Mycotoxicoses in children

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Mycotoxicoses are acute and chronic poisonings caused by mould toxins called mycotoxins. Although acute mycotoxicoses, caused by high mycotoxin levels in food are rare nowadays, they need to be described in order to inform physicians and other health care workers about their symptoms. Children are more sensitive to mycotoxins because of their lower body mass, higher metabolic rate, and underdeveloped organ functions and detoxication mechanisms. Some mycotoxicoses appear only in children, and some are more pronounced in children than in adults. Acute mycotoxicoses in children are reported poorly, mostly because they occur in the tropical regions with poor healthcare coverage. In developed countries healthcare authorities are more concerned about child exposure to low levels of mycotoxins with immunotoxic, genotoxic or carcinogenic properties.

KEY WORDS: 3-NPA; aflatoxins; ergot; fumonisins; ochratoxins; trichothecces; Ustilago maydis toxins; zearalenone

Mycotoxins are the metabolites of moulds (fungi imperfecti) that contaminate food and feed around the world. Humans are continuously exposed to mycotoxins, as not even good agricultural and production practices can entirely prevent their production. Mould growth and the production of mycotoxins depend on the genetic properties of individual mould species and on environmental conditions such as temperature, humidity, and insect infestation. Grains are usually contaminated with several mycotoxins, which may either be the consequence of contamination with several strains of moulds, each producing its own mycotoxin, or of the production of several mycotoxins by one mould species. Out of about 400 known mycotoxins, only a few have been investigated in detail in laboratory animals and even fewer have known toxic and other health-threatening effects in humans.

The main route of exposure in humans is ingestion of contaminated food, but for some mycotoxins inhalation or dermal exposure are also possible. It is very difficult to assess the health risks associated with mycotoxins in children, as reliable exposure data are seldom available, mycotoxins often occur with other mycotoxins or other toxins, other dietary elements and contaminants affect their toxicity, and many diseases induced by mycotoxins have long latency (1).

For most mycotoxins there are no epidemiological data on their carcinogenicity in humans, but for some (such as aflatoxin B1) there is convincing evidence of an association with human hepatocellular carcinoma. Other mycotoxins (such as ochratoxin A, patulin, and fumonisin B1) are suspected to be human carcinogens due to positive results in experimental animals (2). Young animals and children are more sensitive to the adverse effects of mycotoxins than adults because of lower body mass, higher metabolic rate, and underdeveloped organ functions and detoxication mechanisms.

Most mycotoxins may cause acute and chronic mycotoxicoses. Acute mycotoxicoses are serious diseases caused by exposure to very high mycotoxin
levels. They are most common in tropical regions where exposure of children to certain mycotoxins, particularly aflatoxins, is continuously high. Conversely, chronic mycotoxicoses are associated with low-level exposure. Mycotoxicoses are important differential diagnoses and should be taken into consideration when a clinical disorder affects a group of persons in whom it cannot be related to an infectious agent.

Even though clinical symptoms and the outcome are more severe in children than adults, mycotoxicosis reports seldom distinguish the two groups.

This article is an attempt to review what little is known about mycotoxicosis in children and the available data on mycotoxins and biomarkers of exposure to mycotoxins in various biological materials (blood of pregnant women, umbilical cord blood, blood and urine of children).

Aflatoxins

Aflatoxins B1 (AFB1), B2 (AFB2), G1 (AFG1), and G2 (AFG2) are mycotoxins produced by Aspergillus spp. Their names come from the colour of their fluorescence (B - blue, G - green). The most common and also the most toxic is AFB1. In the organism, AFB1 and AFG1 are metabolised to various compounds, including aflatoxin M1 (AFM1) and M2 (AFM2), which appear in human and cow milk, urine, and faeces.

Aflatoxins contaminate nuts, maize, sorghum, millet, beans, cassava, and rice, whereas their metabolites AFM1 and AFM2 contaminate milk and dairy products.

Animal studies show that, apart from being acutely toxic, aflatoxins are immunosuppressive, mutagenic, teratogenic, and carcinogenic (2). The target organ of their toxicity and carcinogenicity is the liver. The International Agency for Research on Cancer (IARC) has classified natural mixtures of AFs as carcinogenic in humans (Group 1) and AFM1 as potentially carcinogenic in humans (Group 2) (2).

Aflatoxins are well absorbed by the gastrointestinal (GI) tract and transported to the liver, where they are metabolised by a number of competitive pathways. They are bioactivated mainly by cytochrome P450 (CYP) 3A enzymes (CYP3A4, CYP3A5, and CYP3A7) in the first oxidative phase. AF activation is also possible in the small intestine due to the presence of CYP3A4 and CYP3A5. About 1-3 % of the absorbed AFs is activated to highly mutagenic exo-epoxide (AFB1-8,9-epoxide), whose half-life (t1/2) is only one second in aqueous buffer but long enough to react with DNA to yield 98 % of the N7-guanyl adduct. Wojnowski et al. (4) have associated high levels of the mutagenic AFB1 exo-8,9-epoxide in humans exposed to dietary AFB1 with the CYP3A5 polymorphism, particularly in individuals with low CYP3A4. AFB1 8,9-epoxides hydrolyse to non-genotoxic dihydrodiol, which is further metabolised to its dialdehyde (5). The amount of AFB1-DNA adducts is greater in the liver than in other organs and generally correlates with AFB1 levels and species susceptibility to hepatocarcinogenesis (6). DNA- and albumin-AFB1 adducts (AFB1-DNA, AFB1-alb) are reliable biomarkers of long-term exposure to AFB1. The major detoxication route for AFB1 metabolites is via conjugation with glutathione (GSH) to form the AFB1-GSH conjugate excreted in urine and bile, and this reaction determines species resistance to aflatoxin toxicity (7).

