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DOI: https://doi.org/10.52091/EVIK-2021/2-2-ENG

Received: March 2021 – Accepted: May 2021

# *Chronic aflatoxin M1 exposure of Hungarian consumers*

**Keywords:** deterministic exposure estimation, chronic aflatoxin M1 exposure, consumer groups at risk, carcinogenic effect, risk of liver cancer

## 1. SUMMARY

**The mycotoxin contamination of foods also appears in the food chain. Aflatoxin is metabolized in animals and its aflatoxin M1 (AFM1) metabolite, which is similarly, but ten times less genotoxic and carcinogenic than aflatoxin B1 (AFB1), is also present in milk, liver and eggs. Of these, the most significant food safety risk is posed by the contamination of milk with AFM1. In our article, the deterministic exposure estimation of Hungarian consumers is presented, based on the AFM1 contamination of milk and dairy products. The results indicate that the exposure of children under three years of age clearly poses a health risk, while the exposure of the 3 to 6 year old age group is borderline. The exposure of older age groups in ng/kg body weight does not pose an immediate health risk due to the increasing body weight. However, it needs to be emphasized that the presence of carcinogenic compounds should be kept to a minimum in all age groups. To this end, we propose an amendment to the regulation regarding the factory inspection of milk.**

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## 2. Introduction

In our previous paper on mycotoxin contamination, we presented the mycotoxin contamination of foods and feeds, the legal regulation of their tolerable maximum concentrations, the limitations of sampling procedures, and the experiences of current domestic practice were analyzed **[1]**. Using the data of the food consumption survey conducted in 2009 and measurement results available from the period 2010-2018, the exposure of Hungarian consumers to DON and aflatoxin M1 was estimated. Based on our preliminary estimates, it was determined that for some consumers, exposure to aflatoxin M1 and DON may exceed the toxicological reference values from time to time due to the yearly variation in food contamination levels, which may pose a risk to human health.

In our present paper, the results of our calculations carried out by a deterministic method and using the latest analytical measurement results and the data of the Hungarian food consumption survey performed in 2018-2019 using the methodology uniformly applied in Europe are presented, which provides information on chronic AFM1 exposure of different consumer age groups.

Intensive research is induced by the expected spread of mycotoxin-producing fungi due to global warming, the increase in food and feed contamination levels caused by the toxins they produce and the health problems and economic damages attributed to them. The large number of reports on the results are made more manageable by research area by the regularly published review articles, such as those on the interaction of mycotoxin-producing *Aspergillus* species with soil microorganisms **[2]**, on human physiological effects of mycotoxin exposure **[3]**, on the application of biocontrol technologies to reduce aflatoxin contamination, on the effect of silage production technology and the microbiota on aflatoxin contamination **[4],** on the sources, occurrence and regulation of mycotoxin contamination **[5, 6]** and on its detection methods **[7]**. A special edition of the journal Frontiers in Microbiology, containing 22 of the latest research articles and summaries, has also been published in the form of a book **[8]**.

In view of the above reviews, only literature publications closely related to the objective of our paper are summarized below.

### *2.1. Occurrence of aflatoxins*

Aflatoxins and other mycotoxins that occur in raw agricultural products (mainly peanuts, maize, rice, nuts, figs, spices and dried fruits) and feeds enter the food chain and can be detected in milk **[9, 10, 11, 12]**, eggs, meat and offal **[6, 13]**. Aflatoxicol has been detected in the liver, kidney and meat of broiler and laying hens **[14, 15]**. Compared to the concentration of AFB1 in the feed, >5700-, >4600- and >3800-fold concentrations were measured in the livers of hens, in egg yolk and in egg white, respectively **[16]**.

In addition to the exposure of the animals to AFB1 (μg/kg body weight), the concentration of AFM1 entering the milk from the feed depends on a number of factors, such as the health status of the cow, its milk yield, the lactation period, etc. **[17]**. The transmission rate is higher in specimens with higher milk yields **[18]**. The results of several studies have been reported in the literature, according to which the rounded transmission rate varied from 0.35 to 6%. Lower transmission rates (0.08%-0.33%) were observed in sheep **[19]**.

There is less research on the transmission of aflatoxin to the liver, meat and eggs, but these report significantly lower transmission rates compared to milk, making milk still the most significant source of aflatoxin among foods of animal origin **[4, 20]**.

When a food contaminated with AFB1 is consumed, AFM1 is excreted in breast milk to a similar extent as in cow's milk **[21, 22, 23, 24, 25, 26]**. Infants and young children who are fed formula or milk drinks based on cow's milk may also be exposed to AFM1. The results of European surveys indicate much lower levels than African or Asian publications **[27]**.

As with all mycotoxins, aflatoxins show significant annual fluctuations in their levels depending on the weather conditions affecting fungal growth and toxin production **[28]**.

