

Biomonitoring of Mycotoxins in Human Breast Milk: Current State and Future Perspectives

Benedikt Warth,^{†,‡} Dominik Braun,[†] Chibundu N. Ezekiel,^{§,||} Paul C. Turner,[⊥] Gisela H. Degen,[#] and Doris Marko^{*,†}

[†]University of Vienna, Faculty of Chemistry, Department of Food Chemistry and Toxicology, Waehringerstraße 38, 1090 Vienna, Austria

[‡]University of Natural Resources and Life Sciences, Vienna (BOKU), Department IFA-Tulln, Konrad-Lorenz-Straße 20, 3430 Tulln, Austria

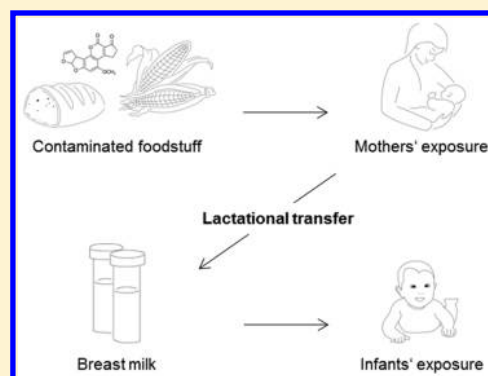
[§]Department of Biosciences and Biotechnology, Babcock University, Ilishan Remo, Nigeria

^{||}Partnership for Aflatoxin Control in Africa, Department of Rural Economy and Agriculture, African Union Commission, Addis Ababa, Ethiopia

[⊥]Maryland Institute for Environmental Health, School of Public Health, University of Maryland, College Park, Maryland 20742, United States

[#]Leibniz-Research Centre for Working Environment and Human Factors (IfAdo), Ardeystraße 67, D-44139 Dortmund, Germany

ABSTRACT: Human breast milk is considered as the best and ideal form of nutrition for infants. However, food contaminants such as mycotoxins, which may be transferred from maternal blood to milk, are poorly described. Mycotoxins are a major group of natural toxins frequently detected in foods. Here, we review the current state-of-the-art in the monitoring of mycotoxins in human breast milk, i.e., knowledge on occurrence, metabolism, and analytical assays utilized for their quantification. We highlight that most of the data captured to date have not been verified with the precision now capable utilizing LC-MS/MS and LC-HRMS approaches. One concern is that some studies may overestimate individual measures, and most cannot capture the patterns and levels of mycotoxin mixtures. We propose accurate assessment as a priority, especially for aflatoxins, fumonisins, ochratoxin A, zearalenone, and deoxynivalenol as well as their major metabolites. However, also so-called emerging toxins such as citrinin, the enniatins, beauvericin, aurofusarin, or *Alternaria* toxins should be considered to evaluate their potential relevance. Key requirements for analytical quality assurance are identified and discussed to guide future developments in this area. Moreover, research needs including investigations of lactational transfer rates, the role of human metabolism for bioactivation or detoxification, and an evaluation of potential combinatory effects of different mycotoxins are pointed out. It is hoped that LC-MS based multianalyte methods will enable more accurate, rapid and affordable human biomonitoring approaches that support informed decisions for maternal and infant health.



CONTENTS

| | |
|---|------|
| Introduction | 1087 |
| Current State of the Art | 1089 |
| Lactational Transfer of Mycotoxins | 1089 |
| Single Toxin Analysis | 1090 |
| Analytical Methods and Sample Preparation Approaches | 1090 |
| Toward Multianalyte Methods | 1090 |
| Future Perspectives and Research Needs | 1091 |
| Targeted and Untargeted Multianalyte Methods and Inclusion of Key Metabolites | 1091 |
| Analytical Quality Issues | 1091 |
| Biomonitoring and Toxicological Aspects | 1092 |
| Concluding Remarks | 1092 |
| Author Information | 1092 |

| | |
|----------------------|------|
| Corresponding Author | 1092 |
| Funding | 1092 |
| Notes | 1092 |
| Biographies | 1092 |
| Abbreviations | 1093 |
| References | 1093 |

INTRODUCTION

Mycotoxins are toxic secondary metabolites produced by a variety of fungal genera such as *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria* that contaminate crops and foodstuff worldwide.

Received: April 14, 2016

Published: June 14, 2016

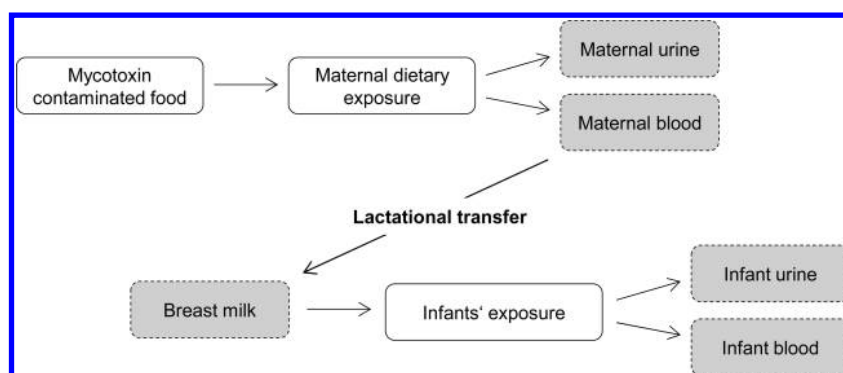


Figure 1. Scheme illustrating maternal and infants' exposure toward mycotoxin contaminated food. Potential sampling points to obtain biological fluids for biomonitoring purpose are indicated by dotted boxes. The scheme was created on the basis of a previously published concept by Muñoz et al.⁴⁶

Several hundred mycotoxins have been described since the aflatoxins (AFs) were first discovered around 1960.^{1–3} Occurrence of these major food contaminants depends on many factors including climate and poor agricultural practice, both pre and postharvest; thus, wealth may be a significant predictor of the level of food contamination.⁴ Climate change is predicted to influence future mycotoxin contamination patterns and levels on a global scale, with consequences for national and international trade, and levels to which consumers may be exposed through their diet.^{5,6} Regions with poorly developed regulatory structures and practices are likely to be most affected by international trade issues around contamination, and populations in such regions will more frequently have higher levels in their diets.

Mycotoxins are diverse with respect to both their chemical structure and properties, and also their effects on human health. Because of their toxicity and frequency as contaminants, AFs, fumonisins (FBs), zearalenone (ZEN), ochratoxin A (OTA), and deoxynivalenol (DON) are of particular concern for public health. Ingestion of these mycotoxins causes animal toxicities including cancer, immune-modulation, inflammation, kidney toxicity, stunted growth, or endocrine changes.^{3,7,8} AFs are proven human liver carcinogens and implicated in immune-modulation and impaired child growth.^{1,7,9,10} FBs interfere with sphingolipid synthesis in animals and humans,¹¹ are suspected human esophageal carcinogens,¹² and have been implicated in the etiology of both neural tube defects and stunting.¹³ OTA is carcinogenic in rodents and nephrotoxic in many animal species, though its role in human disease remains controversial.^{14,15} DON, also referred to as vomitoxin, is the major type-B trichothecene occurring frequently in moderate climate regions. Its primary mode of action is the inhibition of protein translation,¹⁶ whereas ZEN, another *Fusarium* toxin, and its phase-I metabolites exert potent estrogenic effects.^{8,17} Mixtures of *Fusarium* toxins in the diet have been implicated in dozens of acute poisoning incidences, on occasion affecting tens of thousands of individuals.⁶ However, the most often reported mycotoxicoses relate to AFs which can even lead to acute death, for example, in a severe outbreak in Kenya 2004 resulting in 125 deaths.^{7,18}

While the Food and Agriculture Organization (FAO) has estimated about two decades ago that 25% of the world's agricultural crops contain mycotoxins,¹⁹ a large survey measuring the presences of AFs, FBs, DON, ZEN, and OTA in cereal-based feed report both a high frequency of contamination (72% with at least one mycotoxin, $n > 19,000$ samples) and frequent cocontamination with other mycotoxins.²⁰ Similar frequencies

have been reported for cereal based foods, albeit typically at lower levels at least in highly developed world regions.^{19,21} Representative data for most African regions were notably absent at that time, though it was predicted that contamination of particularly AFs and FBs is frequent and at high levels compared to more developed regions.⁷ The application of multianalyte LC-MS/MS methods confirmed frequent cocontamination of cereals and foodstuff in several sub-Saharan countries.^{22–27} Thus, there remains a concern that individuals, particularly in developing regions, are frequently exposed to mixtures of mycotoxins through their regular diet. Recently, these predicted coexposures have been demonstrated in several populations and world regions by innovative biomeasurements of urine.^{28–36}

With respect to infant feeding, there are strict regulatory limits in place for complementary or weaning food in the European Union.³⁷ The maximum residual concentration levels in infant formula, follow-on formula, dietary foods for special medical purposes intended specifically for infants or processed cereal-based foods and baby foods for infants and young children, respectively, have been defined as follows: AFB₁ (0.1 ng/mL), AFM₁ (0.025 ng/mL), OTA (0.5 ng/mL), FB₁ (200 ng/mL), patulin (10 ng/mL), ZEN (20 ng/mL), and DON (200 ng/mL). While these regulations are not based on any clearly established risk from mycotoxins at such low levels in these items, food analysis and exposure assessment showed that baby foods in Europe can be contaminated by multiple mycotoxins and that infants are typically the most exposed population group.^{21,38–41} For newborns and infants, breast milk is regarded as the ideal form of nutrition. In addition, breastfeeding supports health benefits for both mother and child, and it is recommended by the World Health Organization to exclusively breastfeed for 6 months.^{42,43} However, food contaminants, including some mycotoxins, may be transferred from the maternal diet to breast milk (see Figure 1).