In some regions of Africa and Asia the risk of hepatocellular carcinoma (HCC) is very high, particularly in hepatitis B surface antigen-positive (HBsAg+) persons. Without AF exposure, the relative risk of HCC development in HBsAg+ persons is 7.3, but with AFs exposure it soars to about 60 (8). In adults, AFs cause deletional mutations in the P53 tumour-suppressing gene and therefore activate oncogenes. However, in two to five-year-old children in Guinea with high AF-albumin adduct levels Turner et al. (9) found no mutations in P53 tumour-suppressing gene, which suggests that children could be protected from high AF carcinogenicity if vaccinated against HBV infection, as it significantly increases the risk of HCC. In order to decrease the risk of HCC, South Africa has recently introduced vaccination against HBV infection in infants (receiving three shots by their 14th week). In a five-year follow up Tsebe et al. (10) reported that this vaccination decreased significantly the HBsAg+ carrier rate in children younger than five years.

Exposure to AFs may be assessed by measuring parent compound or metabolites in blood and urine, or AFB1-alb and AFB1-DNA adducts in blood. The first method is only reliable for recent exposure to AFs because of their fast metabolism and therefore a tendency to underestimate longer exposure. This is probably why there were no AFB1-positive urine findings in children from Cameroon, even though 11 % of the samples showed the presence of its metabolite AFM1 (11). The second method that measures AF-alb
and AF-DNA adducts is therefore more reliable for longer exposures (2-3 months).

Aflatoxicosis is a clinical condition following exposure to aflatoxins. Acute aflatoxicosis is a severe poisoning that results in serious liver damage, often with fatal outcome. Chronic aflatoxicosis is the consequence of exposure to lower levels of AFs over a longer period and may have chronic nutritional and immunological consequences. In terms of cancer risk, the effects are cumulative.

The first report of acute AF hepatotoxicity in children dates back to 1971 (12), when 20 malnourished children from 1.5 to 5 years of age were hospitalised for symptoms of acute liver damage, 13 of whom were subicteric. They had been eating a peanut protein meal contaminated with aflatoxin (30 μg kg\(^{-1}\)) from five days to one month. At the end of the one-year follow up, 12 had gross hepatomegaly and three had palpable liver with sharp edges. Three children died from hepatic coma after 1.5 years.

Another outbreak of acute aflatoxicosis in India in 1974 practically spared the children under the age of five years, who made about 8 % of the affected patients (13,14). In the most severe aflatoxicosis outbreak in Kenya in 1981, the number of affected children was never established (15), but in the outbreak of 2004, it was half of those whose age data was known (308 of 317 people). Sixty eight children were under the age of five and 90 from five to fifteen (16).

Exposure to AFs in utero in Africa is alarming (Table 1). The findings of AFs in umbilical cord blood from 1988 were the first to evidence transplacental transport of AFs in humans (17). Egyptian researchers established a significant negative correlation between birth weight and AF levels in umbilical cord blood (18, 20). In Gambia, a negative correlation was also found between AF-albumin adduct levels in mother’s blood and weight and height gain in the first year of child’s life (19).

It is estimated that up to 0.43 % of AFs from consumed food is excreted as AFM1 in breast milk (21) and that mothers’ exposure to AFs depends on their socioeconomic status (22, 23). In African countries, Turkey, Iran, and United Arab Emirates the frequency of AF-positive breast milk samples is rather high (Table 2). In Egypt and Gambia the concentrations of AFM1 in breast milk showed great seasonal variations (16-96 %), peaking in the dry season (summer) (26, 41). Breastfed children in Africa are exposed to lower AF concentrations than weaning children because the staple food in the weaning period is maize and cassava, which are often contaminated with AFs (21, 42, 43). In Europe the frequency of AFM1-positive breast milk samples does not exceed 5 %, and the AFM1 levels are much lower than in Africa, but they still exceed the maximum tolerance limit for infant formula, follow-up formula, infant milk, and follow-up milk of 0.025 µg kg\(^{-1}\) in some European countries (44, 45).

Similar to breast milk, the frequency of AF-positive blood and urine samples of African children is high, and AF concentrations peak in the summer (Tables 3 and 4). In children from Gambia AF-albumin adduct levels in the summer are twice as high as in

| Table 1 Reported aflatoxin and AF-alb adduct findings in umbilical cord blood in Africa |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Country**                     | **Positive samples/analysed (N)** | **Positive by toxin**                   | **Ref.**       |
|                                |                               | **B1** | **B2** | **M1** | **M2** | Method       |
| Ghana*                         | 63/188 (34 %)                 | 17/188 (9 %) | 17/188 (9 %) | 21/188 (11 %) | 21/188 (11 %) | HPLC-FD 17 |
| Nigeria                        | 9/78 (11 %)                   | ND     | 1/78 (1 %) | 3/78 (4 %)   | 5/78 (6 %)   | HPLC-FD 17 |
| United Arab Emirates           | 110/201 (57 %)                | 27/201 (13 %) | X=2,040 (228-15,225) | 107/201 (53 %) | 31/201 (15 %) | HPLC-FD 18 |
| Gambia**                       | 49/99 (48 %)                  | 49/99 (48 %) | X=10.1 (5.0-89.6) | 49/99 (48 %) | X=10.1 (5.0-89.6) | ELISA 19 |

ND—not detected; HPLC-FD—High pressure liquid chromatography with fluorescent detection; ELISA—enzyme-linked immunosorbent assay.

*In addition, AFG1 was detected in three samples (611, 1354, and 354 ng L\(^{-1}\)) and AFG2 in one (37 ng L\(^{-1}\)).

**AF-alb adducts (pg mg\(^{-1}\) of protein)
<table>
<thead>
<tr>
<th>Country</th>
<th>No. positive/analysed</th>
<th>No. positive/analysed (range ng L(^{-1}))</th>
<th>Method</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AFM1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AFM2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Africa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cameroon</td>
<td>3/62 (4.8 %)</td>
<td>3/62 (4.8 %)</td>
<td>HPLC-FD</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(5.0-62.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egypt</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apr-Jun</td>
<td>23/37 (62 %)</td>
<td>23/37 (62 %)</td>
<td>HPLC-FD</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>X=0.35 (0.3-2.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sep-Dec</td>
<td>16/45 (35 %)</td>
<td>16/45 (35 %)</td>
<td>HPLC-FD</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>X=0.34 (0.3-1.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jan-May</td>
<td>27/38 (71 %)</td>
<td>27/38 (71 %)</td>
<td>HPLC-FD</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>X=0.3 (0.2-1.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>66/120 (55 %)</td>
<td>248/443 (56 %)</td>
<td>HPLC-FD</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(4.2-889)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>87/125 (70 %)</td>
<td>ELISA</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>X=74.4 (73-328)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ghana</td>
<td>90/264 (34 %)</td>
<td>59/264 (22 %)</td>
<td>HPLC-FD</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(20-1,816)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>18/264 (7 %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(16-2,075)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nigeria</td>
<td>41/50 (82 %)</td>
<td>41/50 (82 %)</td>
<td>HPLC-FD</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(4.62-92.14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sudan*</td>
<td>37/99 (37 %)</td>
<td>26/99 (26 %)</td>
<td>HPLC-FD</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>X=19.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>51/94 (54 %)</td>
<td>51/94 (54 %)</td>
<td>HPLC-FD</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>X=0.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zimbabwe</td>
<td>6/54 (11 %)</td>
<td>6/54 (11 %)</td>
<td>ELISA</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.2-50)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle East</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jordan</td>
<td>100/100 (100 %)</td>
<td>100/100** (100 %)</td>
<td>ELISA</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>X=68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>United Arab Emirates</td>
<td>144/201 (71 %)</td>
<td>107/201 (53 %)</td>
<td>HPLC-FD</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>X=1,108 (210-4,060)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>31/201 (13 %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>X=854 (210-3,700)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iran</td>
<td>8/132 (6 %)</td>
<td>8/132 (6 %)</td>
<td>ELISA</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>X=9.45 (7.1-10.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sari</td>
<td>1/136 (0.7 %)</td>
<td>1/136 (0.7 %)</td>
<td>ELISA</td>
<td>33</td>
</tr>
<tr>
<td>Tabriz (rural)</td>
<td>20/91 (22 %)</td>
<td>20/91 (22 %)</td>
<td>ELISA</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>X=6.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tabriz (urban)</td>
<td>0/91</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Teheran</td>
<td>157/160 (98 %)</td>
<td>157/160 (98 %)</td>
<td>ELISA</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>X=8.2 (0.3-26.7)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
the winter (41). The highest AF-albumin adduct levels were found in children with acute hepatitis B, followed by children with chronic hepatitis, while healthy children had the lowest levels.

Although the immunotoxic effects of AFs in experimental animals are well known, there are no data on their immunotoxicity in children. In adults Jiang et al. (50) found a significant negative correlation between high AFB1-albumin adduct levels and the percentage of activated T and B cells. Denning et al. (51) believe that the immunotoxicity of AFs greatly contributed to acute lower respiratory tract infections in Filipino children, eleven of whom died, whereas Oyelami et al. (52.) found significant concentrations of AFs in the lung tissues of Nigerian children post mortem.

In a study of 479 blood samples taken from children from 9 months to five years in Benin and Togo, 99 % were positive for AF-alb adducts (42). In this and another longitudinal study of 400 children from Benin peak serum AF-alb adducts in fully weaned children was 2.5 times higher than in partially breast-fed children (23, 46). These authors also found negative correlation between AF-alb adducts and children weight and height (46). In another study of children from Sierra Leone, the authors suggested that the failure to thrive may have been caused by exposure to AFs and another mycotoxin - ochratoxin A (OTA) (53).

In tropical countries neonatal jaundice is very frequent. A Nigerian study of 327 neonates with jaundice and 60 controls has demonstrated that glucose-6-phosphate dehydrogenase deficiency and/or the presence of aflatoxins in serum are the risk factors for neonatal jaundice (54). A study performed in Kenya showed that school children with hepatomegaly, which is very frequent in this country, had significantly higher levels of AF-alb adducts than children without hepatomegaly (55).

Some diseases that affect children in tropical regions, such as Rey’s syndrome and kwashiorkor were erroneously attributed to AF exposure (56, 57). Frequent post-mortem findings of AFs in various tissues of children with these diseases were probably the consequence and not the cause of liver injury. It is likely that their damaged liver could not metabolise the AFs.

**Ochratoxin A**

Ochratoxin A (OTA) is the most toxic and most common ochratoxin. Ochratoxins are produced by *Penicillium verrucosum* and several species of *Aspergillus* moulds from all over the world. They contaminate foodstuffs of plant origin (cereals, coffee...
beans, raisins, wine, beer, and grape juice) and commodities of animal origin such as pork and poultry meat, eggs, milk, and dairy products due to the carryover effect. Most data on food contamination with this heat-stable mycotoxin are from Europe (58).