For its most recent risk assessment **[29]**, EFSA used the results of aflatoxin M1 measurements reported by Member States after 2013. Statistical data for some of the major food categories are summarized in ***Table 1***.

*Table 1. AFM1 mean and 95th percentile concentration values based on the 2013-2020 Member State data of EFSA.*

| Food category | N (pc) | % LCD | Mean LB (µg/kg) | Mean UB (µg/kg) | P95 LB (µg/kg) | P95 UB (µg/kg) |
|---|---|---|---|---|---|---|
| Milk | 6020 | 76 | 0.018 | 0.031 | 0.087 | 0.087 |
| Condensed milk/Milk powder | 168 | 81 | 0.037 | 0.044 | 0.018 | 0.087 |
| Cream | 114 | 96 | 0.000 | 0.009 | 0.000 | 0.020 |
| Fermented milk products | 96 | 94 | 0.052 | 0.069 | 1.00 | 1.00 |
| Cheeses | 359 | 53 | 0.097 | 0.107 | 0.415 | 0.415 |
| Baby formula | 354 | 90 | 0.060 | 0.071 | 1.00 | 1.00 |
| Other | 85 | 53 | 0.124 | 0.204 | 1.00 | 1.00 |

*Comment:*
*N: number of measurement results; % LCD (left censored data): ratio of results below the detection/quantification limit; P95: 95th percentile; LB: lower bound – result of substitution with the lowest concentration value; UB: upper bound – result of substitution with the highest concentration value; Other: foods for infants and young children.*

### *2.2. Health effects of aflatoxins*

Aflatoxins (especially AFB1, AFG1 and AFM1) proved to be extremely potent carcinogenic, kidney and liver damaging, genotoxic, malformative, reproductive capacity decreasing, immunosuppressive and nervous system damaging compounds in all experimental animal species, such as fish, ducks, mice, rats and monkeys **[30]**. A recently published study showed that the spores of pathogenic fungi cause severe, fatal infections in various birds **[31]**.

High levels of AFB1, both in humans and animals, can cause fast-action, acute poisoning, during which severe hepatitic failure can lead to death, however, human risk of this in developed countries is negligible. Of aflatoxins, aflatoxin B1 is the most potent carcinogenic and genotoxic compound, and it is the one most commonly found in foods and feeds. Most often, it causes hepatocellular carcinoma (HCC), which is why AFB1 has been classified as a Group 1 human carcinogen by the IARC. After consumption of feed contaminated with AFB1, its hydroxy metabolite, aflatoxin M1, which is also a carcinogenic compound, although with a toxicity that is about one tenth of that of AFB1, is excreted by dairy cows in milk **[32, 33]**.

Aflatoxins are rapidly and extensively adsorbed in the small intestine and, once in the liver, the metabolism of aflatoxin is catalyzed by the cytochrome P450 enzyme system found there. AFB1, AFG1 and AFM1 are converted to a reactive electrophilic epoxide that is capable of covalently binding to both DNA and proteins. Glutathion S-transferases (GST) are able to form a conjugative link with the 8,9-exo epoxide of AFB1, which is no longer able to enter harmful reactions in the body, and is excreted through bile and the kidneys. Polymorphisms among individuals result in high variability in enzymatic processes, and thus sensitivity to aflatoxin also varies from individual to individual **[30, 34, 35]**. In optimal cases, most aflatoxin metabolites are excreted within a few days, however, they have been observed to be present in protein-bound form over a longer period of time (e.g., in the case of aflatoxin-albumin adducts), with a half-life of 30 to 60 days in peripheral circulation **[36]**.

Aflatoxins also damage liver cells directly, as well as indirectly, by altering the expression of genes involved in lipid metabolism. Increased cholesterol, triglyceride and lipoprotein production can cause the disintegration of hepatocytes. Hepatocyte death may lead to acute hepatitis, which can result in liver failure and, in more severe cases death. The disrupted metabolism of hepatitis patients can lead to malnutrition, which indirectly contributes to a general decrease in the antioxidant capacity of hepatocytes, to a loss of liver tissue regeneration capacity and, ultimately, liver failure **[3].**

Based on the opinion of EFSA experts, a key point in the risk assessment of aflatoxins is the evaluation of the role these toxins play in the development of liver cancer. From this point of view, children are particularly sensitive to aflatoxins, because, due to their low body weight, have a higher intake of food per kg body weight, and the risk of developing liver cancer is also higher in individuals infected with the hepatitis B (or C) virus and in the elderly. In people living in areas where both hepatitis B virus (HBV) infection and aflatoxin exposure are common, hepatocellular carcinoma (HCC) samples show a mutation hotspot (G-T transformation) at codon 249 of the p53 gene, which mutation is considered to be a signature of aflatoxin-induced HCC **[37]**. The possible reason for this is that hepatitis infection of the liver alters the expression of genes encoding aflatoxin detoxification enzymes, resulting in, for example, the induction of CYP enzymes or a decreased GST activity, thereby preventing the body from adequately eliminating aflatoxins **[35]**. Due to the immunosuppressive effect of aflatoxins, elderly people with chronic diseases are at particular risk, because in their case the efficiency of cell-level repair mechanisms is inferior, so the elimination of aflatoxins is also less effective. It should be emphasized that aflatoxins are able to cross the placenta, so aflatoxin exposure of pregnant women can also endanger the fetus **[38]**.

### *2.3. The effect of processing on the aflatoxin content in foods*

The common feature of aflatoxins is that they are stable, resistant to processing and heat effects. As a consequence, their presence must also be taken into account in the case of processed foods. Certain processing steps, such as sorting, refining, grinding, cooking, baking, frying in oil, roasting, preservation, flocculating, alkaline cooking, nixtamalisation, extrusion and fermentation, can reduce the concentration of mycotoxins in crops and processed foods, but they are not adequate enough to eliminate all contaminants, so the role of prevention at the very beginning of the food chain is of paramount importance **[20]**. For example, in terms of AFM1 contamination, it is important to reduce the AFM1 contamination of feeds using pre- and post-harvest biotechnological methods as well as toxin binders **[4, 17]**.

Of heat treatment processes, conventional cooking and baking have little effect on mycotoxin contamination, while methods performed at higher temperatures, or possibly using dry heat **[39]**, are more efficient. The breakdown of mycotoxins is enhanced by the presence of sugars, e.g., glucose, during heat treatment **[40]**.

During the wet milling of cereals, such as corn, aflatoxin is distributed among the milling fractions in the following proportions: soaking water: 39–42%, fiber: 30–38%, gluten: 13–17%, germ, 6–10% and starch: 1%. Thus, the total aflatoxin level in the processed products decreases with the proportion remaining in the soaking water. After the dry milling of corn, the groats, bran and flour fractions contain only 6 to 10% of the original aflatoxin content, with most of the aflatoxin entering the germ and husk fractions **[20]**.

Contamination of rice with aflatoxin most often occurs due to improper harvest and storage conditions. Mycotoxins are found primarily in the rice husk and bran layers. Husked brown rice and white rice obtained by polishing are gradually less contaminated **[41]**.

The various heat treatment processes, pasteurization and freezing do not have a significant effect on the aflatoxin content of milk and dairy products **[42]**. The reduction effect of some heat treatment processes on AFM1 expressed in numerical values are as follows: pasteurization: 7.6%-12.9%, boiling: 14.5-23.9% **[43]**, UHT treatment: 32% **[44]**.

Different physical and chemical methods have been used with good efficiency to reduce the AFM1 content of milk or other liquid products: microwave irradiation (52%) **[43],** membrane filtration (81%) **[45]**, biofiltration (81%) **[46]** combination of centrifugation and filtration (83%) **[45]**, ozone treatment **[47]**, the use of adsorbents (85-90%) **[48, 49]**.

Intensive research is underway on the use of microorganisms. Encouraging results for the reduction of AFM1 contamination in milk have been obtained using *Saccharomyces cerevisiae* (90-93%) **[50]**, *S. cerevisise* + *L. rhamnosus, L. delbrueckii spp. bulgaricus, B. lactis* (100%) **[49]**, the mixture of different yeasts (65-69%) **[51]**, heat-treated *L. plantarum* (94,5%) [**44]**, *L. bulgaricus* (58%) **[52]** and in yogurt using *S. thermophilus, L. bulgaricus* and *L. plantrium* strains **[53]**. It remains to be seen how (in the case of using live microbes) changes in organoleptic properties can be eliminated if non-conventional cultures are used, and how the lactic acid bacterium-AFM1 complex formed can be removed from the product **[44]**.

## 3. Data used to estimate consumer exposure

Exposure (g/kg body weight or ng/kg body weight) is calculated by multiplying the amount of food consumed (g/kg body weight) and the contaminant concentrations measured in it (ng/kg). In the deterministic method, we multiply the mean (median), or sometimes an upper percentile (95.0, 97.5) value. This calculation results in a point estimate giving a specific value **[54, 55]**. A more subtle estimate is obtained by probabilistic methods **[56, 57]**, in which the distribution of input data is taken into account and thus a distribution is also obtains for exposure. Care should be taken when considering test results below the limit of quantification (LOQ). If the proportion of samples below the LOQ is between 50 and 80%, a maximum likelihood estimate (MLE) gives the best results **[58]**.

Whichever method is used for the estimation, it is important to take into account the uncertainty of each calculation step, their magnitude, and to evaluate the results obtained in light of their cumulative effect **[59]**. The calculated uncertainty interval includes the true value with a certain level of confidence, i.e., with a certain degree of certainty **[60]**. The amount of contaminant entering the consumer's body (EDI) is compared to the toxicological reference value(s) to determine the expected health risk.

For both short-term and long-term exposure estimation, it is worth examining the consumer groups that are particularly affected by the consumption of the given food/contaminant combination, and comparing the exposure of average consumers and „large consumers" **[61]**.

### *3.1. Reference values for exposure assessment*

Depending on the specific properties of the contaminant, the reference value may be the Acceptable Daily

Intake (ADI), the Provisionally Tolerable Weekly/Monthly Intake (PTW/MI) or the Acute Reference Dose (ARfD). For food contaminants, the reference value is usually the tolerable daily intake (TDI). The benchmark dose (BMD) is the smallest dose that is estimated from the fitted dose-response curve at which a preselected effect level (benchmark response – BMR) can be observed, usually an increase or decrease of 5 or 10% compared to the control group. The lower confidence value of the BMD is the BMDL **[62]**. In the case of aflatoxins, Margin of Exposure (MoE) analysis is used to characterize the risk, as no TDI or other toxicological reference value can be established. In such a case, the value of the BMDL, adjusted by the uncertainty factor, is compared to the estimated exposure. The risk attributed to a contaminant can also be expressed as the ratio of the exposure to other reference values, the Hazard Quotient (HQ) or the Hazard Index (HI), which is the sum of the hazard ratios of substances acting on the same target organ or organ system, usually used for cumulative estimates **[63]**.

EFSA recommends the use of 4 μg/kg body weight/day as the $BMDL_{10}$ value as a benchmark for AFM1 risk characterization **[29]**. The results obtained are considered to be of concern below 10,000, with an MoE of 10,000 or greater indicating little risk to public health.

To characterize the risk of AFM1, the safe dose recommended by Kuiper-Goodmann (0.2 ng/kg body weight/day) can also be used to calculate the hazard index (HI), which is a quotient of a tumor-causing dose in 50% of animals and a safety factor of 50,000 **[64]**.

According to the 2018 calculations of the JECFA **[20],** with an average daily intake of 1 ng/kg body weight AFB1, the probability of developing liver cancer is on average 0.269 per 100,000 persons per year, with the upper limit of the 95% confidence interval of the estimate being 0.562/100,000 persons/year in HBsAg+ (positive for hepatitis B surface antigen) individuals. For HBsAg- (negative for hepatitis B surface antigen) individuals, the mean value was 0.017 cancers/year/100,000 persons, with the upper limit of the 95% confidence interval of the estimate being 0.049/100,000 persons/year. The estimated mean values for AFM1 are one order of magnitude lower: 0.027/100,000 persons for HBsAG+, and 0.002/100,000 persons for HbsAg- individuals **[20]**.

JECFA estimated the risk of hepatocellular carcinoma (HCC) associated with aflatoxin exposure using Equation 1:

$$R_i = [(P_{HBV+} \times HBV+) + (P_{HBV-} \times (1-HBV+))] \text{ x AF input} \quad (1),$$

where $R_i$ is the HCC risk for region i, HBV+ is the prevalence of chronic hepatitis B in the study population, $P_{HBV+}$ is the probability of developing liver cancer in this fraction of the population and $P_{HBV-}$ is the probability of developing liver cancer in the rest of the population.

### 3.2 Food consumption data

The calculations were performed using data from two representative Hungarian food consumption surveys conducted 10 years apart. The three-day survey of 2009 provided food consumption data for 4,992 individuals for a total of 14,976 consumption days, processed by dietitians and broken down into raw materials for the characterization of food consumption habits **[65]**. The ratio of milk and dairy product consumption days is shown in ***Figure 1***.

Of the 14,976 consumption days in the 2009 survey, the consumption frequency [%] of milk, sour cream and cream, cheese and kefir or yogurt was 75.2, 52.8, 46.3 and 19.1, respectively.

The 2018-2020 survey was conducted within the framework of EFSA's Europe-wide EU MENU or "What's on the table in Europe?" project, in accordance with the recommended, uniform methodology **[66, 67]**. Participating persons were selected from the households participating in the Hungarian Central Statistical Office Household Budget and Living Conditions survey. During the program, two consumption days of 2,657 individuals between the ages of 1 and 74 were recorded, with the help of dietitians. On the 5,314 consumption days, the consumption frequencies [%] of milk, sour cream and cream, cheese and kefir or yogurt were 96.8, 54, 60.6 and 24. The ratio of milk and dairy product consumption days is shown in ***Figure 2***.

The distribution of consumers by age group is shown in ***Table 2***.

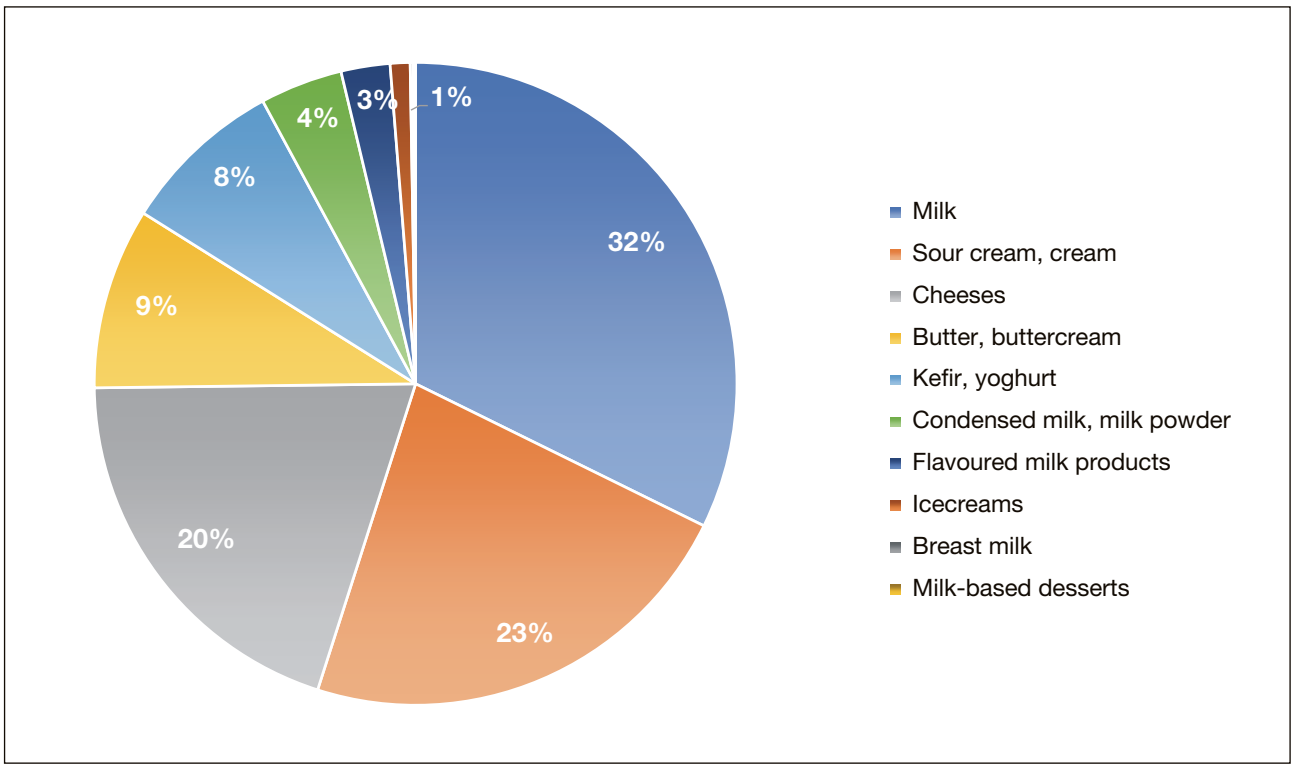


*Figure 1. The proportion of milk and dairy product consumption days by food group in the 2009 survey*

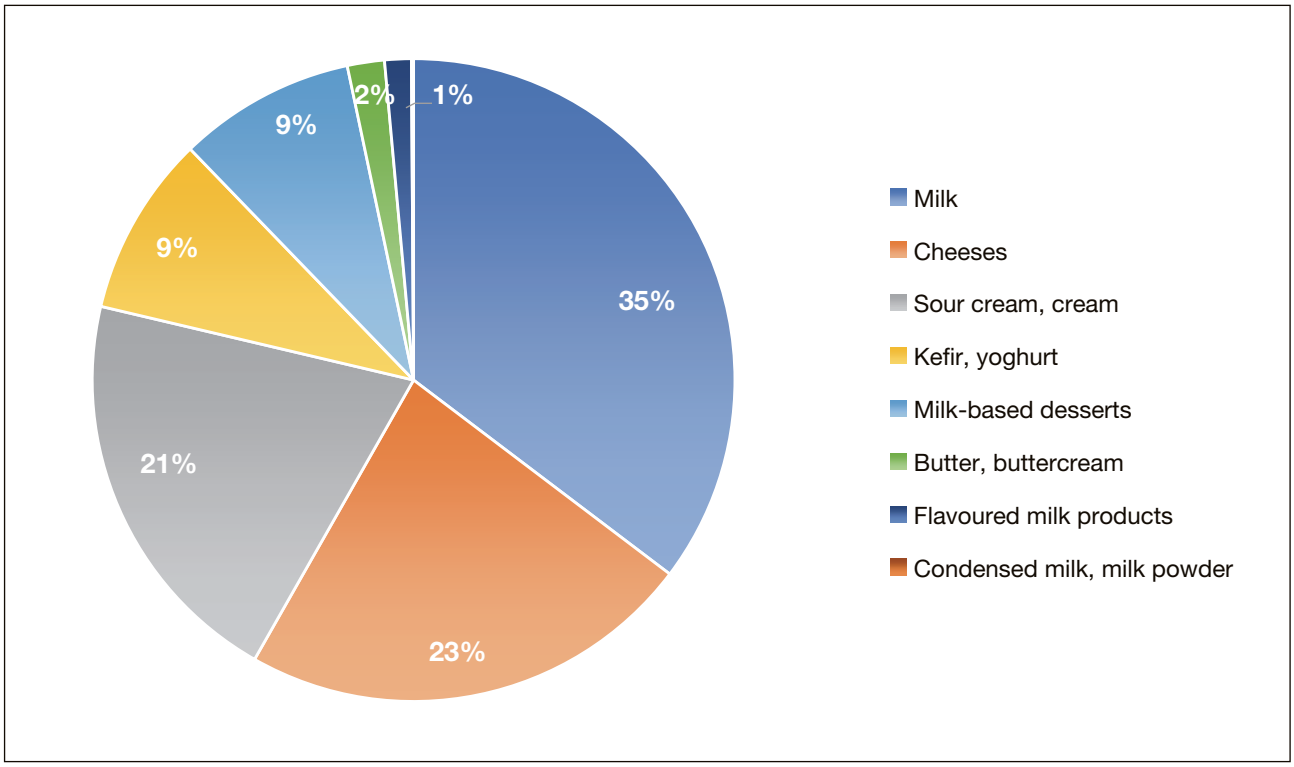


*Figure 2. The proportion of milk and dairy product consumption days by food group in the 2018-2020 survey*

*Table 2. Age groups of the 2009 and 2018-2020 food consumption surveys and the number and proportion of consumers of dairy products by age group*

| Age group | Age (year) | Consumers 2009 (persons) | Dairy product consumers 2009 | | Consumers 2018-2020 (persons) | Dairy product consumers 2018-2020 | |
|---|---|---|---|---|---|---|---|
| | | | persons | % | | persons | % |
| Babies | 0.0-0.9 | 26 | | | 0 | 0 | 0 |
| Toddlers | 1.0-2.9 | 90 | 90 | 100 | 535 | 482 | 90 |
| Children | 3.0-9.9 | 324 | 324 | 100 | 537 | 536 | 100 |
| Adolescents | 10.0-17.9 | 494 | 487 | 98 | 528 | 525 | 99 |
| Adults | 18.0-64.9 | 3360 | 3297 | 98 | 529 | 515 | 97 |
| Elderly | 65.0- | 698 | 691 | 99 | 527 | 509 | 96 |
| Total | | 4966 | 4889 | 98 | 2654 | 2567 | 97 |

Changes in the frequency of consumption of milk and various dairy products were compared using the milk and dairy product consumption days of the 2009 and 2018-2020 food consumption surveys. The numbers of consumption days of the different foods were compared to the total consumption days of the given survey (Figure 3). The frequencies of consumption of the different foods during the survey periods are characterized by the figure. Among the food categories studied, the consumption frequency of milk and milk-based desserts increased by more than 20%. The consumption frequency of cheeses shows an increase of 14%. The consumption frequencies of sour milk products (kefir, yogurt, sour cream), cream and flavored milks remained almost constant (with the former increasing slightly and the latter decreasing to a small degree). The consumption frequencies of condensed milk and milk powder has decreased significantly. Overall, it can be stated that the consumption frequencies of milk and dairy products has increased slightly over the last 10 years.

Based on the change in consumption frequencies over 10 years, an increase in aflatoxin exposure could be expected, however, this effect was offset by the change in the amounts consumed. The average consumption in milk equivalent, calculated with the median value of processing and enrichment factors, was 310.7 g/day in 2009, and this value decreased to 295.3 g/day in 2018-2020.

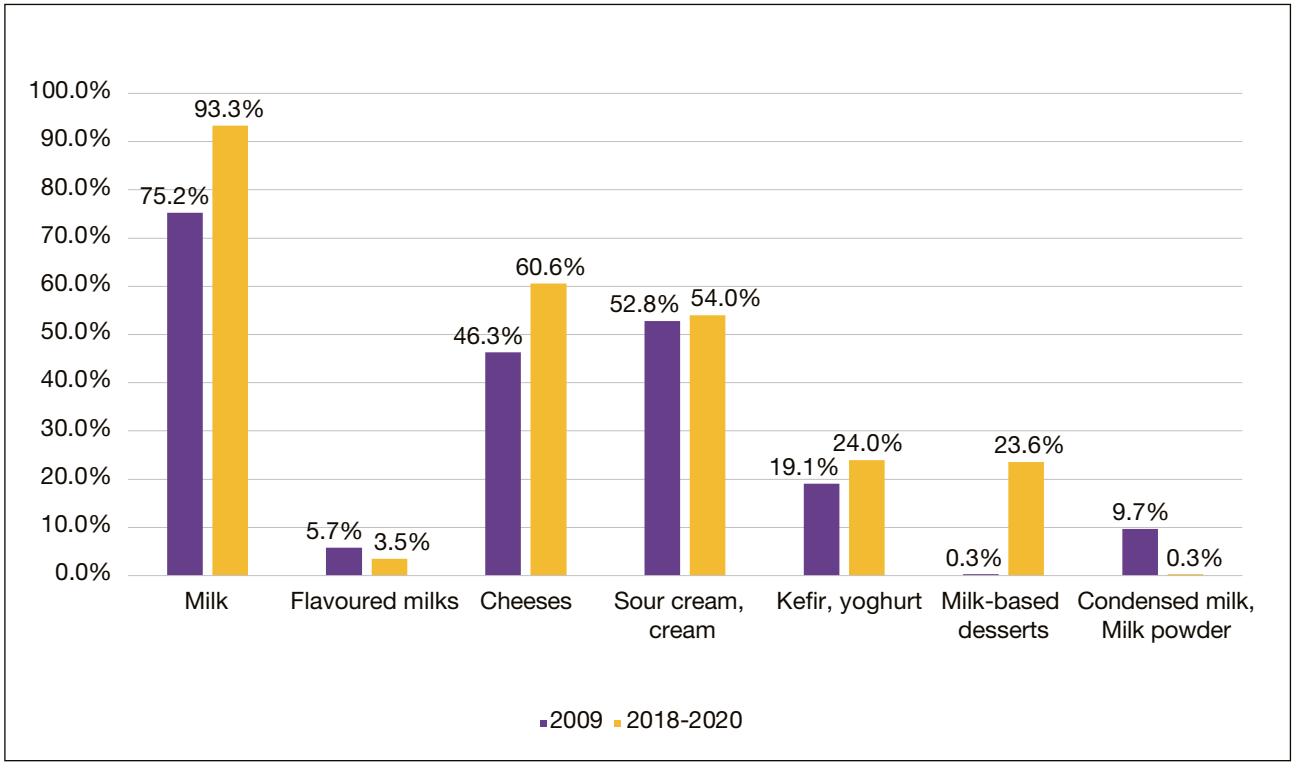


*Figure 3. Proportion of consumption days to total consumption days; changes in consumption frequencies of various food groups based on the results of the 2009 and 2018-2020 food consumption surveys*

Very little data were available on AFM1 concentrations in processed dairy products, so a database of AFM1 processing and enrichment factors for sour milk products (e.g., kefir, yogurt, sour cream) and various cheeses (hard, semi-hard, soft and processed cheeses, fresh cheeses) was compiled on the basis of the latest literature data, and consumer exposure was calculated with the milk equivalent of the consumed quantities of these products.

### *3.3. Aflatoxin concentration data*

AFM1 analytical data are partly derived from NÉBIH's 2011-2020 Hungarian monitoring survey (1,288 data). 40% of the samples contained measurable amounts of AFM1. Most of the measurements were performed by and HPLC methods on samples taken from the milk of dairy farms or private producers and, to a small extent, from commercially available mixed milk. In addition to the large number of items exhibiting contamination below the LOQ (60%), there were also items with very high contamination compared to the average. Values above 100 ng/kg were: 110, 122, 141, 149, 150, 190, 238, 240, 252, 260, 292, 376, 513, 740 and 860 ng/kg, respectively. We were unable to check the correctness of the results, but we saw no reason to omit them either, so the full data set was used in our further calculations. Another 1,177 samples were analyzed by January 2021 within the framework of the joint project of the University of Debrecen and NÉBIH („Determining of the short- and long-term aflatoxin exposure of Hungarian consumers in the dairy product chain and establishing risk management measures"). In the latter case, milk samples taken directly from the transport tankers by the staff of the dairy company at the 9 dairy farms participating in the project were analyzed between 2019 and 2021 by the ELISA method in the laboratory of the Instrument Center of the University of Debrecen. In samples with concentrations above the 20 ng/kg „action level", exact AFM1 concentrations were confirmed by HPLC in the laboratory of NÉBIH. The number of samples with concentrations above the LOQ was 672 (57.1%). In the case of samples with concentrations above 20 ng/kg, the dairy farm was notified and it was recommended that appropriate precautionary measures be taken. As a result of this intervention, it was possible to stop the increase in milk contamination, and the AFM1 contamination of the milk produced was kept below the 50 ng/kg level. Detailed results will be published in the final report of the project.

The number of milk samples examined, broken down by year, is shown in ***Figure 4***.

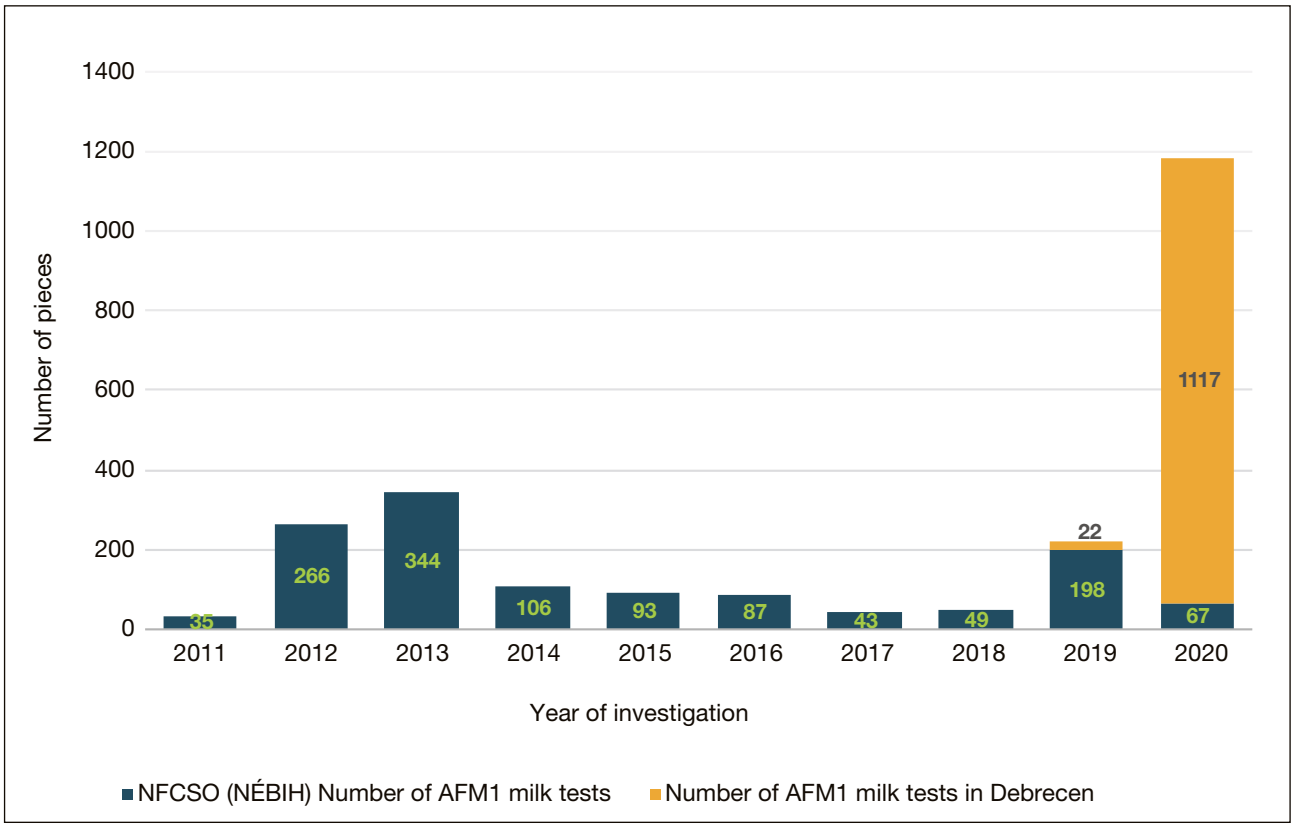


*Figure 4. Yearly test sample numbers from the Hungarian survey of NFCSO (NÉBIH) and from dairy farms participating in the joint project*

To refine our estimate and to compensate for the large number of values below the LOQ, instead of the usual LOQ, LOQ=0 and LOQ/2 approximations, the values of concentration data below the LOQ were also taken into account with the values of data generated with the help of a distribution with an element number identical to that of the number of measurement results. To measurement results above the LOQ, different distributions were fitted using the GAMLSS and GAMLSS.dist packages of the R statistical software using maximum likelihood estimation, then we used the parameters describing the goodness of the fit (AIC – Akaike's Information Criterion, BIC – Bayesian Information Criterion and Global Deviance) to select the distributions that gave the optimal fit. The adequacy of the fits was also evaluated by visual comparison of the histogram made from the data and the distribution obtained, as well as by examining the normality of the differences and using a Q-Q plot. The two best-fit distributions were the two-parameter lognormal (Figure 6) and the four-parameter Box-Cox t-distribution (BCT) (Figure 7), which is suitable for the modeling of slowly decaying, continuously distributed data with positive or negative distortion similar to those of aflatoxins **[68, 69]**. Exposure calculations were performed with a lognormal distribution generated with the assumption of LOQ=5. The selected distributions were then fitted to the entire AFM1 data set, and the evaluation was performed again. