

A Special Focus on Mycotoxin Contamination in Baby Foods: Their Presence and Regulations

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Summary

Food safety is one of the major concerns in researches related to food toxicology. Contaminants present in food and feed are the most attention-drawing subjects in the last decade. Particularly, mycotoxin contamination is of great importance as it is widespread and unpreventable. Mycotoxins are toxic secondary metabolites by different fungi species. These compounds pose a potential threat to human and animal health through the ingestion of food products prepared from these commodities. Mycotoxicosis is the term used for poisoning associated with exposures to mycotoxins. The symptoms of a mycotoxicosis depend on the type of mycotoxin; the concentration and length of exposure; as well as age, health, and sex of the exposed individual. Aflatoxin B1 and ochratoxin A are mutagenic, teratogenic, and carcinogenic in many species where *Fusarium* toxins such as T2 toxin pose a threat as biological warfare agent. Many international agencies are trying to achieve universal standardization of regulatory limits for mycotoxins. Special emphasis must be drawn to mycotoxin contamination of baby foods and infant formulas as babies and small children are the most susceptible population to the effects of these toxins. In this review, the toxic effects of mycotoxins, the regulations in Europe and United States as well as Turkey and particularly the studies and regulations in baby foods will be dwelt upon.

Key Words: mycotoxin, regulatory limits, baby food/infant formula

Received: 26.11.2009

Revised: 25.05.2010

Accepted: 03.07.2010

Bebek Mamalarında Mikotoksin Kontaminasyonuna Bakış: Bulunışları ve Yasal Düzenlemeleri

Özet

Gıda güvenliği gıda toksikolojisi ile ilgili araştırmaların ana konularından biridir. Gıdalarda ve yemlerdeki bulaşıcılar son on yılın en çok ilgi çeken konusu olmuştur. Yaygın ve önlenemez olması nedeniyle özellikle mikotoksin kontaminasyonu çok önemlidir. Mikotoksinler farklı mantar türlerinin toksik sekonder metabolitleridir. Bu bileşikler bunları içeren gıdaların alımıyla insan ve hayvan sağlığı için potansiyel bir tehdittir. Mikotoksikoz mikotoksin maruziyetiyle ortaya çıkan zehirlenme için kullanılan bir terimdir. Mikotoksikozisin semptomları mikotoksin türüne, maruziyetin miktarına ve süresine, bireyin yaşına, sağlık durumuna ve cinsiyetine göre değişmektedir. Aflatoxin B1 ve okratoksin A mutajenik, teratojenik ve karsinojeniktir. *Fusarium* türlerinden T2 biyolojik savaş ajanı olarak bir tehdit oluşturmaktadır. Birçok uluslararası kuruluş mikotoksinlerin limit düzenlemelerine bir standardizasyon getirmeye çalışmaktadır. Bebekler ve küçük çocuklar bu toksinlerin etkilerine daha hassas oldukları için özel olarak bebek mamalarındaki mikotoksin kontaminasyonuna dikkat edilmelidir. Bu derlemede mikotoksinlerin toksik etkilerinden; Avrupa, Amerika Bileşik Devletleri ve Türkiye'deki regülasyonlardan bahsedilecek ve özellikle bebek mamaları üzerindeki çalışmalar ve regülasyonların üzerinde durulacaktır.

Anahtar Kelimeler: mikotoksin, düzenleyici limitler, bebek maması

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INTRODUCTION

A major international focus has been ensuring the safety of food. Toxins present in food and animal feed are of major concern of health care givers and public for decades. A toxin can be defined as a substance that is synthesized by a plant species, an animal, or by microorganisms, that is harmful to another organism. The term 'mycotoxin' is usually reserved for the relatively small (MW ~700), toxic chemical products formed as secondary metabolites by a few fungi that readily colonize crops in the field or after harvest. These compounds pose a potential threat to human and animal health through the ingestion of food products prepared from these commodities. Generally, crops that are stored for more than a few days become a potential target for mould growth and mycotoxin formation. Mycotoxins can occur both in temperate and tropical regions of the world, depending on the species of fungi. Contamination can occur pre- or post-harvest or at the field (1). Favorable conditions such as high humidity and high temperature can increase the content of mycotoxin during storage. Cereals, spices, nuts, grapes, apples, dried fruit, dried vegetables (peas, beans), oil seeds, teas, cocoa and coffees can contain high amount of different mycotoxins. Food-based mycotoxins and their health effects were extensively studied in the last century and there are several regulations based on their presence in different foodstuffs (2-4). Mycotoxins can also enter the human food chain via meat or other animal products such as eggs, milk and cheese as the result of livestock eating contaminated feed (5).