In experimental and domestic animals the main target organs of OTA toxicity are the kidney and liver, but it also affects the heart, blood (causing aberrations in coagulation factors), GI tract, lymphoid tissue, and bone marrow. OTA is readily absorbed by the upper GI tract and persists in the circulation for a long time due to binding to plasma proteins, enterohepatic circulation, and kidney resorption, enhanced by organic anion transporters (59). Its plasma half-life in humans is 35.55 days (60), which is extremely long and makes plasma OTA a good biomarker of exposure. There are several mechanisms involved in OTA toxicity: production of reactive oxygen species, inhibition of mitochondrial respiration, disruption of calcium homeostasis, inhibition of protein synthesis, and DNA damage (61-65). IARC has classified OTA as carcinogenic in experimental animals with limited evidence for its carcinogenicity in humans (Group 2B) (66).

Despite severe acute toxicity in laboratory animals, ochratoxicosis is quite rare in humans (67). So far, no ochratoxicosis has been reported in children. Some researchers believe that OTA is involved in the development of Balkan endemic nephropathy (BEN) and otherwise rare urothelial tumours, whose incidence is high in the endemic regions of the Balkans (68, 69). In several studies performed in the endemic regions of Bulgaria and Croatia the level of OTA or the frequency of OTA-positive food and human blood samples were higher than in control regions (for a more comprehensive review see 70). Low OTA concentrations are frequently found in blood and urine of apparently healthy persons in all countries where it was looked for, with significant geographical and seasonal variations (70-72). Various studies have demonstrated that the blood levels of OTA are higher in patients with chronic renal insufficiency treated with dialysis (for a review see 73).

In Europe the calculated daily human exposure to OTA ranges from 0.7 to 4.7 ng kg\(^{-1}\) body weight (b.w.), which is below the tolerable daily intake of 14 ng kg\(^{-1}\) b.w. proposed by the Joint FAO/WHO Experts Committee on Food Additives and accepted by the European Scientific Committee (ESF) (57, 74). EFSA established the tolerable daily intake of 18 ng kg\(^{-1}\) b.w. (75).

Most data on child exposure to OTA are also from Europe. In Switzerland Zimmerli and Dick (76) reported twice as high OTA concentrations in the umbilical cord as that in maternal blood, indicating active transplacental transport of OTA.

Table 3 Aflatoxin B1-albumin adducts in blood of children

<table>
<thead>
<tr>
<th>Country</th>
<th>Age (years)</th>
<th>No. of positive/analysed</th>
<th>AFBI-alb adducts pg mg(^{-1}) albumin Mean (95 % CI)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benin/Togo</td>
<td>Fully weaned</td>
<td>&gt;3</td>
<td>45.6 (38.8-53.7)</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>Partially breast fed</td>
<td>&lt;3</td>
<td>18.0 (15.2-21.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>0.9-5</td>
<td>475/479 (99 %)</td>
<td>32.8 (5-1064)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.4</td>
<td>13/118 (11 %)</td>
<td>8.7 (5.0-30.3)*</td>
</tr>
<tr>
<td>Gambia</td>
<td>Healthy</td>
<td>3-4</td>
<td>444/444 (100 %)</td>
<td>31.6 (2.2-495)</td>
</tr>
<tr>
<td></td>
<td>Acute HBV infection</td>
<td>6/6</td>
<td>404/404 (100 %)</td>
<td>96.9 (45.2-207.7)</td>
</tr>
<tr>
<td></td>
<td>Chronic HBV infection</td>
<td>34/34 (100 %)</td>
<td>44.9 (32.3-62.5)</td>
<td></td>
</tr>
<tr>
<td>Guinea</td>
<td>HBsAg-</td>
<td>2-5</td>
<td>119/124 (96 %)</td>
<td>9.9 (8.8-11.0)</td>
</tr>
<tr>
<td></td>
<td>HBsAg+</td>
<td>13-15</td>
<td>94</td>
<td>3.14 ±1.05**</td>
</tr>
</tbody>
</table>

Cl-confidence limits; HPLC-FD–high pressure liquid chromatography with fluorescent detection; ELISA–enzyme-linked immunosorbent assay
*range
**standard deviation
OTA is also a frequent contaminant of breast milk. Its concentrations are about one quarter of those in maternal plasma (77) and the exposure of breast-fed children often exceeds the daily limit of 14 ng kg\(^{-1}\) b.w. (Table 5). In colostrum OTA concentrations are much higher than in mature breast milk. Obviously, breast milk OTA contamination is related to maternal dietary habits, which may vary from country to country. In Italy, significantly higher OTA concentrations in breast milk correlate with the consumption of bread, bakery products, and pork meat (36), while in Norway they correlate with the consumption of liver paste (liverwurst, liver paté) and cakes (cookies, fruitcakes, chocolate cakes) (86). An Egyptian study (78) looked at a number of biochemical parameters in blood and urine of breast-fed children with high and low levels of OTA in maternal milk and children’s blood. Elevated OTA levels (in either milk or blood) correlated with a microglobulinuria, which was significantly greater in children with high than with low OTA concentrations and indicated initial kidney lesion. EFSA suggested that infants and children may experience higher rate of exposure than adults (75). It is not possible to link exposure to OTA during early childhood with any known human disease, although Schwartz (89) has put forward the hypothesis that mothers’ consumption of OTA-contaminated food and OTA exposure in early childhood may be the cause of testicular cancer in adulthood. This theory is based on the correlation between the incidence of testicular cancer and per-capita consumption of food items contaminated with OTA (coffee and pig meat) in 19 countries.

In tropical countries children are frequently exposed to OTA and AFs at the same time (44). Any health effect of this combination of mycotoxins, such as growth failure, could only be hypothesised (53). In a recent study in children in Cameroon under five years of age combined exposure to OTA and several other mycotoxins (AFs, fumonisins B1 – FB1, deoxynivalenol – DON, zearalenone – ZEA, α-zearalenol – α-ZEA and β-zearalenol β-ZEA) did not correlate with the degree of malnutrition (11).

**Trichothecenes**

Trichothecenes are a group of about 170 mycotoxins produced mostly by the moulds of the *Fusarium* strains that are common in mild climates. Other strains that produce them include *Trichoderma*, *Trichothecium*, *Myrothecium*, and *Stachybotrys*. Only a few types of trichothecenes are found in grains (wheat, oats, maize, barley) for human and animal consumption. The most common are deoxynivalenol (DON previously called vomitoxin), nivalenol (NIV), and diacetoxyscirpenol (DAS), while T-2 toxin is rare. IARC has designated DON, NIV, and T-2 toxin not classifiable as to carcinogenicity to humans (Group 3) (66). Trichothecenes inhibit protein synthesis and activate mitogen-activated protein kinases (MAPKs) that are involved in immune response and apoptosis signalling (90). The main characteristic of trichothecenes toxicity is immunomodulation. Lower doses increase resistance to pathogens, up-regulate many immune-related genes, and elevate serum IgA levels. Higher doses injure tissues with high cellular turnover such as bone marrow, lymph nodes, thymus, spleen, and intestinal mucosa. The consequence is a weaker immune response. In general, trichothecenes are haematotoxic and immunotoxic without genotoxic and carcinogenic properties (91-94).

Large outbreaks of acute mycotoxicosis caused by T-2 toxin, called alimentary toxic aleukia (ATA), were seen in the USSR in the 1930s and 40s. This disease mostly affected people from 10 to 40 years of age from rural areas because of the ingestion of grains that remained beneath the snow in the fields over the winter (95). Breast-fed infants of mothers affected by ATA were not ill. The first symptoms were local irritation of oral mucosa and painful swallowing. They developed after a few hours of ingestion of contaminated grains and subsided after two to three days if the exposure ceased. If it continued, the disease progressed to the second, leukopenic stage with minimal symptoms, and then to progressive leukopenia, granulocytopenia, lymphopenia, anaemia (low RBC or haemoglobin count), and thrombocytopenia. In case of mild or discontinued exposure, patients would recover completely. The third stage was characterised by pharyngo-haemorrhagic symptoms with severe necrotic pharyngitis and petechial rash, characteristically involving the trunk, inner sides of the arms and hips, and the inguinal fossae. These symptoms were accompanied by nose and mouth bleeds and bleeding in the stomach and intestines. This stage of ATA had a mortality rate of 50%. The fourth stage was the recovery stage when leukocytes counts started to increase, but it was also the stage when bacterial infections such as pneumonia and purulent tonsillitis appeared. Several later outbreaks of trichothecene toxicosis were not so severe. A large outbreak in the Kashmir Valley (India) also affected...
Table 4 Aflatoxins in urine of children

<table>
<thead>
<tr>
<th>Country</th>
<th>Age (years)</th>
<th>No of positive/analysed</th>
<th>No. of positive/analysed Mean concentration (range ng L⁻¹)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>AFB1</td>
<td>AFB2</td>
</tr>
<tr>
<td>Egypt</td>
<td>1-2.5</td>
<td>19/50 (38 %)</td>
<td>1/50 (2 %)</td>
<td>5/50 (10 %)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>189</td>
<td>1.4 (0.8-2.2)</td>
</tr>
<tr>
<td>Guinea</td>
<td>2-4</td>
<td>43/50 (86 %)</td>
<td>8/50 (16 %)</td>
<td>2.682 (179-18,000)</td>
</tr>
<tr>
<td></td>
<td>5-14 (Dry season/Boys)</td>
<td>133/134 (100 %)</td>
<td>47/134 (35 %) (0.6-188)</td>
<td>40/134 (30 %) (0.1-15.5)</td>
</tr>
<tr>
<td>Sierra Leone</td>
<td>Dry season/Girls</td>
<td>110/110 (100 %)</td>
<td>53/110 (49 %) (0.04-319)</td>
<td>18/110 (17 %) (0.2-152)</td>
</tr>
<tr>
<td></td>
<td>Wet season/Boys</td>
<td>95/97 (98 %)</td>
<td>32/97 (33 %) (1.2-115)</td>
<td>9/97 (9 %) (0.2-48)</td>
</tr>
<tr>
<td></td>
<td>Wet season/Girls</td>
<td>91/93 (98 %)</td>
<td>38/93 (41 %) (0.08-127)</td>
<td>19/93 (20 %) (0.1-12)</td>
</tr>
</tbody>
</table>

ND-not detected
the children (96). Symptoms appeared 15 minutes to
one hour after consuming contaminated bread; they
were milder than in ATA and disappeared immediately
after exposure ended. In the GI tract they included
abdominal pain, diarrhoea, blood in the stool, and
vomiting), but the most frequent were irritated throat
and secondary infections of the upper respiratory tract.

Except for the study by Rubert et al. (97) there are
no other reports of NIV and HT-2 toxin contamination
of breast milk.

In animal studies trichothecenes turned out to be
about 40 times more toxic when inhaled than when
ingested (98). The outbreak of idiopathic pulmonary
haemorrhage (IPH) in Cleveland (Ohio, US) between
1993 and 1998 involved 37 children, mostly African
American, who lived in humid houses damaged by
flooding or plumbing and roof leaks (99). IPH was
associated with exposure to satratoxin G and H and
roridin that are produced by Stachybotrys chartarum
on water-soaked cellulose walls. In 12 children the
outcome was fatal, and 60 % of the children that
returned home had recurrent pulmonary haemorrhage.
It is not clear whether the toxins of S. chartarum
caused IPH, as the Center of Disease Control declared
that there was not enough evidence to confirm the
suspicion (100).

Zearalenone

Zearalenone (ZEA) (previously also called F-2
toxin) is mainly produced by Fusarium moulds. ZEA
is a field contaminant of wheat, maize, oats, and barley,
particularly in warm and temperate climates (101). It
can also be found in surface waters close to agricultural
areas (102).

In humans, ZEA is rapidly absorbed by the GI tract
and metabolised to α-ZEA, β-ZEA, α-zearalenol
(α-ZAL), and β-zearalenol (β-ZAL), which are then
conjugated with glucuronic acid and rapidly excreted
in bile and urine. Zearanol, a synthetic derivative of
ZEA, is used as an anabolic agent for sheep and cattle
in the USA, but in Europe it has been banned since
1989.

In experimental animals ZEA is hepatotoxic,
haematotoxic, immunotoxic, and genotoxic (101). It
has low acute toxicity, and there are no reports of acute
human ZEA mycotoxicoses. At chronic levels, ZEA
and its derivatives show oestrogenic effect by binding
to oestrogenic receptors and by modulating the activity
of aldo-keto-reductases involved in the steroid
synthesis. In female experimental and domestic
animals they cause infertility, reduce litter size,
increase embryonic resorption, reduce milk production,
hypertrophy mammal glands, change weight of the
thyroid, pituitary, and adrenal glands, and increase
oestins. In male animals they reduce testis weight,
testosterone production, spermatogenesis, and mating
drive and lead to feminisation.

Studies on experimental animals showed that ZEA
crosses the placental barrier and is also excreted in
breast milk. In a recent Spanish study (97), ZEA was
found in 13 out of 35 samples of human breast milk
(37 %) and its metabolites α-ZAL and β-ZAL in only
one sample (2 %).

Historically, in Puerto Rico thousands of children
experienced puberty, mainly due to environmental
hormone contamination (103). Some of these children
had ZEA in their blood, probably as the consequence
of zearanol use as growth promoting agent in animal
breeding. Since 1989, Hungary has seen an increase
in the number of early telarche/mastopathy patients
(104). Five out of them 36 had blood ZEA levels
between 18.9 and 103.5 ng mL⁻¹.

IARC has classified ZEA as a Group 3 carcinogen
(66), but recent documents classify it and its
metabolites as endocrine disruptors (105).

Fumonisins

Fumonisins are a group of 15 mycotoxins produced
by Fusarium moulds (mostly F. verticilloides and F.
moniliforme). In naturally contaminated grains the
most frequent is fumonisin B1 (FB1), often
accompanied by small amounts of fumonisin B2 (FB2)
and fumonisin B3 (FB3).

Fumonisins contaminate various grains, but are
most common in maize (106). They are poorly
absorbed by the GI tract and quickly eliminated from
plasma with low accumulation in the kidney and liver.
They exert their toxic effects by inhibiting ceramide
synthase, the key enzyme in the sphingolipid
metabolism. This results in lower sphingolipid de novo
synthesis and reuse of sphinganine. Significantly
increased blood sphinganine affects the membrane
transport of folic acid by binding to folate receptors,
which eventually leads to lower folate uptake (107).

Fumonisins target different organs in domestic and
experimental animals: in horses they cause
leukoencephalomalacia, in pig pulmonary oedema, in
rats they are predominantly nephrotoxic, and in mice
they are hepatotoxic and teratogenic, causing neural
tube defects (NTD) (2).

There is a only one report of acute fumonisin-
caused mycotoxicosis that occurred in India (108).
Symptoms appeared after ingestion of contaminated sorghum or maize and included abdominal pain, borborygmi, and diarrhoea. The disease was self-limiting, and preschool children were less affected.

In some regions where maize is staple food (Transkei in Southern Africa, China, and Northern Italy) the high frequency of oesophageal cancers is believed to be related to exposure to fumonisins or their producers (*F. moniliforme*) (109, 110). In the early 1990s, a higher prevalence of NTD (anencephaly and spina bifida) was observed in children born along the Texan and Mexican border by Mexican-American women, and Hendricks et al. (111) suggested that this was caused by fumonisin exposure in the first trimester of pregnancy. High prevalence of NTD was also found in the Transkei region in Southern Africa (112), northern Iran (113), and several regions of China, where it was higher in rural than in urban population (114). In a large controlled study in China (130,142 women taking folic acid vs. 117,689 controls) the incidence of NTD was significantly decreased by folic acid supplementation (115). In Ireland, B12 and folic acid fortification of breakfast cereals decreased the rate of NTD from 47 to 13 per 10,000 births (116).

Table 5 *Ochratoxin A* concentrations in breast milk

<table>
<thead>
<tr>
<th>Country</th>
<th>No. of positive/analysed</th>
<th>Concentration (ng L⁻¹) Mean±SD (range)</th>
<th>Method</th>
<th>LOD (ng L⁻¹)</th>
<th>LOQ (ng L⁻¹)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Africa</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egypt</td>
<td>43/120 (36 %)</td>
<td>21.06±13.7 (5.07-45.01)</td>
<td>HPLC-FD</td>
<td>NI</td>
<td>NI</td>
<td>25.</td>
</tr>
<tr>
<td></td>
<td>36/50 (72 %)</td>
<td>NI</td>
<td>HPLC/Mf</td>
<td>NI</td>
<td>NI</td>
<td>78.</td>
</tr>
<tr>
<td>Sierra Leone</td>
<td>40/113 (35 %)</td>
<td>NI</td>
<td>HPLC-FD</td>
<td>200</td>
<td>NI</td>
<td>79.</td>
</tr>
<tr>
<td><strong>Americas</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brazil</td>
<td>0/224</td>
<td>NI</td>
<td>HPLC-FD</td>
<td>NI</td>
<td>0.0003</td>
<td>39.</td>
</tr>
<tr>
<td>Sao Paolo</td>
<td>2/50 (4 %)</td>
<td>10, 24</td>
<td>HPLC-FD</td>
<td>NI</td>
<td>10</td>
<td>38.</td>
</tr>
<tr>
<td>Ribeirão</td>
<td>66/100 (66 %)</td>
<td>4 (0.3-21)</td>
<td>HPLC-FD</td>
<td>0.3</td>
<td>0.8</td>
<td>40.</td>
</tr>
<tr>
<td>Preto-SP</td>
<td>9/9 (100 %)</td>
<td>106±45 (44-184)</td>
<td>HPLC-FD</td>
<td>NI</td>
<td>NI</td>
<td>77.</td>
</tr>
<tr>
<td>Chile</td>
<td>40/50 (80 %)</td>
<td>52±56 (10-186)</td>
<td>HPLC-FD</td>
<td>10</td>
<td>30</td>
<td>80.</td>
</tr>
<tr>
<td><strong>Asia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iran, Sari</td>
<td>2/136 (1 %)</td>
<td>90, 140</td>
<td>ELISA</td>
<td>NI</td>
<td>NI</td>
<td>33.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HPLC-FD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Australia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Victoria</td>
<td>2/100 (2 %)</td>
<td>3, 3.6</td>
<td>HPLC-FD</td>
<td>1.6</td>
<td>NI</td>
<td>81.</td>
</tr>
<tr>
<td><strong>Europe</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Italy</td>
<td>9/50 (18 %)</td>
<td>4189 (1700-6,600)</td>
<td>HPLC-FD</td>
<td>200</td>
<td>NI</td>
<td>82.</td>
</tr>
<tr>
<td></td>
<td>22/111 (20 %)</td>
<td>(100-12,000)</td>
<td>HPLC-FD</td>
<td>100</td>
<td>NI</td>
<td>83.</td>
</tr>
<tr>
<td></td>
<td>74/85 (75 %)</td>
<td>NI</td>
<td>HPLC-FD</td>
<td>20</td>
<td>NI</td>
<td>84.</td>
</tr>
<tr>
<td></td>
<td>61/82 (74 %)</td>
<td>30.43±66.9 (5-405)</td>
<td>HPLC-FD</td>
<td>2</td>
<td>5</td>
<td>36.</td>
</tr>
<tr>
<td></td>
<td>198/231 (86 %)</td>
<td>6.01±8.31 (1-57)</td>
<td>HPLC-FD</td>
<td>0.5</td>
<td>NI</td>
<td>35.</td>
</tr>
<tr>
<td></td>
<td>38/115 (33 %)</td>
<td>(10-130)</td>
<td>HPLC-FD</td>
<td>NI</td>
<td>10</td>
<td>85.</td>
</tr>
<tr>
<td></td>
<td>17/80 (21 %)</td>
<td>30</td>
<td>HPLC-FD</td>
<td>NI</td>
<td>10</td>
<td>86.</td>
</tr>
<tr>
<td>Norwegian</td>
<td>23/76 (30 %)</td>
<td>(2.3-60.3)</td>
<td>HPLC-FD</td>
<td>4.8</td>
<td>14.4</td>
<td>87.</td>
</tr>
<tr>
<td>Slovakian</td>
<td>23/40 (58 %)</td>
<td>(10-40)</td>
<td>HPLC-FD</td>
<td>10</td>
<td>40</td>
<td>88.</td>
</tr>
<tr>
<td>Switzerland</td>
<td>4/40 (10 %)</td>
<td>7.2 (5-14)</td>
<td>HPLC-FD</td>
<td>NI</td>
<td>5</td>
<td>76.</td>
</tr>
<tr>
<td>Turkey</td>
<td>75/75 (100 %)</td>
<td>(620-13,111)</td>
<td>HPLC-FD</td>
<td>10</td>
<td>NI</td>
<td>37.</td>
</tr>
</tbody>
</table>

NI—not indicated

*LC/Mf: liquid chromatography with microfluorimetric detection
order to decrease the incidence of NTD, the US Food and Drug Administration (FDA) mandated fortification of grain products with folic acid (117). This resulted in lower NTD incidence in the general population, but not in several ethnic groups. There are reports of a 58% drop in NTD occurrence rate and of an even more significant (95%) drop in NTD recurrence rate when folate supplementation was applied starting one month before conception (116). However, some studies reported that folate supplementation was not that effective or not effective at all, and the disturbance of folate metabolism by fumonisins has not been confirmed, although there is no doubt about their neurotoxicity in experimental animals (118-120).

IARC has classified fumonisins as carcinogenic in experimental animals with limited evidence of its carcinogenicity in humans (Group 2B carcinogen) (66).