It is predicted that the health benefits of exclusive breastfeeding likely far surpass the putative health risk from modest levels of transfer, especially in highly regulated world regions. However, while the limits of these toxins in infant food are very restrictive and controlled by surveillance programs, breast milk is comparably rarely evaluated. Accurate assessment of toxins or toxicants in breast milk, however, is complex. Key aspects include the timing within any given feed, time of day of feed, duration of breastfeeding, birth order, stability of toxins, and the analytical accuracy of measurement tools.⁴⁴ Despite this, several studies report data on levels and frequency of contamination (see Table 1) though not all provide sufficient details related to the accuracy of collection and importantly the

Table 1. Summary of Case Studies and Analytical Methods Used for the Determination of Aflatoxins, Ochratoxin A, Zearalenone, and Fumonisin B₁ in Breast Milk 2006–2016^a

| country | mycotoxin | milk type | dietary information | sample number: positive/total (%) | concentration range (ng/L) | mean conc. (ng/L) | LOD/LOQ (ng/L) | sample preparation | analytical method | ref |
|----------|-------------------------------|--------------------------|---------------------|-----------------------------------|----------------------------|----------------------|-----------------|-----------------------|--------------------|-----|
| Brazil | AFM ₁ ^b | NI ^c | NI ^c | 2/100 (2) | LOD – LOQ ^a | | 0.3/0.8 | IAC ^d | LC-FD ^e | 96 |
| Brazil | OTA ^f | NI | NI | 66/100 (66) | LOQ - 21 | | 0.3/0.8 | IAC | LC-FD | 96 |
| Cameroon | AFM ₁ | mature | FQ ^g | 3/62 (5) | 5–625 | | NI ^c | LLE ^h | LC-FD | 97 |
| Chile | OTA | All | FQ | 27/37 (73) | LOD - 186 | 86 ± 59 ⁱ | 10/30 | LLE | LC-FD | 46 |
| Chile | OTA | colostrum | NI | 9/9 (100) | 44–184 | 106 ± 45 | 10/30 | LLE | LC-FD | 98 |
| Chile | OTα ^j | colostrum | NI | 9/9 (100) | LOQ - 100 | 40 ± 30 | 20/40 | LLE | LC-FD | 98 |
| Columbia | AFM ₁ | NI | FQ | 45/50 (90) | LOD - 19 | 5.2 ± 2.1 | 1/2 | IAC | LC-FD | 88 |
| Egypt | AFB ₁ ^k | mature | NI | 24/50 (48) | NI | 1900 ± 600 | NI | SkM ^l | ELISA ^m | 47 |
| Egypt | AFM ₁ | all | FQ | 138/388 (36) | 6–5131 | | 4/NI | SPE ⁿ | LC-FD | 48 |
| Egypt | AFM ₁ | NI | FQ | 248/443 (56) | 6–497 ^o | | 4/NI | SPE | LC-FD | 99 |
| Egypt | AFM ₁ | NI | FQ | 248/443 (56) | LOD – 108 ^p | | 4/NI | SPE | LC-FD | 99 |
| Egypt | AFM ₁ | mature | NI | 98/150 (65) | 200–19000 | 7100 ± 500 | NI | SkM | ELISA | 100 |
| Egypt | AFM ₁ | all | FQ | 87/125 (70) | 7–328 | 74 ± 7 | NI | SkM | ELISA | 101 |
| Egypt | OTA | mature | NI | 36/50 (72) | NI | 1890 ± 980 | NI | SkM | ELISA | 102 |
| Germany | OTA | all | FQ | 45/90 (50) | LOD - 100 | | 10/30 | LLE | LC-MS ^q | 55 |
| Iran | AFM ₁ | NI | FQ | 157/160 (98) | LOD - 27 | 8 ± 5 | 5/NI | SkM | ELISA | 103 |
| Iran | AFM ₁ | NI | FQ | 8/132 (6) | 7–10 | 9.5 ± 1.5 | 5/NI | SkM | ELISA | 104 |
| Iran | AFM ₁ | mature | FQ | 20/182 (11) | LOD - 8 | 7 ± 1 | 5/NI | SkM | ELISA | 105 |
| Iran | AFM ₁ | NI | FQ | 85/85 (100) | 2–10 | 5.9 ± 2 | NI | SkM | ELISA | 106 |
| Iran | AFM ₁ | mature | FQ | 1/80 (1) | 6.8 | | 5/NI | SkM | ELISA | 107 |
| Iran | AFM ₁ | NI | NI | 24/87 (28) | 0.13–5 | 0.56 ± 1.23 | NI | SkM | ELISA | 108 |
| Iran | AFM ₁ | mature | FQ | 1/136 (1) | 20 | | NI | LLE | LC-FD | 109 |
| Iran | OTA | mature | FQ | 2/136 (3) | 90–140 | | NI | LLE | LC-FD | 109 |
| Iran | OTA | NI | NI | 84/87 (97) | 2–60 | 25 ± 14 | NI | SkM | ELISA | 110 |
| Italy | AFM ₁ | mature | FQ | 4/82 (5) | 7–140 | 55 ± 59 | 3/7 | IAC | LC-FD | 90 |
| Italy | OTA | mature | FQ | 61/82 (74) | 5–405 | 30 ± 67 | 2/5 | IAC | LC-FD | 90 |
| Italy | OTA | colostrum | FQ | 45/57 (79) | 1–75 | 10 ± 16 | 0.5/1 | IAC | LC-FD | 89 |
| Italy | ZEN ^s | mature | FQ | 47/47 (100) | 260–1780 | 1130 ± 340 | 60/NI | IAC | ELISA and LC-FD | 53 |
| Jordan | AFM ₁ | mature | NI | 80/80 (100) | 10–137 | 68 ± 5 | NI | SkM | ELISA | 111 |
| Kuwait | AFM ₁ | NI | NI | 5/12 (42) | 9–15 | 9.7 | 5/NI | SkM | ELISA | 112 |
| Nigeria | AFM ₁ | NI | FQ | 41/50 (82) | 4–92 | | 10/50 | IAC | LC-FD | 113 |
| Nigeria | AFM ₁ | NI | NI | 5/28 (18) | LOD - 4000 | | 2000 | NI | TLC ^r | 114 |
| Nigeria | AFM ₁ | NI | NI | 17/120 (14) | 2000–187000 | 44000 | NI | LLE | LC-FD | 115 |
| Poland | OTA | colostrum | NI | 5/13 (38) | 6–17 | | 5/15 | IAC | LC-FD | 91 |
| Serbia | AFM ₁ | mature | NI | 6/10 (60) | 5–50 | | 1.5/5 | SkM | ELISA | 116 |
| Slovakia | OTA | mature | NI | 23/76 (30) | LOQ - 60 | | 5/14 | LLE, IAC | LC-FD | 117 |
| Sudan | AFM ₁ | NI | NI | 51/94 (54) | LOD - 2561 | 401 ± 525 | 13/NI | LLE | LC-FD | 118 |
| Tanzania | FB ₁ ^t | colostrum/ transitory | NI | 58/131 (44) | 6570–47105 | | 5500/19500 | LLE, SAX ^u | LC-FD | 51 |
| Tanzania | AFM ₁ | all | FQ | 143/143 (100) | 10–550 | | 5/NI | | LC-FD | 119 |
| Turkey | AFB ₁ | NI | NI | 75/75 (100) | 95–4123 | | 5/NI | LLE | LC-FD | 120 |
| Turkey | AFM ₁ | NI | NI | 75/75 (100) | 61–300 | | 5/NI | LLE | LC-FD | 120 |
| Turkey | AFM ₁ | NI | FQ | 18/73 (25) | 1–6 | 3 ± 1.4 | 10/NI | SkM | ELISA | 121 |
| Turkey | AFM ₁ | NI | NI | 8/61 (13) | 5–7 | 5.7 ± 0.6 | NI | IAC | LC-FD | 122 |
| Turkey | AFM ₁ | mature | | 9/70 (13) | | 5730 ± 740 | | | ELISA | 123 |
| Turkey | OTA | mature | | 34/70 (49) | | 140 ± 30 | | | ELISA | 123 |
| Turkey | OTA | NI | NI | 75/75 (100) | 620–13111 | >1500 | 10/NI | LLE | LC-FD | 124 |

^aLOD, limit of detection; LOQ, limit of quantification. ^bAflatoxin M₁. ^cNo information provided. ^dImmuno-affinity cartridge. ^eLiquid chromatography with fluorescence detector. ^fOchratoxin A. ^gFood questionnaire. ^hLiquid–liquid extraction. ⁱIn colostrum. ^jOchratoxin α. ^kAflatoxin B₁. ^lSkM, skimmed milk. Centrifugation and analysis of aqueous phase. ^mEnzyme-linked immunosorbent assay. ⁿSolid phase extraction. ^oSummer month. ^pWinter month. ^qLiquid chromatography mass spectrometry. ^rThin layer chromatography. ^sZearalenone. ^tFumonisin B₁. ^uStrong anion exchange.

demonstrated accuracy of the analytical methodology. This remains a concern, particularly if inaccurate data discourage breastfeeding of infants. Here, we propose that more accurate tools to better understand patterns, levels, and frequency of contamination are required.