Mycotoxin Types

Aflatoxins

Aflatoxins (AFs) are naturally occurring highly toxic mycotoxins that are produced as secondary metabolites of different widespread *Aspergillus* species (*Aspergillus flavus*, *Aspergillus parasitius*, *Aspergillus nomius*) and they may be present in groundnuts, other edible nuts, dried fruits, spices, figs and cereals (especially maize) (2, 6-10). Sources of AF contamination in animal feedstuffs may vary geographically. Contamination of agricultural crops with AFs is a worldwide problem not limited to developing countries, where both climatic and technological conditions stimulate aflatoxin

formation (11). *Aspergillus flavus* produces AFB1 and AFB2, while two other species produce AFG1 and AFG2 (10). The diseases caused by AF consumption are called aflatoxicosis. Acute aflatoxicosis results in death; chronic aflatoxicosis results in cancer, immune suppression, and other "slow" pathological conditions (12).

AFB1 is the most known potent natural carcinogen. The activation of AFB1 by Phase I enzymes namely, cytochrome P450 (CYPs) isoenzymes CYP1A2 and CYP3A4, produces AF1-8,9-epoxide which is highly carcinogenic in humans. It forms DNA adducts and albumin adducts (13). A reactive glutathione S-transferase (GST) system found in the cytosol and microsomes catalyzes the conjugation of activated aflatoxins with reduced glutathione, leading to the excretion of aflatoxin (14). Variations in the level of the GST system as well as variations in the CYP system are thought to contribute to the differences observed in interspecies aflatoxin susceptibility (15).

AFB1 is listed as Group I agent by International Agency for Research on Cancer (IARC). The principle target organ of AFB1 is liver. It is known that the reactive aflatoxin epoxide binds to the N7 position of guanines (16). AFB1 causes mutation of p53 gene at third base of codon 249, and takes the form of G >T transversions. This mutation may inactivate p53 and the detection of TP53 mutant DNA plasma is a biomarker of both AFB1 exposure and hepatocellular carcinoma (17, 18). AFB1 alters the activation of p53 in CYP450-expressing human lung cells (19). Long-term exposure to AFB1 produces liver enlargement and hepatocellular carcinoma (HCC), which is one of the most common cancers worldwide, causing millions of deaths annually (2, 13, 17, 20). There are several studies conducted on animals indicating the carcinogenic potency of AFB1. Mice and hamster are protected against AFB1-induced HCCs in *in vivo* and *in vitro* conditions. Mouse liver is protected as a consequence of the impermeability of mitochondrial membrane to the toxin. On the other hand, it is a more complex process including both a permeability barrier and a possible scavenging system in hamsters (21). Moreover, AFB1 causes colon and kidney cancers in rats, lung adenomas in mice, cholangiocellular cancer

in hamsters, osteogenic sarcoma, adenocarcinoma of gall bladder and pancreas cancer in monkeys (22).

Antibodies to AFB1 have been reported in humans and they are considered to be an indicator of exposure (23). Besides, AFs cause increased levels of tumor necrosis factor alpha (TNF- α) and changes in serum lactate dehydrogenase activity (24). AFs are also suspected to cause Reye-like syndrome with multiple symptoms. Moreover, AFs target kidney and cause renal cortex changes (25).

AF exposure cause changes in oxidative phosphorylation, which subsequently cause changes in the structure of mitochondria (abnormal mitochondrial structure, and elevation in mitochondrial enzymes) (25-30). The changes in mitochondria are important in aflatoxin-induced hepatocarcinogenesis as AFs preferably attack mitochondrial DNA (mtDNA) three to four times higher than nuclear DNA (30). Aflatoxins also cause mitochondria-directed apoptosis (31).

The “no observed adverse effect level (NOAEL)” for AFB1 in male CD-1 mice, male BALB/c mice and male C57B1/6 mice is found to be 30 $\mu\text{g}/\text{kg}$ b.w. and in male weanling rats it is 60 $\mu\text{g}/\text{kg}$ b.w. (32-34). In studies performed on Gambian children, considering the impairment in host resistance to infections, the NOAEL value was found to be 30 $\mu\text{g}/\text{kg}$ b.w. (35, 36). However, the European Food Safety Authority (EFSA) panel on mycotoxins could not establish a NOAEL as a point of departure for the risk assessment (37).