Ergot alkaloids are a group of about 40 toxins produced mostly by the fungal species of the genus Claviceps that contaminate rye, oats, and pearl millet. They are also produced by some strains of Penicillium, Aspergillus, and Rhizopus spp. (121). These toxins may be divided in three groups: lysergic acid derivatives (e.g. ergotamine and ergocristine), isolysergic acid derivatives (e.g. ergotaminine), and dimethylergoline derivatives (e.g. agroclavine) (122). Ergot is the name of sclerotia, a dark fungal mass that replaces the seed or kernel of the infected plant. Ergot poisoning is called ergotism and was common in European history (123).

The clinical presentation of acute ergotism depends on the type of toxins that are produced by particular strains of the genus Claviceps.

Ergot alkaloids are absorbed by the GI tract, distributed readily in plasma, and metabolised by CYP3A4. Some of them are conjugated with glucuronic acid and eliminated by biliary excretion (124).

Claviceps purpurea produces toxins from the group of lysergic acid derivatives (ergotamine and ergocristine), which cause severe vasoconstriction. Poisoning symptoms include severe pain in the legs, loss of pulse, and oedema. Paraesthesia is followed by gangrene around the tendons, with painless demarcation. In the ergotism outbreak in Wollo (Ethiopia) in 1977-78 four children lost one or both legs (125).

Clavine alkaloids produced by Claviceps fusiformis can cause the convulsive type of ergotism that occurs 1-48 hours after ingestion of contaminated food. It starts with gastrointestinal symptoms (nausea, vomiting, dizziness) and continues with the nervous system symptoms (crawling sensation in the skin, tingling in the fingers, vertigo, headache drowsiness, prolonged sleepiness, painful muscular contractions leading to convulsions, blindness, and paralysis). Mental disturbances may appear such as mania, psychosis, and delirium. One notable historical case presented by Caporeal (126) were the teenage girls accused of witchcraft in Salem in 1692.

3-nitropropionic acid

3-nitropropionic acid (3-NPA) is produced by Arthrinium moulds and causes the so called mouldy sugar cane disease (127). This disease appears in the late winter months (February and March) in 13 northern Chinese counties and affects children who consume sugar cane contaminated with Arthrinium moulds stored for at least two months. From 1978 to 1988, 884 persons were involved in a series of small epidemics (involving usually up to five children), and 88 (10%) died (128). The first symptoms appeared two to three hours after the consumption of sugar cane and included vomiting, general fatigue, convulsions, carpopedal spasms, and coma. Dystonia appeared in 10-50% of the cases as the consequence of basal ganglia necrosis. These last symptoms can be predicted if the basal ganglia are scanned with computerised tomography (CT) (129). In adults, the symptoms of 3-NPA poisoning include mild disturbances of the GI tract and brain lesions, but these are very rare. Since 1995, there have been no further reports on mouldy sugar cane disease in the available scientific literature. However, Chinese health authorities keep issuing warnings in daily newspapers, which suggests that this disease is still a threat.

Ustilago maydis toxins

Historically, mycotoxicoses that appeared in Croatia between the two world wars were suspected to be caused by corn smut (Ustilago maydis). Children, infants, and toddlers, suspected of ustilaginism fell ill between March and May after a winter of almost exclusive consumption of corn flour from fields infested with corn smut. The clinical presentation was either acute - with acropathic symptoms including hand and sole pruritus, oedema and erythema - or...
chronic - with frequent relapses of acute symptoms and abundant skin desquamation (130, 131). At the time, it was believed that the cause were corn smut spores at a specific window of maturity. If the corn was consumed before or after that window, it was considered harmless. This corn smut hypothesis has never been confirmed, and the only argument in its favour is that the patient’s condition would improve as soon (several days to a fortnight) as the corn flour was removed from the diet. No similar phenomena have been reported since 1945.

CONCLUSION

Acute mycotoxicoses in children are serious diseases, mostly diagnosed only when an epidemics breaks out, affecting several children. They should be suspected when the disease cannot be explained by infection with a known microorganism. Acute mycotoxicoses are more frequent in tropical regions but are no stranger to temperate climates as well. Chronic mycotoxicoses may appear all over the world and paediatricians should keep in mind that their clinical manifestations vary a lot.

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**Sažetak**

**Mikutoksikoze u djece**

Mikutoksikoze su akutne i kronične trovanja toksinima pljesni koji se nazivaju mikotoksini. Odrasle osobe i djeca stalno su izloženi niskim koncentracijama mješavine mikotoksina, većinom putem kontaminirane hrane. Akutne toksikoze koje uzrokuje izloženost velikim količinama mikotoksina danas su rijetke te su opisane kako bi se liječnici i drugo zdravstveno osoblje informirali o njihovim simptomima koji mogu oponašati druge bolesti. Djeca su osjetljivija na toksični učinak mikotoksina zbog toga što je njihova tjelesna masa manja, a neki sustavi za detoksikaciju nisu u potpunosti razvijeni. Neke se mikotoksikoze javljaju samo u djece ili su u djece simptomi jače izraženi. Podrobnih podataka o epidemiologiji mikotoksikoza u dječjoj dobi nema jer one pogotovo prvenstveno prvenstveno najsromašnije, uglavnom u zemljama tropskoga pojasa gdje je zdravstvena služba nedostatna, a potrebe stanovništva za liječenjem velike. U tim se zemljama češće javljaju i kronične mikotoksikoze, no ima ih i u zemljama s umjereno klimom. U razvijenim su zemljama zdravstvene vlasti više zabrinite zbog izloženosti djece niskim koncentracijama mikotoksina koji imaju imunotoksična, genotoksična i kancerogena svojstva.

**KLJUČNE RIJEČI:** 3-NP; aflatoxini; ergot; fumonizini; okratoxini; trihoteceni; toksini Ustilago maydis; zearalenon

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