■ CURRENT STATE OF THE ART

Lactational Transfer of Mycotoxins. There are significant knowledge gaps regarding the uptake and transfer of mycotoxins and their metabolites to breast milk. The transfer of mycotoxins

from food to breast milk may depend on dietary diversity and maternal hydration, physicochemical properties of the toxicant for both uptake and distribution, frequency of infant feeding, and probably breast infections that may occur as a consequence of breast milk production and feeding. However, our knowledge of these processes in humans is scarce or nonexistent with respect to mycotoxins. The few studies that have been conducted to determine lactational transfer rates for AFs and OTA from blood to breast milk were previously reviewed by Degen et al.⁴⁵ and may vary through different stages of breastfeeding. Muñoz et al.⁴⁶ demonstrated that milk to blood plasma (M/P) ratios for OTA changed from 0.40 ± 0.26 during the first days (colostrum) to 0.15 ± 0.26 in the later stages (15 days–four months). For AFM₁, a M/P ratio of 0.21 was derived from levels in milk and blood of nursing Egyptian mothers,⁴⁷ though AFM₁ levels in plasma tend to be transient and thus less informative than OTA. It was also shown that AFM₁ concentrations in breast milk are higher at the early stages of lactation.^{45,48} Moreover, estimates of the percentage of aflatoxins in the diet excreted as AFM₁ in breast milk ranged from 0.09 to 0.43% in a study from The Gambia.⁴⁹ It should also be noted that besides the major metabolite, AFM₁, the parent toxin AFB₁ as well as other AFs and their metabolites may be present in breast milk, although no transfer rates are published to the best of our knowledge.

Since knowledge on transfer rates in humans is limited, the carry-over from feed to milk in dairy cattle can give a rough approximation of what to expect.⁴⁵ However, it is important to consider that (a) bacterial conversion in ruminants is different from that in monogastric species and that this may heavily impact the disposition of a given mycotoxin and that (b) the milk production rates/kg bw in dairy herds far exceeds that of a breastfeeding mother, significantly modifying the distribution of systemically available toxicants. With those caveats, in brief, for FB₁, DON, and NIV, very low carryover to dairy milk is described (<0.1%) and is sometimes nondetectable, whereas about 0.7% of ZEN and its metabolites are transferred. Carryover of AFB₁ as AFM₁ is reported at about 2–6% and of OTA and metabolites at about 6%.⁵⁰ However, new reported data on FB₁ concentrations in human breast milk (max. 471 ng/mL)⁵¹ obtained from Tanzanian mothers raises significant concern. This observation may reflect (i) very high transfer rates in humans that would be counter to the physicochemical properties of FB₁, (ii) extraordinary high FB₁ exposure in the mothers' diet, or (iii) issues around peak identification or quantification in the HPLC-FD methodology with FB₁-conjugates. The physicochemical properties of FBs mean that they are poorly taken up from the gut of humans¹⁰ and at least in dairy herds have suggested transfers to milk of only up to 0.05%.⁵²

Clearly data on transfer rates are limited on the families of mycotoxins of public health concern. In addition to lactational transfer rates, the knowledge on potential correlations between mycotoxin concentrations in breast milk and in the urine of both the mother and/or the infant would be useful. Sample collection of infants' urine could enable a combined exposure assessment for both the infant and the mother where contributions from complementary food may also be relevant. Where exclusive breastfeeding is conducted, it could be argued that breast milk measures may be easier and more informative of infant exposures compared to estimates from urinary biomesures of the infant. For the latter, clear dose–response relationships between infant exposure and the urinary measure are lacking, compared to the direct measure from breast milk. More research efforts are required for correlating maternal dietary exposure, blood plasma,

and breast milk levels in a proper way utilizing advanced analytical methodologies and robust experimental designs including proper sampling procedures⁴⁴ and longitudinal data collection.

Single Toxin Analysis. In order to assess putative risks related to mycotoxin exposure of infants via breast milk, many groups have established analytical methods to determine AFs and OTA in breast milk, starting in the 1980s,⁹ with more recent contributions for FB₁⁵¹ and ZEN.^{53,54} The current state of the art is still the monitoring of single or a single class of mycotoxins (e.g., different AFs) in human breast milk.⁴⁵ There is particular concern where some of these methods lack the specificity of LC-MS identification of analytes but are reporting high levels of toxins in breast milk, with known low uptake and transfer rates,⁵⁰ as recently reported for FBs.⁵¹ To date, around 98% of all data generated do not involve the specificity of the LC-MS techniques in primary measurements or as a confirmatory tool for data generated by more rapid approaches that can remain useful in primary screening. An overview of methods, case studies, and the analytical methodologies employed in studies published during the last 10 years is presented in Table 1.

Most of the generated data reported concentrations of AFM₁ and OTA in breast milk. When looking at the geographical variation, especially for AFM₁, exposure is apparently very low or negligible in most European countries (low risk countries), whereas critical concentrations can be reached in sub-Saharan Africa and other tropical or subtropical regions as well as in Egypt and Turkey. OTA shows a wide range of concentrations in samples from various countries, with more than 100-fold higher levels in breast milk from Egypt, Turkey, and Sierra Leone than in European or South American countries (Table 1). The extremely limited data on Fusarium toxins FB₁ and ZEN do not allow for a conclusion regarding breast milk contamination.

Analytical Methods and Sample Preparation Approaches. Published methods used to detect mycotoxins after sample preparation are mainly based on liquid chromatography coupled to a fluorescence detector (LC-FD) or by enzyme-linked immunosorbent assays (ELISA). These methods enabled very low detection limits even before the advent of highly sensitive triple quadrupole mass spectrometers during the last 15 years. However, published LC-FD methods may require tedious sample preparation, some with derivatization steps, while the disadvantage of ELISA methods is the potential cross-reactivity with metabolites of the target compounds or with matrix components. LC-MS/MS based methods have been introduced in this field just recently.⁵⁵ Liquid–liquid extraction (LLE), immune-affinity cartridges (IAC), and solid phase extraction (SPE) are the most widely used cleanup procedures that have been applied in the past (see Table 1). Moreover, when formation of phase-II metabolites (i.e., glucuronides and sulfates) is expected, an enzymatic hydrolysis with β -glucuronidase/arylsulfatase prior to sample cleanup is recommended (unless reference compounds for all conjugated forms are available) to cover both conjugated and parent forms of a mycotoxin.

Toward Multianalyte Methods. Development of multi-mycotoxin analytical tools has been a priority in recent years to better capture potential mixtures in a single assay. Approaches include extraction of toxins from different matrices, e.g., various foodstuffs, feed, and biological fluids.^{5,20,34,56} Such multimethods are also available for measuring mycotoxins in cow's milk.^{57,58} However, only very few methods measuring more than one mycotoxin species in parallel in human breast milk have been

reported to date. Andrade et al.⁵⁹ optimized a method analyzing AFB₁, B₂, G₁, G₂, M₁, and OTA by LC-FD with recoveries from 73 to 100% and limits of quantification (LOQ) ranging from 5 to 30 ng/L. Human breast milk samples from Brazil were tested with only 2 out of 224 samples positive for AFB₂, the toxin with the lowest LOQ in this assay.⁵⁹ A screening method using LC coupled to high resolution mass spectrometry (LC-HRMS) was reported by Rubert et al.⁶⁰ Thirty-five human mature milk samples (i.e., 30 days after birth) from Spain were analyzed in a pilot survey. ZEN was found in 37% of the samples in concentrations ranging from 2–14 ng/mL; its metabolites (α - and β -zearalenol) were only found in one sample in which the parent compound was not detected.⁶⁰ Of concern was the fact that some other toxins were reported at surprisingly high concentrations (HT-2 toxin, neosolaniol, enniatins, and nivalenol (NIV)). From a biological point of view, these findings remain controversial as T2/HT-2 toxin (produced mainly by *Fusarium sporotrichioides* and *F. langsethiae*) is typically not a frequent contaminant in Spanish food and undergoes fast and abundant metabolism in humans.⁶¹ This group used an LC-HRMS approach employing Orbitrap technology and, in contrast to many other surveys in Europe (see Table 1), did not detect OTA in any sample. Also, the reported⁶⁰ high concentrations of ZEN (2–14 ng/mL) require urgent confirmation by more targeted methods which have been cross-validated between different laboratories.

FUTURE PERSPECTIVES AND RESEARCH NEEDS

Targeted and Untargeted Multianalyte Methods and Inclusion of Key Metabolites. A clear trend toward the development and application of multianalyte methods with high end LC-MS instruments is obvious in the area of food and feed safety^{62–67} and, more specifically, in the field of human biomonitoring.^{31,35,56,68} We propose that this trend will also take off in the analysis for mycotoxins in human breast milk utilizing advanced LC-MS/MS and LC-HRMS instrumentation. Besides the regulated, major mycotoxins, this will be particularly true for representatives of the group of so-called “emerging mycotoxins” such as beauvericin, the enniatins, aurofusarin, or *Alternaria* toxins. Also other toxins which have received little or no attention in breast milk so far (e.g., nivalenol or citrinin) will be of importance. Citrinin and its main metabolite, for example, were frequently detected in urine samples from Germany and Bangladesh recently.^{69,70} Yet, the main sources of exposure are unknown and may differ between countries. Moreover, multi-methods may additionally allow evaluating the occurrence and metabolism of key metabolites. The chemical or biochemical synthesis of glucuronide and/or sulfate conjugates of ZEN,^{71–73} zearalenols,^{71,74} DON,^{75–78} or T-2/HT-2^{76,79} enabled already the development of targeted analytical methods and subsequently a better insight into the metabolism of mycotoxins in *in vitro* studies^{80–83} and biomonitoring in human urine.^{28,29,31,32,84–87} Integration of these metabolites into multianalyte methods for the analysis of breast milk may aid in a better understanding of exposure and metabolite pattern. Also, other (commercially available) mycotoxin metabolites which may be of relevance in breast milk, such as aflatoxicol, ochratoxin- α , dihydrocitrinone, α - and β -zearalenol, and deepoxy-DON, should be preliminarily evaluated to inform if any of those might occur in relevant concentrations. Furthermore, co-occurring secondary metabolites which might not be toxic per se but may potentially modulate the toxicokinetics of a mycotoxin by, e.g.,

competing for detoxification cosubstrates could be included in these multimethods.