AFM1 is the metabolite of AFB1 in milk of cattle fed on contaminated foods. AFM1 may be present in animal organs and tissues, e.g. kidneys, and in animal products, e.g. milk, milk powder, cheese, butter and other dairy products after consumption of AFB1-contaminated feeds by animals (11). A tolerable daily intake (TDI) of 0.2 ng/kg b.w. for AFM1 was calculated by Kuiper-Goodman (38) and it has been categorized by the IARC as a Group IIB, a possible human carcinogen. In the assessment of carcinogenicity, the infants are more exposed to the risk because the milk is a major constituent of their diet. It must also be considered that young animals have been found to

be more susceptible to AFB1 (and so probably AFM1) than adults. Therefore, the presence of AFM1 in milk and milk products is considered to be undesirable (39-43). AFM1 is cytotoxic, as shown in human hepatocytes *in vitro* and its acute toxicity in several species is similar to that of AFB1. AFM1 can also cause DNA damage, gene mutation, chromosomal anomalies and cell transformation in mammalian cells *in vitro*, in insects, lower eukaryotes and bacteria. However, AFM1 is less mutagenic, and genotoxic than AFB1 (44-47).

Ochratoxins

Ochratoxins A, B, and C are mycotoxins produced by some *Aspergillus* species and *Penicillium* species, like *Aspergillus ochraceus* or *Penicillium viridicalum*, with OTA as the most prevalent and relevant fungal toxin of this group. The mostly debated toxin of this group of mycotoxins is OTA (48). OTA was discovered as a metabolite of *Aspergillus ochraceus* in 1965 (48). OTA is known to occur in commodities like cereals, coffee, dried fruit and red wine. Besides, OTA is of special interest as it can be accumulated in the meat of animals. OTA-contaminated feed has its major economic impact on the poultry industry. Chickens, turkeys and ducklings are susceptible to this toxin. OTA has been detected in blood and other animal tissues and in milk, including human milk (49). The biological effects of OTA are well documented. IARC has classified OTA as a possible carcinogen (Group 2B) (50). There have been reports on its immuno-suppressive nature (51), teratogenicity (52), reproductive toxicity (55), mutagenicity and carcinogenicity (52, 56). OTA is a nephrotoxin to all animal species studied to date and is most likely toxic to humans, who have the longest half-life for its elimination of any of the species examined (57). OTA disturbs cellular physiology in multiple ways, but it seems that the primary effects are associated with the enzymes involved in phenylalanine metabolism, mostly by inhibiting the enzyme involved in the synthesis of the phenylalanine-tRNA complex (58, 59). In addition, it inhibits mitochondrial ATP production (58) and stimulates lipid peroxidation (61). It has also been hypothesized that the heterozygous gene pattern for phenylketonuria might occur in relatively high frequency and it is an advantage in

the ochratoxin poisoning (62) and that OTA might be a risk factor for testicular cancer (63).

Exposure to OTAs through diet can cause acute nephrotoxicity, and may be carcinogenic (55). Several studies in literature have suggested a correlation between exposure to OTA and Balkan endemic nephropathy (BEN), a chronic tubulointerstitial disease, found between 0.5 to 4.4 % (in some places as high as 20%) in South-Eastern Europe (Serbia, Bosnia and Herzegovina, Croatia, Romania, and Bulgaria). More specifically, BEN is most likely to occur among those living along the confluence of the Danube River, a region in which the plains and low hills generally have high humidity and rainfall. These conditions seem to contribute to high occurrence of OTA in food and feed. A high frequency of urothelial atypia, occasionally culminating in tumors of the renal pelvis and urethra, is associated with this disorder (56).

The most comprehensive studies on OTA toxicity in rats have been performed within the US National Toxicology Program (US-NTP) (63). The overall NOAEL level derived from these studies was found to be 21 µg/kg b.w. per day for 5 days/week, equivalent to 15 µg/kg b.w. per day (64, 65). On the other hand, pigs were found to be more susceptible to OTA toxicity and the NOAEL level in female pigs was reported as 8 µg/kg b.w. per day. In consideration of these findings, the EFSA Panel in year 2006 tried to find the lowest observed adverse effect level (LOAEL) and NOAEL for OTA in humans by applying a set of uncertainty factors; however, these levels have not yet been set for humans (66).

Patulin

Patulin is a mycotoxin produced by a variety of molds, particularly *Aspergillus* and *Penicillium*. It is commonly found in rotting apples, and the amount of patulin in apple products is generally viewed as a measure of the quality of the apples used in production. It is not a particularly potent toxin, but a number of studies have shown that it is genotoxic, which has led to some theories stating that it may be a carcinogen, though animal studies have remained inconclusive. IARC has classified patulin as a Group III carcinogen (a compound for which there is not

enough data to allow its classification) (67). Several countries have instituted patulin restrictions in apple products. The World Health Organization (WHO) recommends a maximum concentration of 50 µg/L in apple juice (68). The maximum provisional tolerable daily intake (PTDI) of 0.43 µg/kg b.w./day. NOAEL value in rats was found to be 0.3 mg/kg b.w./day in rats (69). Several studies have been conducted on patulin content of apple juices marketed in Turkey. In a study performed by Gökmen and Acar, the researchers found that throughout the years the concentrations of patulin in apple juices decreased. Percentages of concentrates exceeding the maximum permitted concentration of 50 µg/L were 52%, 34%, 8% and 8% for 1996, 1997, 1998 and 1999, respectively (70). In another study performed by Yurdun et al., 40% of the apple juice samples had patulin contamination levels higher than 50 µg/L (71).

