marko, D. (2017). Dual effectiveness of Alternaria but not Fusarium mycotoxins against human topoisomerase II and bacterial gyrase. *Archives of Toxicology, 91*(4), 2007-2016.

Juan, C., Manes, J., Raiola, A., & Ritieni, A. (2013). Evaluation of beauvericin and enniatins in Italian cereal products and multicereal food by liquid chromatography coupled to triple quadrupole mass spectrometry. *Food Chemistry, 140*(4), 755-762.

Juan, C., Raiola, A., Manes, J., & Ritieni, A. (2014). Presence of mycotoxin in commercial infant formulas and baby foods from Italian market. *Food Control, 39*, 227-236.

Knutsen, H. K., Barregard, L., Bignami, M., Bruschweiler, B., Ceccatelli, S., Cottrill, B., Dinovi, M., Edler, L., Grasl-Kraupp, B., Hogstrand, C., Hoogenboom, L., Nebbia, C. S., Petersen, A., Rose, M., Roudot, A. C., Schwerdtle, T., Vleminckx, C., Vollmer, G., Wallace, H., Dall'Asta, C., Gutleb, A. C., Humpf, H. U., Galli, C., Metzler, M., Oswald, I. P., Parent-Massin, D., Binaglia, M., Steinkellner, H., Alexander, J., & Chain, E. P. C. F. (2018). Appropriateness to set a group health-based guidance value for fumonisins and their modified forms. *EFSA Journal, 16*(2), e05172.

LaKind, J. S., Lehmann, G. M., Davis, M. H., Hines, E. P., Marchitti, S. A., Alcala, C., & Lorber, M. (2018). Infant Dietary Exposures to Environmental Chemicals and Infant/Child Health: A Critical Assessment of the Literature. *Environmental Health Perspectives, 126*(9), 96002.

Lehmann, G. M., LaKind, J. S., Davis, M. H., Hines, E. P., Marchitti, S. A., Alcala, C., & Lorber, M. (2018). Environmental Chemicals in Breast Milk and Formula: Exposure and Risk Assessment Implications. *Environmental Health Perspectives, 126*(9), 96001.

Liu, S., Fan, J., Huang, X., Jin, Q., & Zhu, G. (2016). Determination of Sterigmatocystin in Infant Cereals from Hangzhou, China. *Journal of AOAC International, 99*(5), 1273-1278.

Lombaert, G. A., Pellaers, P., Roscoe, V., Mankotia, M., Neil, R., & Scott, P. M. (2003). Mycotoxins in infant cereal foods from the Canadian retail market. *Food Addit Contam, 20*(5), 494-504.

Lu, H., Ruiz Leal, M. J., Míguez, M. P., & Fernández-Franzón, M. (2013). Detection of seven trichothecene mycotoxins in infant cereal foods by QuEChERS extraction and liquid chromatography coupled to tandem mass spectrometry. *Rev. Toxicol., 30*, 203-208.

Magan, N., Aldred, D., Mylona, K., & Lambert, R. J. (2010). Limiting mycotoxins in stored wheat. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess, 27*(5), 644-650.

Mahnine, N., Meca, G., Elabidi, A., Fekhaoui, M., Saoiabi, A., Font, G., Manes, J., & Zinedine, A. (2011). Further data on the levels of emerging Fusarium mycotoxins enniatins (A, A1, B, B1), beauvericin and fusaproliferin in breakfast and infant cereals from Morocco. *Food Chemistry, 124*(2), 481-485.

Mateo, J. J., Mateo, R., & Jimenez, M. (2002). Accumulation of type A trichothecenes in maize, wheat and rice by Fusarium sporotrichioides isolates under diverse culture conditions. *Int J Food Microbiol, 72*(1-2), 115-123.

Meucci, V., Razzuoli, E., Soldani, G., & Massart, F. (2010). Mycotoxin detection in infant formula milks in Italy. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess, 27*(1), 64-71.

Miller, J. D., Schaafsma, A. W., Bhatnagar, D., Bondy, G., Carbone, I., Harris, L. J., Harrison, G., Munkvold, G. P., Oswald, I. P., Pestka, J. J., Sharpe, L., Sumarah, M. W., Tittlemier, S. A., & Zhou, T. (2014). Mycotoxins that affect the North American agri-food sector: state of the art and directions for the future. *World Mycotoxin Journal, 7*(1), 63-82.

Puntscher, H., Kutt, M. L., Skrinjar, P., Mikula, H., Podlech, J., Frohlich, J., Marko, D., & Warth, B. (2018). Tracking emerging mycotoxins in food: development of an LC-MS/MS method for free and modified Alternaria toxins. *Analytical and Bioanalytical Chemistry, 410*(18), 4481-4494.

Sartori, A. V., de Moraes, M. H. P., Santos, R., Souza, Y. P., & da Nobrega, A. W. (2017). Determination of Mycotoxins in Cereal-Based Porridge Destined for Infant Consumption by Ultra-High Performance Liquid Chromatography-Tandem Mass Spectrometry. *Food Analytical Methods, 10*(12), 4049-4061.

Scott, P. M., Zhao, W., Feng, S., & Lau, B. P. (2012). Alternaria toxins alternariol and alternariol monomethyl ether in grain foods in Canada. *Mycotoxin Res, 28*(4), 261-266.

Tanaka, H., Takino, M., Sugita-Konishi, Y., Tanaka, T., Leeman, D., Toriba, A., & Hayakawa, K. (2010). Determination of Fusarium mycotoxins by liquid chromatography/tandem mass spectrometry coupled with immunoaffinity extraction. *Rapid Communications in Mass Spectrometry, 24*(16), 2445-2452.

Vejdovszky, K., Hahn, K., Braun, D., Warth, B., & Marko, D. (2017). Synergistic estrogenic effects of Fusarium and Alternaria mycotoxins in vitro. *Archives of Toxicology, 91*(3), 1447-1460.

Warth, B., Preindl, K., Manser, P., Wick, P., Marko, D., & Buerki-Thurnherr, T. (2019). Transfer and Metabolism of the Xenoestrogen Zearalenone in Human Perfused Placenta. *Environmental Health Perspectives, 127*(10), 107004.

WHO. (2006). World Health Organization Multicentre Growth Reference Study Group. WHO Child Growth Standards: Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: Methods and development. In *World Health Organization* (pp. 312.).

Zhang, K., Flannery, B. M., Oles, C. J., & Adeuya, A. (2018). Mycotoxins in infant/toddler foods and breakfast cereals in the US retail market. *Food Additives & Contaminants, Part B: Surveillance, 11*(3), 183-190.

Zhang, K., Wong, J. W., Hayward, D. G., Vaclavikova, M., Liao, C. D., & Trucksess, M. W. (2013). Determination of mycotoxins in milk-based products and infant formula using stable isotope dilution assay and liquid chromatography tandem mass spectrometry. *Journal of Agricultural and Food Chemistry, 61*(26), 6265-6273.

Figures

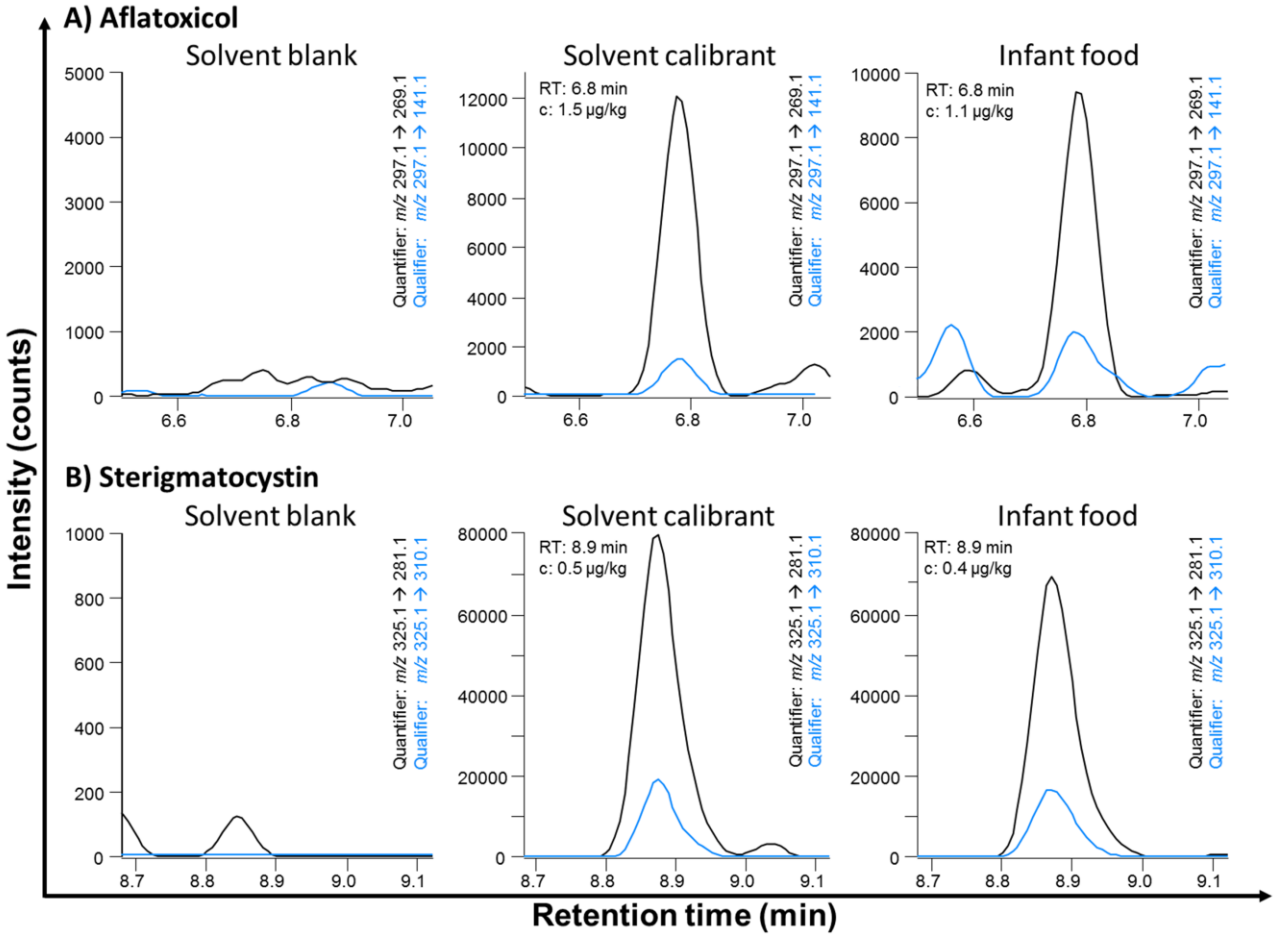

Figure 1: MRM-chromatograms of aflatoxicol (A) and sterigmatocystin (B). For both toxins a solvent blank, solvent calibrant and a naturally contaminated infant food sample are shown, respectively.

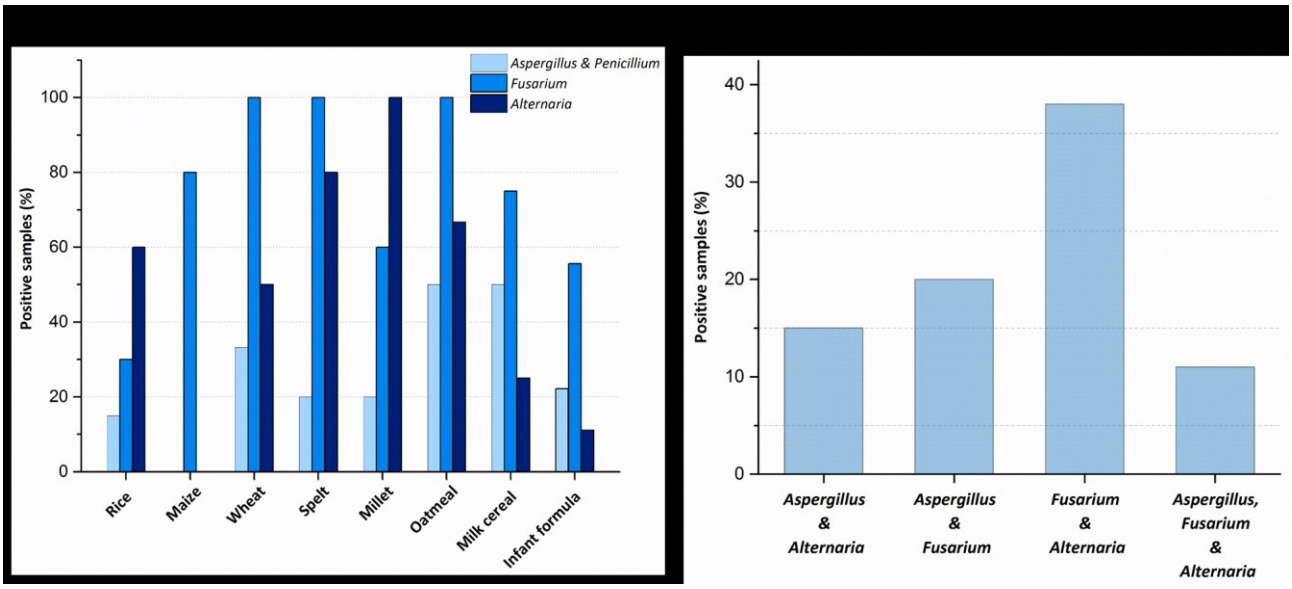

Figure 2: Occurrence of multiple mycotoxins in infant food and raw flour samples (n=59) based on food categories (A) and co-occurrence of mycotoxins based on the producing fungal species (B).

Tables

Table 1: Method performance characteristics of 17 detected mycotoxins in infant cereal and formulae samples.[a]

Analyte	Spiking level[b]	Limit of detection (LOD)	Limit of quantification (LOQ)	Calibration range[c]	R^2	Apparent recovery (R_A ± RSD)[d]				
						Rice	Maize	Wheat[e,f]	Oatmeal[f]	Infant formulae[g]
	[µg/kg sample]	[µg/kg]	[µg/kg]	[ng/mL]		[%]	[%]	[%]	[%]	[%]
Method 1: Multi-mycotoxin method										
Aflatoxicol	15	0.1	0.25	0.01- 50	0.9976	34 ± 5	45 ± 5	66 ± 11	61 ± 9	98 ± 9
Aflatoxin B_1	3.0	0.15	0.3	0.01 - 10	0.9905	60 ± 6	52 ± 3	71 ± 26	86 ± 17	102 ± 16
Sterigmatocystin	1.5	0.05	0.1	0.015 - 5	0.9946	78 ± 11	70 ± 15	99 ± 5	70 ± 2	108 ± 2
Zearalenone	6.0	0.3	0.6	0.02 - 20	0.998	54 ± 3	55 ± 6	41 ± 11	91 ± 17	80 ± 8
Deoxynivalenol	45	10	20	0.15 - 150	0.9717	25 ± 7	36 ± 2	52 ± 19	21 ± 32	20 ± 1
Nivalenol	80	8.0	16	0.27 - 267	0.9854	49 ± 18	53 ± 12	81 ± 31	53 ± 20	79 ± 17
T-2 toxin	6.0	0.3	0.6	0.02 - 20	0.9927	97 ± 11	77 ± 19	56 ± 29	77 ± 20	78 ± 9
Beauvericin	0.6	0.2	0.4	0.02 - 20	0.9686	117 ± 6	153 ± 21	232 ± 39	n.a.	289 ± 16
Enniatin A	0.6	0.2	0.4	0.02 - 20	0.9763	158 ± 14	129 ± 10	210 ± 5	208 ± 9	208 ± 4
Enniatin A_1	0.6	0.2	0.4	0.02 - 20	0.9702	122 ± 8	90 ± 11	168 ± 4	n.a.	167 ± 26
Enniatin B	0.6	0.2	0.4	0.02 - 20	0.9531	89 ± 10	82 ± 21	n.a.	n.a.	165 ± 15
Enniatin B_1	0.6	0.2	0.4	0.02 - 20	0.9624	74 ± 20	80 ± 18	n.a.	n.a.	69 ± 17
Fumonisin B_1	75	3.5	7.0	0.25 - 250	0.9695	120 ± 2	166 ± 26	156 ± 42	134 ± 37	129 ± 9
Method 2: Multi-Alternaria toxin method										
Alternariol monomethyl ether	6.0	0.3	0.6	0.02 - 20	0.9962	90 ± 10	77 ± 5	62 ± 8	59 ± 6	93 ± 6
Tenuazonic acid	360	12	24	1.2 - 1200	0.9962	81 ± 10	72 ± 5	74 ± 3	66 ± 6	65 ± 7
Tentoxin	9.6	0.5	1.0	0.03 - 9.6	0.9705	120 ± 38	98 ± 22	75 ± 3	73 ± 10	119 ± 4
Alterperylenol	30	5.0	10	0.1 - 100	0.9843	114 ± 27	74 ± 10	112 ± 27	62 ± 12	113 ± 6

[a]Non-detected mycotoxins (n=29) are listed in Supplementary Table S1.

[b]Spiking levels were identical for all matrices, each matrix was spiked in triplicate.

[c]Concentration range covered by calibration standards.

[d]Combined evaluation of extraction recovery and matrix effects, calculated as the ratio of measured analyte concentration of the spiked samples and the known spiking level. Average and relative standard deviation of three independent experiments.

[e]A wheat-based infant cereal sample (99% wheat) was chosen for the evaluation.

[f]Recovery was not calculated for analytes where no blank material was available and the spiked matrix was contaminated ("n.a.").

[g]Breast milk substitute products based on milk powder, whey and vegetable oils as main ingredients.

530 **Table 2:** Mycotoxins detected in cereal-based infant foods, infant formulae and raw flours of rice (n=11) and maize (n=4).

Analyte		Processed cereal-based infant foods (n=35)			Infant and follow-on formulae[a] (n=9)			Raw flour samples[b] (n=15)		
	Limit of quantification (LOQ) [µg/kg]	Incidence n (%)	Range [µg/kg]	Mean[c] [µg/kg]	Incidence n (%)	Range [µg/kg]	Mean[c] [µg/kg]	Incidence n (%)	Range [µg/kg]	Mean[c] [µg/kg]
Method 1: Multi-mycotoxin method										
Aflatoxicol	0.25	2 (6%)	<LOQ - 1.1	-	-	-	-	-	-	-
Aflatoxin B_1	0.3	-	-	-	-	-	-	2 (13%)	<LOQ - 0.4	-
Sterigmatocystin	0.1	8 (23%)	<LOQ - 0.5	0.24	2 (22%)	<LOQ - 0.2	-	-	-	-
Zearalenone	0.6	1 (3%)	1.2	-	2 (22%)	<LOQ - 0.7	-	2 (13%)	2.2 - 2.3	-
Deoxynivalenol	20	2 (6%)	25 - 62	43	-	-	-	2 (13%)	126 - 131	-
Nivalenol	16	2 (6%)	<LOQ - 20	-	2 (22%)	<LOQ - 19	-	-	-	-
T-2 toxin	0.6	9 (26%)	0.8 - 3.0	1.5	-	-	-	1 (5%)	0.9	-
Beauvericin	0.4	5 (14%)	<LOQ - 3.1	1.9	-	-	-	5 (33%)	0.6 - 2.0	1.4
Enniatin A	0.4	1 (3%)	<LOQ	-	-	-	-	-	-	-
Enniatin A_1	0.4	4 (11%)	<LOQ - 2.1	0.7	-	-	-	-	-	-
Enniatin B	0.4	21 (60%)	<LOQ - 40	5.9	-	-	-	2 (13%)	3 - 5	-
Enniatin B_1	0.4	9 (26%)	<LOQ - 10	3.9	1 (11%)	<LOQ	-	1 (5%)	2	-
Fumonisin B_1	7.0	7 (20%)	<LOQ - 8.3	4.8	3 (33%)	<LOQ	3.5	5 (33%)	<LOQ - 39	27
Method 2: Multi-Alternaria toxin method										
Alternariol monomethyl ether	0.6	7 (20%)	<LOQ - 1.1	0.6	1 (22%)	<LOQ	-	2 (13%)	<LOQ	-
Tenuazonic acid	24	11 (31%)	<LOQ - 124	48	-	-	-	6 (40%)	<LOQ - 54	37
Tentoxin	1.0	12 (34%)	<LOQ - 1.5	0.9	-	-	-	1 (6%)	<LOQ	-
Alterperylenol	10	8 (23%)	<LOQ - 20	11	-	-	-	-	-	-

531 [a]Infant formulae powder based on skim milk, whey products and vegetable oils.
532 [b]Intended for the production of infant foods.
533 [c]Mean values were calculated for toxins with more than two positive samples. Samples below the LOQ were substituted with the respective LOQ/2 value.
534

535 **Table 3:** Preliminary exposure assessment using an upper bound worst case scenario and comparison to an infant corrected tolerable daily intake (TDI)

Analyte	LOD	LOQ	Maximum concentration	TDI		hUBI[b]
			Infant food	Adults	Infant corrected[a]	
	[µg/kg]	[µg/kg]	[µg/kg]	[µg/kg bw/d]	[µg/kg bw/d]	[µg/kg bw/d]
Method 1: Multi-mycotoxin method						
Aflatoxicol	0.1	0.25	1.1			0.014
Aflatoxin B_1	0.15	0.3	0.4			0.005
Aflatoxin M_1	0.3	0.6	n.d.			0.004
Beauvericin	0.2	0.4	3.1			0.039
Citrinin	0.15	0.3	n.d.	0.2[c]	0.067	0.002
Deoxynivalenol	10	20	131	1[d]	0.33	1.6
Enniatin A	0.2	0.4	0.4			0.005
Enniatin A_1	0.6	0.4	2			0.025
Enniatin B	0.6	0.4	40			0.50
Enniatin B_1	0.6	0.4	10			0.13
Fumonisin B_1	3.5	7	39	2[e]	0.67	0.49
Nivalenol	8	16	20	1.2[f]	0.40	0.25
Ochratoxin A	0.4	0.8	n.d.	0.017[g]	0.006	0.005
Sterigmatocystin	0.05	0.1	0.5			0.006
T-2 toxin	0.3	0.6	3			0.038
Zearalenone	0.3	0.6	2.3	0.25[h]	0.083	0.029
Method 2: Multi-Alternaria toxin method						
Alternariol monomethyl ether	0.3	0.6	1.1			0.014
Tenuazonic acid	12	24	124			1.6
Tentoxin	0.5	1	1.5			0.019
Alterperylenol	5	10	20			0.25

536 [a]According to the EFSA guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age the adult TDI was age-corrected (infant corrected
537 TDI = TDI/3)(EFSA, 2017). [b]For calculation of the hypothetical upper bound intake (hUBI): Assuming contamination at the LOD for mycotoxins not detected, values <LOQ were
538 estimated at the LOQ level or maximum concentration in infant food was used. This upper bound value was multiplied by the intake rate (IR, 100 g/d; as stated on the package leaflet
539 for infant formulae) and divided by the average infant body weight (bw, 8 kg). [c]According to EFSA (2012). [d]According to EFSA (2013a). [e]According to Knutsen, et al. (2018). [f]According
540 to EFSA (2013b). [g]TDI calculated as 120 ng/kg bw per week (EFSA, 2006) divided by 7. [h]According to EFSA (2011).