Here, we suggest that for routine surveillance fast and simple high throughput approaches such as ELISA or lateral flow devices (once validated for breast milk) are valuable but also that highly accurate LC-MS/MS measurements will be valuable tools to support such biomonitoring approaches. While LC-MS/MS offers great promise in terms of specificity and accuracy of analyte identification, with additional capabilities of multianalyte and metabolite quantification, there are still outstanding issues. To date, often highly specific but expensive IAC cleanup was required in order to reach acceptable sensitivity.^{88–91} These antibody-based sample preparation approaches can be discriminative when it comes to metabolic products. For example, several IACs were reported to not quantitatively retain glucuronides of DON and ZEN.³⁵ When using the latest state-of-the-art mass spectrometers, it seems even realistic to omit time and cost intensive sample preparation and just “defat and dilute” the breast milk samples. This would not only speed up analysis time but also avoid the loss of metabolites during the cleanup process. This will be important once high resolution mass spectrometers (HR-MS) find their way into this field of research as a routine technique. It is speculated that during the next decade more laboratories will utilize HR-MS instruments such as TOFs or Orbitraps in the full scan mode for biomonitoring studies instead of triple quadrupole instruments which are the leading type in the field currently. This is caused by the increasing sensitivity of new HR-MS instruments, their superb mass accuracy, and the great advantage that the acquired data can always be evaluated on new compounds retrospectively. This approach is already applied in other areas of food safety,^{62–64,66} and we believe that the number of HR-MS applications in the biomarker/food safety arena will continuously increase.

Analytical Quality Issues. Breast milk is a complex biological matrix, and it is part of the nature of any multianalyte method to accept compromises during sample extraction and cleanup, chromatographic separation, and setting ionization and detector parameters. Furthermore, the way and timing of sample collection will greatly influence contaminant levels in breast milk. To overcome analytical issues and ensure the generation of comparable, high-quality data sets, the following analytical needs have been identified: (1) Stability testing of analytes in breast milk and robust sampling procedures including proper documentation will be of crucial importance. Milk can be obtained either by a mechanic or electric pump (complete breast-milk expression) or by hand expression. The removal of all milk from the breasts at one time point utilizing a pump has been the preferred method in several studies.⁴⁴ While this gives no information about the milk which the baby would have consumed, this approach supports uniform sampling as the condition of the milk changes during one breastfeed. As the fat content is typically much higher during a later time point (hind milk), this might have an effect on the contaminant concentration according to its lipophilicity. Large variations in fat content might also considerably influence matrix effects during electrospray ionization. For exposure assessment, it is suggested to collect several spot samples over a time of, e.g., 24 h or even beyond and combine to pooled samples. In addition, transport and storage conditions of breast milk should be considered and reported accordingly. We are aware of course that this ideal desired collection may not always be possible in many situations, especially for low-income populations and because of cultural and nutritional issues.

(2) The establishment and thorough validation of robust sample preparation protocols minimizing both analyte losses during extraction and cleanup as well as matrix effects. Priority should be given to reduce the contamination of the chromatographic column and the mass spectrometer interface in order to ensure constant performance throughout long sequences and to reduce the cost of maintenance. Innovative concepts such as stable isotope dilution assays (SIDA), multitoxin IACs, QuEChERS, or new SPE materials should be considered and evaluated carefully. It is important to keep in mind that the composition of the milk varies through different stages of breastfeeding (i.e., colostrum versus mature milk) and also within a specific feed (fore milk vs hind milk). Consequently, proper validation should include an in-depth evaluation of possible effects caused by the varying matrix on the methods' performance.

(3) For accurate quantification, especially in challenging biological matrices, the use of suitable, ideally isotope-labeled (^2H or ^{13}C), internal standards is highly recommended. In recent years, ^{13}C -labeled reference standards have become commercially available for all regulated mycotoxins. However, only for very few key metabolites (e.g., AFM_1) internal standards are available. It is our hope that many of these metabolite standards will become routinely available. Also, recent achievements in the synthesis of nonlabeled reference standards of phase II metabolites should be continued. It is likely that some of these conjugates such as ZEN-14-glucuronide also occur in breast milk in the case of maternal exposure to the parent toxin and thus could aid in the characterization of infant exposure.

(4) Lastly, the critical evaluation of analytical performance parameters of single as well as multianalyte methods using well-defined reference materials and interlaboratory comparisons will be of high relevance. Yet, these initiatives are not established in the mycotoxin biomonitoring field in general. Only one study comparing two multibiomarker and two single biomarker methods in human urine was published by Solfrizzo et al.⁹² The results showed feasible z-score values for most analytes but also highlighted several issues leading the authors to the recommendation of further studies in this area.

Biomonitoring and Toxicological Aspects. There is limited information on either the pattern of mycotoxins or their metabolites that will occur in breast milk. Even for the AFs, the class for which by far most data is available, little is known besides the classical representatives AFB_1 , AFB_2 , AFG_1 , AFG_2 , and AFM_1 . However, even if a toxicological relevance of other metabolites such as aflatoxinol or sterigmatocystin seems unlikely, an evaluation of their potential impact and other co-occurring mycotoxins through plausible toxicological pathways should be considered. Hence, we recommend investigating typical patterns of mycotoxins and key metabolites beyond AFs and OTA in different populations (e.g., West African vs European vs Asian) and mycotoxin susceptible subgroups such as vegetarians. In parallel, it will be crucial to investigate mycotoxin exposure in infants who are not breastfed and thus consume postweaning food which is likely to result in far higher contaminant exposures as compared to exclusively breastfed infants.⁴⁵

Once the proposed multibiomarker methods for breast milk are established, we further suggest their application to correlate maternal dietary exposure to plasma and breast milk levels. To date, such dose–response relationships remain basically unexplored. Furthermore, innovative biomonitoring in breast milk alongside urine and/or plasma analysis may support evaluation of

intervention strategies to reduce the mycotoxin burden in the most exposed rural settings in Africa or Asia.

When looking at the potential impact of mycotoxins and their metabolites in breast milk, several aspects require attention. First, an improved characterization of the mycotoxin patterns and levels in breast milk samples from various continents is required. From what is known already from food contaminant analysis, it is predicted that the pattern and levels will vary considerably also in human breast milk samples from different countries. Once such information is available, it could provide some guidance for studies focusing on combined effects of naturally occurring mixtures.^{93–95}

■ CONCLUDING REMARKS

To date, we are not aware of any reported adverse health outcome related to maternal transfer of mycotoxins to infants, though some reported levels would exceed recommended TDIs for some toxins. However, it remains important that potential sources of contamination are measured and evaluated accurately. There is no doubt that breast milk is the ideal initial diet for infants from both nutrition and immune standpoints. This may be particularly true for populations in economically less developed regions where alternatives will lack essential nutrients found in breast milk; and importantly, these breast milk alternatives may be less hygienic, contain fewer non-nutrient beneficial components, and likely be contaminated with mycotoxins at significantly higher levels than breast milk. To date, little is known about the pattern of mycotoxins and their metabolites in breast milk as well as lactational transfer rates or potential combinatory effects. In this perspective, we propose that recent advances in mass spectrometry and the toxicological evaluation of contaminant mixtures will shed light on these issues. Such data will improve risk communication and inform policy makers. It is hoped that this outline of anticipated research activities will support the scientific community to aid those who are most susceptible toward the negative effects of mycotoxins and other contaminants, and where encouragement of methods to protect mothers and infants is crucial.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +43-1-4277-70800. E-mail: doris.marko@univie.ac.at.

Funding

This work was supported by the City of Vienna Jubilee Funds for the University of Natural Resources and Life Sciences, Vienna (BOKU Research Funding, project *MycoMarker*).

Notes

The authors declare no competing financial interest.

Biographies

Dr. Benedikt Warth holds a Senior Scientist position at the Department of Food Chemistry and Toxicology, University of Vienna since 2015. His Ph.D. at BOKU University (Vienna, Austria) focused on innovative mass spectrometry based methods for biomonitoring of mycotoxins in human urine. His research interest is at the edge of analytical toxicology, food science, and metabolomics and aims to better understand the role of bioactive food constituents and contaminants. He has had research stays at Linköping University (Sweden, 2007–2008) and The Scripps Research Institute (La Jolla, CA, 2016–ongoing). So far, he has received seven scientific awards and is an author or coauthor of 35 publications.

Dominik Braun is a project assistant at the Department of Food Chemistry and Toxicology, University of Vienna. He obtained his

diploma in Food Chemistry from the Technical University of Kaiserslautern (Germany) in 2015. His current focus is on the development of modern LC-MS/MS based assays to assess infants' exposure to mycotoxins. Dominik Braun is a member of the Austrian Society of Toxicology and is a participant in the EUROTOX associated Master's program of Toxicology for Postgraduates in Vienna.

Dr. Chibundu N. Ezekiel holds a Ph.D. in mycology/mycotoxicology and has been working on mycotoxins and mycotoxigenic fungi in food and feed for the past 10 years. His recent interest is in biomonitoring and exposure assessment of mycotoxins in humans. He has published several articles on mycotoxins in high impact peer reviewed journals. He is currently Technical Advisor (on secondment) for the Partnership for Aflatoxin Control in Africa based at the African Union Commission, Addis Ababa, where he provides technical backstopping on aflatoxin mitigation activities on the continent. Prior to joining the AUC, he was Senior Lecturer in Food Microbiology/Mycotoxicology at Babcock University, Nigeria.

Paul C. Turner, Ph.D. is an Assistant Professor at the University of Maryland, College Park. He is an applied environmental toxicologist involved in developing programs and tools to assess and intervene in environmental causes of disease. His focus is the development and use of exposure biomarkers for mycotoxins, with a global research portfolio including parts of Africa, South America, Asia, and Europe. Dr. Turner has >50 publications, including ground breaking research from West Africa demonstrating links between early life exposure to aflatoxin and stunting. Recent initiatives seek better understanding of the relationship between infant growth, mycotoxin exposure, nutrition, and hygiene to inform and develop targeted interventions.

Dr. Gisela H. Degen, trained as a Food Chemist, graduated in Toxicology (Ph.D.) at the University of Würzburg, Germany, then worked as Postdoctoral Fellow at the NIEHS (USA) in the Laboratories of Reproductive and Developmental Toxicology. Back in Germany, she completed her "Habilitation", received formal admission as lecturer in 1990, and in 1997 the title apl. Prof. in Biochemical Toxicology. She has been Head of Research Unit "Chemical Risks" at Leibniz-Institut für Arbeitsforschung an der TU Dortmund since 1992 and is now retired. Dr. Degen is the author of numerous original research papers and some reviews or book chapters on mycotoxins and other topics.

Doris Marko is Professor for Food Chemistry and head of the Department of Food Chemistry and Toxicology at the University of Vienna, Austria. Educated in food chemistry and toxicology, she is accredited as Registered European Toxicologist (EUROTOX) serving in several national and international commissions on food safety. Doris Marko is known for innovative studies on molecular mechanisms of food constituents. She has >120 publications including ground-breaking studies on genotoxic mechanisms of emerging mycotoxins. In interdisciplinary approaches combining food science, state-of-the art analytics and toxicology, current studies aim to unravel combinatory effects of naturally occurring mycotoxin mixtures on mammalian cells.

■ ABBREVIATIONS

AFs, aflatoxins; DON, deoxynivalenol; ELISA, enzyme-linked immunosorbent assay; FAO, Food and Agriculture Organization; FBs, fumonisins; HPLC-FD, high pressure liquid chromatography coupled to a fluorescence detector; IAC, immune-affinity cartridges; LC-FD, liquid chromatography coupled to a fluorescence detector; LC-HRMS, liquid chromatography coupled to high resolution mass spectrometry; LCL, lower calibration limit; LC-MS/MS, liquid chromatography tandem mass spectrometry; LC-MS, liquid chromatography-mass spectrometry; LLE, liquid-liquid extraction; LOD,

limits of detection; LOQ, limits of quantification; M/P, milk to plasma; ND, not detected; NIV, nivalenol; OTA, ochratoxin A; QuEChERS, quick, easy, cheap, effective, rugged and safe; SAX, strong anion exchange; SIDA, stable isotope dilution assay; SPE, solid phase extraction; TLC, thin layer chromatography; TOF, time-of-flight; ZEL, zearalenol; ZEN, zearalenone

■ REFERENCES

- (1) Kensler, T. W., Roebuck, B. D., Wogan, G. N., and Groopman, J. D. (2011) Aflatoxin: A 50-year Odyssey of mechanistic and translational toxicology. *Toxicol. Sci.* 120, S28–S48.
- (2) Blount, W. (1961) Turkey "X" disease. *Turkeys* 9, 52–55.
- (3) Bennett, J. W., and Klich, M. (2003) Mycotoxins. *Clin. Microbiol. Rev.* 16, 497–516.
- (4) Shephard, G. S. (2008) Impact of mycotoxins on human health in developing countries. *Food Addit. Contam., Part A* 25, 146–151.
- (5) Uhlig, S., Eriksen, G. S., Hofgaard, I. S., Krška, R., Beltran, E., and Sulyok, M. (2013) Faces of a changing climate: semi-quantitative multi-mycotoxin analysis of grain grown in exceptional climatic conditions in Norway. *Toxins* 5, 1682–1697.
- (6) CAST (2003) *Mycotoxins: Risks in Plant Animal and Human Systems*, Task Force Report No. 139, Council for Agricultural Science and Technology, Ames, IA.
- (7) Turner, P. C., Flannery, B., Isitt, C., Ali, M., and Pestka, J. (2012) The role of biomarkers in evaluating human health concerns from fungal contaminants in food. *Nutr. Res. Rev.* 25, 162–179.
- (8) Metzler, M., Pfeiffer, E., and Hildebrand, A. A. (2010) Zearalenone and its metabolites as endocrine disrupting chemicals. *World Mycotoxin J.* 3, 385–401.
- (9) Smith, L. E., Prendergast, A. J., Turner, P. C., Mbuya, M. N. N., Mutasa, K., Kembo, G., and Stoltzfus, R. J. (2015) The Potential Role of Mycotoxins as a Contributor to Stunting in the SHINE Trial. *Clin. Infect. Dis.* 61, S733–S737.
- (10) IARC (2002) Some Traditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 82, pp 1–556, World Health Organization, International Agency for Research on Cancer, Lyon, France.
- (11) Riley, R. T., Torres, O., Matute, J., Gregory, S. G., Ashley-Koch, A. E., Showker, J. L., Mitchell, T., Voss, K. A., Maddox, J. R., and Gelineau-van Waes, J. B. (2015) Evidence for fumonisin inhibition of ceramide synthase in humans consuming maize-based foods and living in high exposure communities in Guatemala. *Mol. Nutr. Food Res.* 59, 2209–2224.
- (12) Wang, X., Wu, Q., Wan, D., Liu, Q., Chen, D., Liu, Z., Martínez-Larrañaga, M. R., Martínez, M. A., Anadón, A., and Yuan, Z. (2016) Fumonisin: oxidative stress-mediated toxicity and metabolism in vivo and in vitro. *Arch. Toxicol.* 90, 81–101.
- (13) Wu, F., Groopman, J. D., and Pestka, J. J. (2014) Public health impacts of foodborne mycotoxins. *Annu. Rev. Food Sci. Technol.* 5, 351–372.
- (14) O'Brien, E., and Dietrich, D. R. (2005) Ochratoxin A: The continuing enigma. *Crit. Rev. Toxicol.* 35, 33–60.
- (15) Mally, A. (2012) Ochratoxin a and mitotic disruption: Mode of action analysis of renal tumor formation by ochratoxin A. *Toxicol. Sci.* 127, 315–330.
- (16) Pestka, J. (2010) Deoxynivalenol: mechanisms of action, human exposure, and toxicological relevance. *Arch. Toxicol.* 84, 663–679.
- (17) Mally, A., Solfrizzo, M., and Degen, G. H. (2016) Biomonitoring of the mycotoxin Zearalenone: current state-of-the art and application to human exposure assessment. *Arch. Toxicol.* 90, 1281–1292.
- (18) Lewis, L., Onsongo, M., Njapau, H., Schurz-Rogers, H., Lubber, G., Kieszak, S., Nyamongo, J., Backer, L., Dahiye, A. M., Misore, A., DeCock, K., Rubin, C., Nyikal, J., Njuguna, C., Langat, A., Kilei, I. K., Tetteh, C., Likimani, S., Oduor, J., Nzioki, D., Kamau, B. W., Onsongo, J., Slutsker, L., Mutura, C., Mensah, P., Kessel, F., Park, D. L., Trujillo, S., Funk, A., Geiseler, K. E., Azziz-Baumgartner, E., and Gupta, N. (2005) Aflatoxin contamination of commercial maize products during an outbreak of

acute aflatoxicosis in eastern and central Kenya. *Environ. Health Perspect.* 113, 1763–1767.

(19) Smith, J. E., and Solomons, G. L. (1994) Mycotoxins in Human Nutrition and Health, European Commission, Directorate-General for Research and Innovation, retrieved from <http://bookshop.europa.eu/en/mycotoxins-in-human-nutrition-and-health-pbCGNA16048/>.

(20) Schatzmayr, G., and Streit, E. (2013) Global occurrence of mycotoxins in the food and feed chain: facts and figures. *World Mycotoxin J.* 6, 213–222.

(21) SCOOP (2003) *Collection of Occurrence Data of Fusarium Toxins in Food and Assessment of Dietary Intake by the Population of EU Member States*, Scientific Cooperation on Questions Relating to Food.

(22) Njumbe Ediage, E., Diana Di Mavungu, J., Monbaliu, S., Van Peteghem, C., and De Saeger, S. (2011) A Validated Multianalyte LC–MS/MS Method for Quantification of 25 Mycotoxins in Cassava Flour, Peanut Cake and Maize Samples. *J. Agric. Food Chem.* 59, 5173–5180.

(23) Abia, W. A., Warth, B., Sulyok, M., Krska, R., Tchana, A. N., Njobeh, P. B., Dutton, M. F., and Moundipa, P. F. (2013) Determination of multi-mycotoxin occurrence in cereals, nuts and their products in Cameroon by liquid chromatography tandem mass spectrometry (LC-MS/MS). *Food Control* 31, 438–453.

(24) Ezekiel, C. N., Sulyok, M., Warth, B., Odebode, A. C., and Krska, R. (2012) Natural occurrence of mycotoxins in peanut cake from Nigeria. *Food Control* 27, 338–342.

(25) Shephard, G. S., Burger, H. M., Gambacorta, L., Krska, R., Powers, S. P., Rheeder, J. P., Solfrizzo, M., Sulyok, M., Visconti, A., Warth, B., and Van Der Westhuizen, L. (2013) Mycological analysis and multi-mycotoxins in maize from rural subsistence farmers in the former Transkei, South Africa. *J. Agric. Food Chem.* 61, 8232–8240.

(26) Warth, B., Parich, A., Atehnkeng, J., Bandyopadhyay, R., Schuhmacher, R., Sulyok, M., and Krska, R. (2012) Quantitation of Mycotoxins in Food and Feed from Burkina Faso and Mozambique Using a Modern LC-MS/MS Multitoxin Method. *J. Agric. Food Chem.* 60, 9352–9363.