***Fusarium* Toxins**

A variety of *Fusarium* fungi, which are common soil fungi, produce a number of different mycotoxins of the class of trichothecenes: T-2 toxin, HT-2 toxin, deoxynivalenol and nivalenol and some other toxins zearalenone and fumonisins. The *Fusarium* fungi are probably the most prevalent toxin-producing fungi of the northern temperate regions and are commonly found on cereals grown in the temperate regions of America, Europe and Asia. *Fusarium* toxins have been shown to cause a variety of toxic effects in both experimental animals and livestock. In some occasions, toxins produced by *Fusarium* species have also been suspected to have caused toxicity in humans (72).

a. Fumonisin

Fusarium verticillioides and the related *Fusarium proliferatum* are the only fungi that produce significant quantities of fumonisins. Fumonisin occurs in sorghum, asparagus, rice, beer and mung beans infrequently (72). Conditions favoring fumonisin production appear to include a period of drought during the growing season with subsequent cool, moist conditions during pollination and kernel formation. Equine leukoencephalomalacia, caused by high exposure to fumonisins, is a disease of the central nervous system (CNS) that affects horses, mules, and donkeys (73).

Fumonisin are classified as Fumonisin B1, Fumonisin B2 and Fumonisin B3 where Fumonisin B1 is the most prevalent member and Fumonisin B3 is relatively There is inadequate evidence in humans for the carcinogenicity of fumonisins. There is sufficient evidence in experimental animals for the carcinogenicity of fumonisin B1. It is classified as “possibly carcinogenic to humans (Group IIB)” by IARC (74). Fumonisin B1 is hepatotoxic and nephrotoxic in all animal species tested (68). Fumonisin were not found to be genotoxic (72). Concerning chronic exposure when liver toxicity is taken as the end point, the NOAEL level is given as 0.6 mg/kg b.w./day for rats. When kidney toxicity is the end point in rats, NOAEL level was given as 0.25 mg/kg b.w./day by US-NTP (75).

b. Trichothecenes

The trichothecenes constitute a family of more than sixty sesquiterpenoid metabolites produced by a number of fungal genera, including *Fusarium*, *Myrothecium*, *Phomopsis*, *Stachybotrys*, *Trichoderma*, *Trichothecium*, and others (76, 77). The term trichothecene is derived from trichothecin, which was the one of the first members of the family identified. They are commonly found as food and feed contaminants (78-80). The symptoms produced by various trichothecenes include effects on almost every major system of the vertebrate body; many of these effects are due to secondary processes that are initiated by often poorly understood metabolic mechanisms related to the inhibition of protein synthesis (81). Of the naturally occurring trichothecenes, T-2 and diacetoxyscirpenol appear to be the most potent in animal studies. In addition to their cytotoxic activity, they have an immunosuppressive effect that results in decreased resistance to infectious microbes (82, 83). They cause a wide range of gastrointestinal, dermatological, and neurologic symptoms (84). (DON and T-2 are the best studied of the trichothecenes produced by *Fusarium* species.

c. Deoxynivalenol

Deoxynivalenol (DON) is a common mycotoxin found in grains. DON may have adverse health effects after acute, short-term, or long-term administration. After acute administration, deoxynivalenol produces two

characteristic toxicological effects: decrease in feed consumption (anorexia) and emesis (vomiting) (78). When ingested in high doses by agricultural animals, it causes nausea, vomiting, and diarrhea; at lower doses, pigs and other farm animals exhibit weight loss and food refusal (83). For this reason, DON is sometimes called “vomitoxin” or “food refusal factor”. Although less toxic than many other major trichothecenes, it is the most prevalent and is commonly found in barley, corn, rye, safflower seeds, wheat, and mixed feeds (85). In 1993, IARC placed deoxynivalenol in Group III, not classifiable as to its carcinogenicity to humans (86). The no-observed-effect level (NOEL) is 0.1 mg/kg b.w./per day (85). On the other hand, NOAEL for DON is found as 100 µg/kg b.w./ per day (87).

d. T-2 Toxin

Among the naturally-occurring trichothecenes found in food and feed, T-2 toxin is the most potent and toxic mycotoxin. Corn, wheat, barley, oats, rice, rye and other crops have been reported to contain T-2 toxin (88). Toxin production is greatest with moisture and temperatures and adequate storage with low moisture and insect control will minimize further fungal growth and T-2 toxin production (89). The major effect of T-2 toxin and other trichothecenes is that they inhibit protein synthesis which is followed by a secondary disruption of DNA and RNA synthesis. It affects the actively dividing cells such as those lining the gastrointestinal tract, skin, lymphoid and erythroid cells. It can decrease antibody levels, immunoglobulins and certain other humoral factors such as cytokines (90, 91). The manifestations of disease include signs of weight loss or poor weight gains, bloody diarrhea, dermal necrosis or beak and mouth lesions, hemorrhage and decreased production of milk and eggs. Besides, it characteristically causes aleukia which is an absence or extreme reduction in the number of white blood cells in circulating blood (92, 93). No effect (NOAEL) was observed in rats was found to be 0.5 mg/kg b.w./day by Sirkka et al (1992) et al. (94). On the other hand, for mice the NOAEL value was found to be 0.23 mg/kg b.w./per day (1994). The EFSA panel in 2001 after applying a set of uncertainty factors set the NOAEL for T-2 toxin as 0.06 µg/kg b.w./day (95).

e. Zearalenone

Zearalenone is also named as *RAL*, *FES*, *Compound F-2* or *Toxin F2* and is a potent estrogenic metabolite produced by some *Giberella* species. Zearalenone is the primary toxin causing infertility, abortion or other breeding problems, especially in swine (96-99). Studies of the pharmacokinetics and metabolism of zearalenone demonstrate that it is extensively metabolized by intestinal tissue in pigs, and possibly in humans, during its absorption, with the formation of α - and β -zearalanol, which are subsequently conjugated with glucuronic acid. Zearalenone has been tested for genotoxicity in a variety of test systems and the results were negative, except for the induction of chromosomal aberrations after exposure of mammalian cells *in vitro* to very high concentrations. Hepatocellular adenomas and pituitary tumors were observed in studies of long-term toxicity and carcinogenicity in mice, but only at doses greatly in excess of the concentrations that have hormonal effects, i.e. at 8-9 mg/kg b.w./per day or more. These tumors were a consequence of the estrogenic effects of zearalenone (96-99). The NOAEL value of zearalenone was given as 4 mg/kg/per day in rats (100).

Regulations for Mycotoxins in Food

Risk Assessment for Mycotoxins and Worldwide Mycotoxin Regulations

The number of countries regulating mycotoxins has increased significantly over the years. Regulations have become more diverse and detailed with newer requirements with regard to official procedures for sampling and analytical methodology (101). At least 99 countries had mycotoxin regulations for food and/or feed in 2003, an increase of approximately 30% compared with 1995. All countries with

mycotoxin regulations in 2003 had regulatory limits for at least AFB1 or the sum of AFB1, B2, G1, and G2 in food and/or feed. Specific regulations also exist for several other mycotoxins such as AFM1; the trichothecenes DON, diacetoxyscirpenol, T-2 toxin and HT-2 toxin; the fumonisins B₁, B₂, and B₃; agaric acid; the ergot alkaloids; OTA; patulin; phomopsins; sterigmatocystin, and zearalenone. Most of the limits are set for human foods. Typically higher regulatory levels are used for animal feed (101).

Risks associated with mycotoxins depend on both hazard and exposure. Exposure throughout the world is at different levels, because of different levels of contamination and dietary habits in the various parts of the world. Food and Drug Administration (FDA) action levels for total AFs in food and feed are presented in **Table 1**. European Union regulations for AFs for human food and feed are presented in **Table 2**. EU regulations for OTs are showed in **Table 3**.

Mycotoxin Studies in Baby Foods and Infant Formulas

There are several studies in literature on the levels of several mycotoxins in infant formulas and baby foods. These studies are largely based on AFs and OTA and only few of them consider exposure to other mycotoxins through baby foods.

In a study performed in Czechoslovakia using enzyme-linked immunosorbent assay (ELISA) technique, AFM1 was measured in 376 samples of raw milk from farms in the area of a new dairy plant producing milk baby foods. 87.8 % of the samples contained no AFM1 (detection limit 0.025 µg/L) and only 2 samples (0.5%) possessed higher concentration

Table 1. FDA action levels for total aflatoxins in food and feed

Commodity	AF (µg/kg)
All products, except milk, designated for humans	20
All other feedstuffs	20
Peanuts and Peanut products	20
Pistachio nuts	20
Milk	0.5 (for AFM1)
Foods	20

Table 2. EU regulations for aflatoxins

Human food	AFB1 (µg/kg)	AFB1, B2, B3, B4 (µg/kg)	AFM1 (µg/kg)
Groundnuts, dried fruit and processed products thereof	2	4	–
Groundnuts subjected to sorting or phys. treating	8	15	–
As above but for nuts and dried fruits	5	10	–
Cereals (including maize) and processed products thereof	2	4	–
Milk	–	–	0.05

Table 3. EU regulations for ochratoxin.