(27) Mngqawa, P., Shephard, G. S., Green, I. R., Ngobeni, S. H., de Rijk, T. C., and Katerere, D. R. (2016) Mycotoxin contamination of home-grown maize in rural northern South Africa (Limpopo and Mpumalanga Provinces). *Food Addit. Contam., Part B* 9, 38–45.

(28) Ezekiel, C. N., Warth, B., Ogara, I. M., Abia, W. A., Ezekiel, V. C., Atehnkeng, J., Sulyok, M., Turner, P. C., Tayo, G. O., Krska, R., and Bandyopadhyay, R. (2014) Mycotoxin exposure in rural residents in northern Nigeria: A pilot study using multi-urinary biomarkers. *Environ. Int.* 66, 138–145.

(29) Gerding, J., Cramer, B., and Humpf, H.-U. (2014) Determination of mycotoxin exposure in Germany using an LC-MS/MS multi-biomarker approach. *Mol. Nutr. Food Res.* 58, 2358–2368.

(30) Heyndrickx, E., Sioen, I., Huybrechts, B., Callebaut, A., De Henauw, S., and De Saeger, S. (2015) Human biomonitoring of multiple mycotoxins in the Belgian population: Results of the BIOMYCO study. *Environ. Int.* 84, 82–89.

(31) Huybrechts, B., Martins, J. C., Debongnie, P., Uhlig, S., and Callebaut, A. (2015) Fast and sensitive LC–MS/MS method measuring human mycotoxin exposure using biomarkers in urine. *Arch. Toxicol.* 89, 1993–2005.

(32) Shephard, G. S., Burger, H.-M., Gambacorta, L., Gong, Y. Y., Krska, R., Rheeder, J. P., Solfrizzo, M., Srey, C., Sulyok, M., Visconti, A., Warth, B., and van der Westhuizen, L. (2013) Multiple mycotoxin exposure determined by urinary biomarkers in rural subsistence farmers in the former Transkei, South Africa. *Food Chem. Toxicol.* 62, 217–225.

(33) Solfrizzo, M., Gambacorta, L., and Visconti, A. (2014) Assessment of multi-mycotoxin exposure in southern Italy by urinary multi-biomarker determination. *Toxins* 6, 523–538.

(34) Warth, B., Sulyok, M., and Krska, R. (2013) LC-MS/MS-based multi-biomarker approaches for the assessment of human exposure to mycotoxins. *Anal. Bioanal. Chem.* 405, 5687–5695.

(35) Warth, B., Sulyok, M., Fruhmann, P., Mikula, H., Berthiller, F., Schuhmacher, R., Hametner, C., Abia, W. A., Adam, G., Fröhlich, J., and Krska, R. (2012) Development and validation of a rapid multi-biomarker liquid chromatography/tandem mass spectrometry method

to assess human exposure to mycotoxins. *Rapid Commun. Mass Spectrom.* 26, 1533–1540.

(36) Warth, B., Petchkongkaew, A., Sulyok, M., and Krska, R. (2014) Utilising an LC-MS/MS-based multi-biomarker approach to assess mycotoxin exposure in the Bangkok metropolitan area and surrounding provinces. *Food Addit. Contam., Part A* 31, 2040–2046.

(37) European Commission (2006) Commission Regulation (EC) No. 1881/2006 of 19 December 2006 Setting Maximum Levels for Certain Contaminants in Foodstuffs (Consolidated Version 2010-07-01), in *Off. J. Eur. Union*, L364, 5–24.

(38) Cano-Sancho, G., Ramos, A. J., Marín, S., and Sanchis, V. (2012) Occurrence of fumonisins in Catalonia (Spain) and an exposure assessment of specific population groups. *Food Addit. Contam., Part A* 29, 799–808.

(39) Assunção, R., Vasco, E., Nunes, B., Loureiro, S., Martins, C., and Alvito, P. (2015) Single-compound and cumulative risk assessment of mycotoxins present in breakfast cereals consumed by children from Lisbon region, Portugal. *Food Chem. Toxicol.* 86, 274–281.

(40) Alvito, P. C., Sizoo, E. A., Almeida, C. M. M., and van Egmond, H. P. (2010) Occurrence of aflatoxins and ochratoxin A in baby foods in Portugal. *Food Anal. Meth.* 3, 22–30.

(41) Raiola, A., Tenore, G. C., Manyes, L., Meca, G., and Ritieni, A. (2015) Risk analysis of main mycotoxins occurring in food for children: An overview. *Food Chem. Toxicol.* 84, 169–180.

(42) Horta, B. L., Bahl, R., Martines, J. C., and Victora, C. G. (2007) *Evidence on the Long-Term Effects of Breastfeeding: Systematic Reviews and Meta-Analyses*, World Health Organization, Geneva, Switzerland, retrieved from http://whqlibdoc.who.int/publications/2007/9789241595230_eng.pdf.

(43) WHO (2015) Infant and Young Child Feeding, Fact Sheet No. 342, retrieved from <http://www.who.int/mediacentre/factsheets/fs342/en/>.

(44) Renfrew, M. J., Hay, A. M., Shelton, N., Law, G., Wallis, S., Madden, S., Shires, S., Sutcliffe, A., and Woolridge, M. W. (2008) Assessing levels of contaminants in breast milk: methodological issues and a framework for future research. *Paediatric and Perinatal Epidemiology* 22, 72–86.

(45) Degen, G., Muñoz, K., and Hengstler, J. (2013) Occurrence of Mycotoxins in Breast Milk, in *Handbook of Dietary and Nutritional Aspects of Human Breast Milk* (Zibadi, S., Watson, R. R., and Preedy, V. R., Eds.) pp 813–831, Wageningen Academic Publishers, The Netherlands.

(46) Muñoz, K., Blaszkewicz, M., Campos, V., Vega, M., and Degen, G. (2014) Exposure of infants to ochratoxin A with breast milk. *Arch. Toxicol.* 88, 837–846.

(47) Hassan, A. M., Sheashaa, H. A., Fatah, M. F. A., Ibrahim, A. Z., and Gaber, O. A. (2006) Does aflatoxin as an environmental mycotoxin adversely affect the renal and hepatic functions of Egyptian lactating mothers and their infants? A preliminary report. *Int. Urol. Nephrol.* 38, 339–342.

(48) Polychronaki, N., Turner, P. C., Mykkänen, H., Gong, Y., Amra, H., Abdel-Wahhab, M., and El-Nezami, H. (2006) Determinants of aflatoxin M1 in breast milk in a selected group of Egyptian mothers. *Food Addit. Contam.* 23, 700–708.

(49) Zarba, A., Wild, C. P., Hall, A. J., Montesano, R., Hudson, G. J., and Groopman, J. D. (1992) Aflatoxin M1 in human breast milk from the Gambia, West Africa, quantified by combined monoclonal antibody immunoaffinity chromatography and HPLC. *Carcinogenesis* 13, 891–894.

(50) Fink-Gremmels, J. (2008) Mycotoxins in cattle feeds and carry-over to dairy milk: A review. *Food Addit. Contam., Part A* 25, 172–180.

(51) Magoha, H., De Meulenaer, B., Kimanya, M., Hipolite, D., Lachat, C., and Kolsteren, P. (2014) Fumonisin B1 contamination in breast milk and its exposure in infants under 6 months of age in Rombo, Northern Tanzania. *Food Chem. Toxicol.* 74, 112–116.

(52) Becker-Algeri, T. A., Castagnaro, D., de Bortoli, K., de Souza, C., Drunkler, D. A., and Badiale-Furlong, E. (2016) Mycotoxins in Bovine Milk and Dairy Products: A Review. *J. Food Sci.* 81, R544–R552.

- (53) Massart, F., Micillo, F., Rivezzi, G., Perrone, L., Baggiani, A., Miccoli, M., and Meucci, V. (2016) Zearalenone screening of human breast milk from the Naples area. *Toxicol. Environ. Chem.* 98, 128–136.
- (54) Choi, M. H., Kim, K.-R., Hong, J. K., Park, S. J., and Chung, B. C. (2002) Determination of non-steroidal estrogens in breast milk, plasma, urine and hair by gas chromatography/mass spectrometry. *Rapid Commun. Mass Spectrom.* 16, 2221–2228.
- (55) Muñoz, K., Wollin, K., Kalhoff, H., and Degen, G. (2013) Occurrence of the mycotoxin ochratoxin A in breast milk samples from Germany. *Gesundheitswesen* 75, 194–197.
- (56) Malachová, A., Sulyok, M., Beltrán, E., Berthiller, F., and Krska, R. (2014) Optimization and validation of a quantitative liquid chromatography–tandem mass spectrometric method covering 295 bacterial and fungal metabolites including all regulated mycotoxins in four model food matrices. *J. Chromatogr. A* 1362, 145–156.
- (57) Huang, L. C., Zheng, N., Zheng, B. Q., Wen, F., Cheng, J. B., Han, R. W., Xu, X. M., Li, S. L., and Wang, J. Q. (2014) Simultaneous determination of aflatoxin M1, ochratoxin A, zearalenone and α -zearalenol in milk by UHPLC–MS/MS. *Food Chem.* 146, 242–249.
- (58) Flores-Flores, M. E., and González-Peñas, E. (2015) Development and validation of a high performance liquid chromatographic–mass spectrometry method for the simultaneous quantification of 10 trichothecenes in ultra-high temperature processed cow milk. *J. Chromatogr. A* 1419, 37–44.
- (59) Andrade, P. D., da Silva, J. L. G., and Caldas, E. D. (2013) Simultaneous analysis of aflatoxins B1, B2, G1, G2, M1 and ochratoxin A in breast milk by high-performance liquid chromatography/fluorescence after liquid–liquid extraction with low temperature purification (LLE–LTP). *J. Chromatogr. A* 1304, 61–68.
- (60) Rubert, J., León, N., Sáez, C., Martins, C. P. B., Godula, M., Yusà, V., Mañes, J., Soriano, J. M., and Soler, C. (2014) Evaluation of mycotoxins and their metabolites in human breast milk using liquid chromatography coupled to high resolution mass spectrometry. *Anal. Chim. Acta* 820, 39–46.
- (61) Yang, W., Yu, M., Fu, J., Bao, W., Wang, D., Hao, L., Yao, P., Nüssler, A. K., Yan, H., and Liu, L. (2014) Deoxynivalenol induced oxidative stress and genotoxicity in human peripheral blood lymphocytes. *Food Chem. Toxicol.* 64, 383–396.
- (62) Martínez-Domínguez, G., Romero-González, R., and Garrido Frenich, A. (2016) Multi-class methodology to determine pesticides and mycotoxins in green tea and royal jelly supplements by liquid chromatography coupled to Orbitrap high resolution mass spectrometry. *Food Chem.* 197, 907–915.
- (63) Reinholds, I., Pugajeva, I., and Bartkevcis, V. (2016) A reliable screening of mycotoxins and pesticide residues in paprika using ultra-high performance liquid chromatography coupled to high resolution Orbitrap mass spectrometry. *Food Control* 60, 683–689.
- (64) De Dominicis, E., Commissati, I., Gritti, E., Catellani, D., and Suman, M. (2015) Quantitative targeted and retrospective data analysis of relevant pesticides, antibiotics and mycotoxins in bakery products by liquid chromatography–single-stage Orbitrap mass spectrometry. *Food Addit. Contam., Part A* 32, 1617–1627.
- (65) Kaklamanos, G., Vincent, U., and Von Holst, C. (2013) Multi-residue method for the detection of veterinary drugs in distillers grains by liquid chromatography–Orbitrap high resolution mass spectrometry. *J. Chromatogr. A* 1322, 38–48.
- (66) Senyuva, H. Z., Gökmen, V., and Sarikaya, E. A. (2015) Future perspectives in Orbitrap-high-resolution mass spectrometry in food analysis: a review. *Food Addit. Contam., Part A* 32, 1568–1606.
- (67) Steinborn, A., Alder, L., Michalski, B., Zomer, P., Bendig, P., Martinez, S. A., Mol, H. G. J., Class, T. J., and Costa Pinheiro, N. (2016) Determination of Glyphosate Levels in Breast Milk Samples from Germany by LC-MS/MS and GC-MS/MS. *J. Agric. Food Chem.* 64, 1414–1421.
- (68) Solfrizzo, M., Gambacorta, L., Lattanzio, V. M., Powers, S., and Visconti, A. (2011) Simultaneous LC–MS/MS determination of aflatoxin M1, ochratoxin A, deoxynivalenol, de-epoxydeoxynivalenol, α and β -zearalenols and fumonisin B1 in urine as a multi-biomarker method to assess exposure to mycotoxins. *Anal. Bioanal. Chem.* 401, 2831–2841.
- (69) Ali, N., Blaszkewicz, M., Alim, A., Hossain, K., and Degen, G. H. (2015) Urinary biomarkers of ochratoxin A and citrinin exposure in two Bangladeshi cohorts: follow-up study on regional and seasonal influences. *Arch. Toxicol.*, DOI: 10.1007/s00204-015-1654-y.
- (70) Ali, N., Blaszkewicz, M., and Degen, G. H. (2015) Occurrence of the mycotoxin citrinin and its metabolite dihydrocitrinone in urines of German adults. *Arch. Toxicol.* 89, 573–578.
- (71) Stevenson, D. E., Hansen, R. P., Loader, J. I., Jensen, D. J., Cooney, J. M., Wilkins, A. L., and Miles, C. O. (2008) Preparative enzymatic synthesis of glucuronides of zearalenone and five of its metabolites. *J. Agric. Food Chem.* 56, 4032–4038.
- (72) Mikula, H., Hametner, C., Berthiller, F., Warth, B., Krska, R., Adam, G., and Fröhlich, J. (2012) Fast and reproducible chemical synthesis of zearalenone-14- β -D-glucuronide. *World Mycotoxin J.* 5, 289–296.
- (73) Mikula, H., Weber, J., Svatunek, D., Skrinjar, P., Adam, G., Krska, R., Hametner, C., and Fröhlich, J. (2014) Synthesis of zearalenone-16- β -D-glucoside and zearalenone-16-sulfate: A tale of protecting resorcylic acid lactones for regiocontrolled conjugation. *Beilstein J. Org. Chem.* 10, 1129–1134.
- (74) Mikula, H., Weber, J., Lexmüller, S., Bichl, G., Schwartz, H., Varga, E., Berthiller, F., Hametner, C., Krska, R., and Fröhlich, J. (2013) Simultaneous preparation of α/β -zearalenol glucosides and glucuronides. *Carbohydr. Res.* 373, 59–63.
- (75) Fruhmann, P., Warth, B., Hametner, C., Berthiller, F., Horkel, E., Adam, G., Sulyok, M., Krska, R., and Fröhlich, J. (2012) Synthesis of deoxynivalenol-3- β -D-O-glucuronide for its use as biomarker for dietary deoxynivalenol exposure. *World Mycotoxin J.* 5, 127–132.
- (76) Fruhmann, P., Skrinjar, P., Weber, J., Mikula, H., Warth, B., Sulyok, M., Krska, R., Adam, G., Rosenberg, E., Hametner, C., and Fröhlich, J. (2014) Sulfation of deoxynivalenol, its acetylated derivatives, and T2-toxin. *Tetrahedron* 70, 5260–5266.
- (77) Uhlig, S., Ivanova, L., and Fæste, C. K. (2013) Enzyme-Assisted Synthesis and Structural Characterization of the 3-, 8-, and 15-Glucuronides of Deoxynivalenol. *J. Agric. Food Chem.* 61, 2006–2012.
- (78) Wu, X., Murphy, P., Cunnick, J., and Hendrich, S. (2007) Synthesis and characterization of deoxynivalenol glucuronide: its comparative immunotoxicity with deoxynivalenol. *Food Chem. Toxicol.* 45, 1846–1855.
- (79) Welsch, T., and Humpf, H.-U. (2012) HT-2 Toxin 4-Glucuronide as New T-2 Toxin Metabolite: Enzymatic Synthesis, Analysis, and Species Specific Formation of T-2 and HT-2 Toxin Glucuronides by Rat, Mouse, Pig, and Human Liver Microsomes. *J. Agric. Food Chem.* 60, 10170–10178.
- (80) Pfeiffer, E., Hildebrand, A., Mikula, H., and Metzler, M. (2010) Glucuronidation of zearalenone, zeranol and four metabolites in vitro: formation of glucuronides by various microsomes and human UDP-glucuronosyltransferase isoforms. *Mol. Nutr. Food Res.* 54, 1468–1476.
- (81) Maul, R., Warth, B., Schebb, N. H., Krska, R., Koch, M., and Sulyok, M. (2015) In vitro glucuronidation kinetics of deoxynivalenol by human and animal microsomes and recombinant human UGT enzymes. *Arch. Toxicol.* 89, 949–960.
- (82) Maul, R., Warth, B., Kant, J.-S., Schebb, N. H., Krska, R., Koch, M., and Sulyok, M. (2012) Investigation of the hepatic glucuronidation pattern of the Fusarium mycotoxin deoxynivalenol in various species. *Chem. Res. Toxicol.* 25, 2715–2717.
- (83) Frizzell, C., Uhlig, S., Miles, C. O., Verhaegen, S., Elliott, C. T., Eriksen, G. S., Sørlie, M., Ropstad, E., and Connolly, L. (2015) Biotransformation of zearalenone and zearalenols to their major glucuronide metabolites reduces estrogenic activity. *Toxicol. In Vitro* 29, 575–581.
- (84) Warth, B., Sulyok, M., Berthiller, F., Schuhmacher, R., and Krska, R. (2013) New insights into the human metabolism of the Fusarium mycotoxins deoxynivalenol and zearalenone. *Toxicol. Lett.* 220, 88–94.
- (85) Šarkanj, B., Warth, B., Uhlig, S., Abia, W. A., Sulyok, M., Klapac, T., Krska, R., and Banjari, I. (2013) Urinary analysis reveals high

deoxynivalenol exposure in pregnant women from Croatia. *Food Chem. Toxicol.* 62, 231–237.

(86) Abia, W. A., Warth, B., Sulyok, M., Krska, R., Tchana, A., Njobeh, P. B., Turner, P. C., Kouanfack, C., Eyongetah, M., Dutton, M., and Moundipa, P. F. (2013) Bio-monitoring of mycotoxin exposure in Cameroon using a urinary multi-biomarker approach. *Food Chem. Toxicol.* 62, 927–934.

(87) Warth, B., Sulyok, M., Fruhmann, P., Berthiller, F., Schuhmacher, R., Hametner, C., Adam, G., Fröhlich, J., and Krska, R. (2012) Assessment of human deoxynivalenol exposure using an LC-MS/MS based biomarker method. *Toxicol. Lett.* 211, 85–90.

(88) Diaz, G. J., and Sánchez, M. P. (2015) Determination of aflatoxin M1 in breast milk as a biomarker of maternal and infant exposure in Colombia. *Food Addit. Contam., Part A* 32, 1192–1198.

(89) Biasucci, G., Calabrese, G., Di Giuseppe, R., Carrara, G., Colombo, F., Mandelli, B., Maj, M., Bertuzzi, T., Pietri, A., and Rossi, F. (2011) The presence of ochratoxin A in cord serum and in human milk and its correspondence with maternal dietary habits. *Eur. J. Nutr.* 50, 211–218.

(90) Galvano, F., Pietri, A., Bertuzzi, T., Gagliardi, L., Ciotti, S., Luisi, S., Bognanno, M., La Fauci, L., Iacopino, A. M., Nigro, F., Li Volti, G., Vanella, L., Giammanco, G., Tina, G. L., and Gazzolo, D. (2008) Maternal dietary habits and mycotoxin occurrence in human mature milk. *Mol. Nutr. Food Res.* 52, 496–501.

(91) Postupolski, J., Karlowski, K., and Kubik, P. (2006) Ochratoxin A in maternal and foetal blood and in maternal milk. *Roczniki Państwowego Zakładu* 57, 23.

(92) Solfrizzo, M., Gambacorta, L., Warth, B., White, K., Srey, C., Sulyok, M., Krska, R., and Gong, Y. Y. (2013) Comparison of single and multi-analyte methods based on LC-MS/MS for mycotoxin biomarker determination in human urine. *World Mycotoxin J.* 6, 355–366.

(93) Alassane-Kpembé, I., Schatzmayr, G., Taranu, I., Marin, D., Puel, O., and Oswald, I. P. (2016) Mycotoxins co-contamination: Methodological aspects and biological relevance of combined toxicity studies. *Crit. Rev. Food Sci. Nutr.*, DOI: 10.1080/10408398.2016.1140632.

(94) Vajdovszky, K., Warth, B., Sulyok, M., and Marko, D. (2016) Non-synergistic cytotoxic effects of Fusarium and Alternaria toxin combinations in Caco-2 cells. *Toxicol. Lett.* 241, 1–8.

(95) EFSA (2013) European Food Safety Authority. Human Risk Assessment of Combined Exposure to Multiple Chemicals. *EFSA J.* 11, 3313.

(96) Iha, M. H., Barbosa, C. B., Heck, A. R., and Trucksess, M. W. (2014) Aflatoxin M1 and ochratoxin A in human milk in Ribeirão Preto-SP, Brazil. *Food Control* 40, 310–313.

(97) Tchana, A. N., Moundipa, P. F., and Tchouanguép, F. M. (2010) Aflatoxin contamination in food and body fluids in relation to malnutrition and cancer status in Cameroon. *Int. J. Environ. Res. Public Health* 7, 178–188.

(98) Muñoz, K., Campos, V., Blaszkewicz, M., Vega, M., Alvarez, A., Neira, J., and Degen, G. H. (2010) Exposure of neonates to ochratoxin A: first biomonitoring results in human milk (colostrum) from Chile. *Mycotoxin Res.* 26, 59–67.

(99) Polychronaki, N., West, R. M., Turner, P. C., Amra, H., Abdel-Wahhab, M., Mykkänen, H., and El-Nezami, H. (2007) A longitudinal assessment of aflatoxin M1 excretion in breast milk of selected Egyptian mothers. *Food Chem. Toxicol.* 45, 1210–1215.

(100) Tomerak, R. H., Shaban, H. H., Khalafallah, O. A., and El Shazly, M. N. (2011) Assessment of exposure of Egyptian infants to aflatoxin M1 through breast milk. *J. Egypt. Public Health Assoc.* 86, 51–55.

(101) El-Tras, W. F., El-Kady, N. N., and Tayel, A. A. (2011) Infants exposure to aflatoxin M1 as a novel foodborne zoonosis. *Food Chem. Toxicol.* 49, 2816–2819.

(102) Hassan, A. M., Sheashaa, H. A., Fattah, M. F. A., Ibrahim, A. Z., Gaber, O. A., and Sobh, M. A. (2006) Study of ochratoxin A as an environmental risk that causes renal injury in breast-fed Egyptian infants. *Pediatr. Nephrol.* 21, 102–105.

(103) Sadeghi, N., Oveisi, M. R., Jannat, B., Hajimahmoodi, M., Bonyani, H., and Jannat, F. (2009) Incidence of aflatoxin M1 in human breast milk in Tehran, Iran. *Food Control* 20, 75–78.

(104) Ghiasian, S. A., and Maghsood, A. H. (2012) Infants' Exposure to Aflatoxin M1 from Mother's Breast Milk in Iran. *Iran. J. Public Health* 41, 119–126.

(105) Mahdavi, R., Nikniaz, L., Arefhosseini, S., and Jabbari, M. V. (2010) Determination of aflatoxin M1 in breast milk samples in Tabriz–Iran. *Maternal and Child Health Journal* 14, 141–145.

(106) Maleki, F., Abdi, S., Davodian, E., Haghani, K., and Bakhtiyari, S. (2015) Exposure of Infants to Aflatoxin M1 from Mother's Breast Milk in Ilam, Western Iran. *Osong Public Health and Research Perspectives* 6, 283–287.

(107) Jafarian-Dehkordi, A., and Pourradi, N. (2013) Aflatoxin M1 contamination of human breast milk in Isfahan, Iran. *Adv. Biomed. Res.* 2, 86.

(108) Rafiei, H., Dehghan, P., Pakshir, K., Pour, M. C., and Akbari, M. (2014) The concentration of aflatoxin M(1) in the mothers' milk in Khorrambid City, Fars, Iran. *Adv. Biomed. Res.* 3, 152.

(109) Afshar, P., Shokrzadeh, M., Kalhori, S., Babae, Z., and Saravi, S. S. (2013) Occurrence of Ochratoxin A and Aflatoxin M1 in human breast milk in Sari, Iran. *Food Control* 31, 525–529.

(110) Dehghan, P., Pakshir, K., Rafiei, H., Chadeganipour, M., and Akbari, M. (2014) Prevalence of Ochratoxin A in Human Milk in the Khorrambid Town, Fars Province, South of Iran. *Jundishapur J. Microbiol.* 7, e11220.

(111) Omar, S. S. (2012) Incidence of aflatoxin M1 in human and animal milk in Jordan. *J. Toxicol. Environ. Health, Part A* 75, 1404–1409.

(112) Dashti, B., Al-Hamli, S., Alomirah, H., Al-Zenki, S., Abbas, A. B., and Sawaya, W. (2009) Levels of aflatoxin M1 in milk, cheese consumed in Kuwait and occurrence of total aflatoxin in local and imported animal feed. *Food Control* 20, 686–690.

(113) Adejumo, O., Atanda, O., Raiola, A., Somorin, Y., Bandyopadhyay, R., and Ritieni, A. (2013) Correlation between aflatoxin M1 content of breast milk, dietary exposure to aflatoxin B1 and socioeconomic status of lactating mothers in Ogun State, Nigeria. *Food Chem. Toxicol.* 56, 171–177.

(114) Atanda, O., Oguntubo, A., Adejumo, O., Ikeorah, J., and Akpan, I. (2007) Aflatoxin M1 contamination of milk and ice cream in Abeokuta and Odeda local governments of Ogun State, Nigeria. *Chemosphere* 68, 1455–1458.

(115) Oluwafemi, F. T. (2012) Aflatoxin M1 levels in lactating mothers in two Nigerian cities. *Arch. Clin. Microbiol.* 3, 1–6.

(116) Kos, J., Lević, J., Đuragić, O., Kokić, B., and Miladinović, I. (2014) Occurrence and estimation of aflatoxin M1 exposure in milk in Serbia. *Food Control* 38, 41–46.

(117) Dostal, A., Jakusova, L., Cajdova, J., and Hudeckova, H. (2007) Results of the first studies of occurrence of ochratoxin A in human milk in Slovakia. *Bratisl. Lek. Listy* 109, 276–278.

(118) Elzupir, A. O., Abas, A. r. A., Fadul, M. H., Modwi, A. K., Ali, N. M. I., Jadian, A. F. F., Ahmed, N. A. A., Adam, S. Y. A., Ahmed, N. A. M., Khairy, A. A. A., and Khalil, E. A. G. (2012) Aflatoxin M1 in breast milk of nursing Sudanese mothers. *Mycotoxin Res.* 28, 131–134.

(119) Magoha, H., Kimanya, M., De Meulenaer, B., Roberfroid, D., Lachat, C., and Kolsteren, P. (2014) Association between aflatoxin M1 exposure through breast milk and growth impairment in infants from Northern Tanzania. *World Mycotoxin J.* 7, 277–284.

(120) Gürbay, A., Sabuncuoğlu, S. A., Girgin, G., Şahin, G., Yiğit, Ş., Yurdakök, M., and Tekinalp, G. (2010) Exposure of newborns to aflatoxin M1 and B1 from mothers' breast milk in Ankara, Turkey. *Food Chem. Toxicol.* 48, 314–319.

(121) Atasever, M., Yildirim, Y., Atasever, M., and Tastekin, A. (2014) Assessment of aflatoxin M1 in maternal breast milk in Eastern Turkey. *Food Chem. Toxicol.* 66, 147–149.

(122) Keskin, Y., Başkaya, R., Karsli, S., Yurdun, T., and Özyaral, O. (2009) Detection of aflatoxin M1 in human breast milk and raw cow's milk in Istanbul, Turkey. *J. Food Prot.* 72, 885–889.

(123) Uyar, B. B., Karaağaoğlu, N., Girgin, G., Gürbay, A., and Karaağaoğlu, E. (2014) Aflatoxin M1 and ochratoxin A levels in breast milk in Ankara, Turkey (1017.8). *FASEB J.* 28, 1017–1018.

(124) Gürbay, A., Girgin, G., Sabuncuoğlu, S. A., Şahin, G., Yurdakök, M., Yiğit, Ş., and Tekinalp, G. (2010) Ochratoxin A: is it present in breast milk samples obtained from mothers from Ankara, Turkey? *J. Appl. Toxicol.* 30, 329–333.