Product	OTA (µg/kg)
Raw cereal grains	5
All products derived from cereals intended for direct human consumption	3
Dried vine fruit (currants, raisins and sultanas)	10
Baby food	0.5

than 0.1 µg/L, which represents the tolerance limit for AFM1 in baby milk foods admitted in the country (102). In another study performed in São Paulo, Brazil AFM1 was surveyed in 300 samples of whole milk powder consumed by infants at municipal schools and nurseries. The analyses were performed by ELISA. Results showed 11 % of the samples were positive for AFM1 at levels of 0.10-1.00 ng/ml (mean: 0.27 ± 0.20 ng/ml) (103). In a study performed in Turkey, the risk of exposure to aflatoxin in infants fed by breast milk and formula was investigated. For this purpose, AFB1 was determined in the serum of both breast-fed and formula-fed infants. Serum AFB1 availability was significantly higher in the formula-feeding (F) group than the breast-feeding (B) group (42.8 vs 8.5 %). The AFB1 concentration in different commercial formulas was also determined. AFB1 was found in seven of the eight newly opened packages of different brands of formula. The concentrations showed a statistically significant increase at the 30th day after the opening of the packages. It was again concluded that for infants, human milk was safer than commercial formulas because of the lower contamination risk of AF (104).

In an Italian study performed during 1995, 159 samples of milk, 97 samples of dry milk for

infant formula, and 114 samples of yogurt were randomly collected in supermarkets and drug stores in four large Italian cities and checked for AFM1 by immunoaffinity column extraction and high performance liquid chromatography (HPLC). AFM1 was detected in 136 (86 %) of the milk samples (in amounts ranging from <1 ng/L to 108.5 ng/L; mean level: 10.19 ng/L), in 81 (84 %) of the dry milk samples (in amounts ranging from <1-101.3 ng/kg; mean level: 21.77 ng/kg), and in 91 (80 %) of the yogurt samples (in amounts ranging from <1 ng/L to 496.5 ng/L; mean level: 18.08 ng/L). Altogether, only two samples of milk, two samples of yogurt, and one sample of dry milk had levels of AFM1 exceeding the Swiss legal limits, which are the most restrictive limits in the world. AFM1 contamination levels in milk and yogurt samples collected in the period of November to April were four times as high as those in samples collected in the period of May to October (105). In another study performed by the same working group in year 1996, 161 samples of milk, 92 samples of dry milk for infant formula and 120 samples of yoghurt, were randomly collected in supermarkets and drug stores in four big Italian cities, and checked for AFM1 by HPLC. AFM1 was detected in 125 (78 %) of milk samples (ranging from < 1-23.5 ng/L; mean

level: 6.28 ng/L), in 49 (53 %) of dry milk samples (ranging from <1-79.6 ng/kg; mean level: 32.2 ng/kg) and in 73 (61 %) of yoghurt samples (ranging from <1-32.1 ng/kg; mean level: 9.06 ng/kg). In both of the studies, evaluating all of the samples analyzed, the researchers concluded that during 1996, despite the widespread occurrence of AFM1, mean contamination levels in dairy products sold in Italy were not a serious human health hazard (105).

In a Korean study, the occurrence of AFM1 in pasteurized milk and dairy products was investigated by ELISA and HPLC. Among a total of 180 samples collected the incidence of AFM1 in pasteurized milk, infant formula, powdered milk and yoghurt was 76, 85, 75, and 83 %, respectively, with a mean concentration of 18, 46, 200, and 29 pg/g, respectively (106). In another study performed in Kuwait, as a part of the program on monitoring of environmental contaminants in food stuff in the country, 54 samples of fresh full cream and skimmed milk, powdered milk, yoghurt, and infant formulae were analyzed for AFM1 by HPLC. Of the samples analyzed, 28 % were contaminated with AFM1 with 6 % being above the maximum permitted limit of 0.2 µg/L (107).

A survey of AFs, OTA and patulin in a variety of foods for infants and young children was carried out by the Food Standards Agency between November 2003 and April 2004 in the U.K. Total 199 foods, including breakfast/rusk products, baby rice, savory products and desert/cereal bar/biscuits were sampled. Of these, 169 were analyzed for AFs and OTA. A further 14 products were analyzed for patulin as well and 16 products, including apple-based drinks and apple fruit products, were tested for patulin only, as patulin is much more likely to occur in these products compared with the other mycotoxins studied. Mycotoxins were not detectable in 90 % of the products analyzed. In those samples where mycotoxins were detectable, levels were very low and regulatory limits were not exceeded in any of them. Data from the survey were used to assess the exposure of infants to mycotoxins and these do not raise a concern for

infant health (108). There are also several studies performed on apple-based baby foods. In a study performed in Tunisia, the researchers did not find any patulin contamination in 21 infant fruit purees (109). In an Italian study, of 10 apple-based baby foods, two samples were contaminated with 17.7 and 13.1 µg/L and both were labeled as “organic food” (110). In another study performed in Italy, patulin was detected (<1 µg/kg) in only 3 of the 23 fruity baby food samples tested (homogenized fruits, 11 conventional and 12 organic) (111).

In a survey performed in Canada on breakfast and infant cereals for AFs B1, B2, G1 and G2, 349 breakfast and infant cereal samples (rice-, soy-, barley-based and mixed-grain infant cereals, corn-, wheat-, and rice-based and mixed-grain breakfast cereals) were collected at retail level across the country from 2002 to 2005. Results showed that 50 % of both breakfast and infant cereals had detectable levels (limit of detection = 0.002 ng/g) of AFB1. The levels found varied from 0.002 to 1.00 ng/g for AFB1, from 0.002 to 0.14 ng/g for AFB2, from 0.008 to 0.27 ng/g for AFG1, and from 0.008 to 0.048 ng/g for AFG2. Only 4 % of the breakfast cereals and 1 % of the infant cereals had AFB1 levels exceeding 0.1 ng/g, which is the European Union maximum limit for AFB1 in baby foods and processed cereal-based foods for infants and young children (112). In another Canadian study demonstrated on three hundred and sixty-three samples of cereal-based infant foods, soy-based cereals (which usually contain corn) exhibited the highest incidences of deoxynivalenol (100%), zearalenone (46%) and fumonisins (75%). Overall, deoxynivalenol was the most frequently detected mycotoxin--it was detected in 63% of samples analyzed (113).

In another study performed in Russia, OTA content in baby foods was determined. The analysis was performed by immunoaffinity column clean-up and HPLC. OTA was detected in 22.5 % of 40 samples up to 1.2 mg/kg. Mean level was 0.15 and 0.31 mg/kg. OTA level was higher in oat-based samples. Calculations made on the basis of the obtained means showed that the daily OTA dietary intake were up to 1.72 ng/kg. b.w. (114).

Mycotoxin Studies in Baby Foods Performed in Turkey

In a study conducted by Baydar et al., 63 infant formulae, follow on formulae and baby foods were randomly collected from pharmacies and supermarkets in Ankara, Turkey. AFB₁, AFM₁, and OTA levels were assessed by ELISA. AFB₁, AFM₁ and OTA levels were found in 87, 36.5 and 40 % of the samples between 0.10-6.04 ppb, 0.06-0.32 ppb and 0.27-4.50 ppb, respectively (115). Another study performed in Turkey on 24 cereal-based baby foods using immunoaffinity column (IAC) clean-up and HPLC determined that OTA was present in 17% of cereal-based baby food samples. OTA levels ranged from 0.122 to 0.374 ng/ml and the levels were much lower below the limit recommended by European Commission Regulation (116).

Recently, Gürbay et al. have indicated OTA levels in 75 Turkish mother breast milk samples ranging from 0.62 to 13 ng/L (117). Besides, the same group performed another study to determine the levels of AFB₁ and AFM₁ in breast milk. The level of AFM₁ were in the ranges of 60.90-299.99 ng/L, and AFB₁ were in the ranges of 94.50-4123.80 ng/L (118). Since there is no limit value for AFM₁ and AFB₁ in mother's breast milk neither in Turkey nor European Union, making a comparison of these results with limit values could not be possible. However, when limit value of AFM₁ for animal milk (50 ng/l) accepted by Turkey and European Union is considered, it has been shown that all samples analyzed, contained AFM₁ above this limit. Moreover, as there is not any limit for AFB₁ concerning animal milk, it is not feasible to compare these results of AFB₁. On the other hand, The Joint FAO/WHO Expert Committee on Food Additives (JEFCA) does not establish a tolerable daily intake (TDI) for aflatoxins, but strongly recommends that the level of aflatoxin should be as low as possible (119). As a result, the authors concluded that breast milk AFM₁ and B₁ levels determined in this study should be considered seriously high and detrimental to human health.

Another study performed on human breast milk and row cow's milk using ELISA and HPLC, demonstrated that AFM₁ was present in 8 (13.1%) of 61 human breast milk samples examined (5.68±0.62

ng/L; ranged between 5.10- 6.90 ng/L) and 20 (33.3%) of 60 raw cow's milk samples (ranged between 5.40 -300.20 ng/L). Five (8.3%) of the positive raw cow's milk samples had AFM₁ levels (153.52 ±100.60 ng/L; ranged between 61.20-300.20 ng/L). The levels in these samples were higher than the maximum tolerance limit (0.05 ppb) stipulated by regulations in Turkey and some other countries (120).

Regulations in Turkey

For baby foods and infant formulas, the permissible levels for total aflatoxin contamination (AFB₁+B₂+G₁+G₂) is 2 ppb as indicated in "Announcement for Aflatoxin Control" by Turkish Ministry of Agriculture, Forestry and Rural Affairs, published in Turkish Republic Official Paper on May 2, 1990 (121). Turkish Ministry of Agriculture and Rural Affairs set different limits for different food stuff in 2002 and published "The Regulation Stating a Change in the Regulation of the Turkish Food Codex" in Turkish Republic Official Paper No 24885, September 23, 2002. These regulations set by Turkish Ministry of Agriculture and Rural Affairs are summarized in **Table 4**.

Is The Mycotoxin Contamination In Baby Foods A Treat?

The reaction of infants and young children differ from that of adults against many drugs and toxins and, in most cases, they are more susceptible. Furthermore, infants and young children eat and drink more relative to their size than adults. The fact that most mycotoxins are toxic in very low concentrations and they can be present in infant formulas and baby foods as a result of contamination or bad storage, there is the need for flexible, reliable, accurate, inexpensive, rapid and reproducible methods for detection and quantification. Due to the varied structures of these compounds, it is not possible to use one standard technique to detect all mycotoxins, as each will require a different method. What works well for some molecules could be inappropriate for others of similar properties or for the same molecule in a different environment/matrix. As baby foods and infant formulas have complex matrices, practical requirements for high-sensitivity detection and the need for a specialist laboratory setting create

Table 4. Maximum permissible levels for mycotoxins in Turkey.

Product	Total Aflatoxin (B1+B2+G1+G2) (ppb)	AFM1 (ppb)	AFB1 (ppb)	OTA (ppb)
Baby foods/infant formula (milk based)	-	0.05	-	-
Baby foods/infant formula	2	-	1	-
Spice	10	-	5	-
Milk	0.5	0.05	-	-
Milk powder	-	0.5	-	-
Cheese	-	0.25	-	-
Agricultural products	20	-	-	-
Animal feed	50	-	-	-
Other foodstuff	10	-	5	-
Nuts, ground nuts and dried oily fruits, oily seeds, dried fruits including fig and grape, foodstuff prepared from the procession of these	10	-	5	-
Cereals (including black wheat <i>Fagopyrum</i>) and all products prepared using cereal	4	-	2	3
Processed cereal seproducts	-	-	-	5
Dried grape	-	-	-	10
Apple juice, fruit juices including apple juice, vinegars	-	-	-	50

challenges for routine analysis. Therefore, depending on the physical and chemical properties, procedures have been developed around existing analytical techniques, which offer flexible and broad-based methods of detecting compounds. Among other methods, analytical liquid chromatography linked with mass spectroscopy is gaining popularity (122).

WHO recommends exclusive breast-feeding for the first six months of life. Breast milk remains the best source of nutrition for infants, and mothers need to be motivated to continue with it as long as possible. However, when breast-feeding is not possible or enough, many different infant formulae and baby foods are available for infants and young children. Preparing or buying safe and proper food for infants and young children is essential for the health of the child. Although the qualities of these products are strictly regulated, contamination is inevitable. Most mycotoxins are chemically stable so they tend to survive in storage and processing, even when cooked at quite high temperatures such as those reached

during bread baking or breakfast cereal production. This makes it important to avoid the conditions that lead to mycotoxin formation. Mycotoxins are notoriously difficult to remove and the best method of control is prevention (123).

Mycotoxin contamination is a very important issue as the possible outcomes in the exposure to these toxins may be the cause of serious problems experienced in the first years or later periods of life such as poor growth, suppressed immune system, and cancer. An accurate prediction of the possible health impact of individual mycotoxins in foods for the vulnerable group is difficult; possible additive and synergistic effects of multiple mycotoxins make the task even more complex and the long-term effects are beyond foresight. Therefore, infant foods must be routinely tested for the mycotoxins presence at every step of manufacturing and marketing (116). Therefore, strict law enforcement is required in each step of the production of infant formulas/baby foods and the public consciousness must be provided for the risks

of mycotoxins. Parents should be well-informed for the right choice and for the use of the product they bought (how to preserve, how to prepare etc) and governments should pursue the production of each food produced for children.

Conclusion

It can be suggested that manufacturers of foods for infants and young children should give an extreme importance to mycotoxin content. The manufacturers, pediatrician, health-care personnel and parents should be provided with enough information and training to minimize health hazards and to form the public policies. In order to protect public health, it is essential to keep contaminants at levels toxicologically acceptable. Ultimately, surveillance should be continuous, widespread and must be conducted by the government and related ministries as the quality of the end product depend on the precise controlling at every step of the production

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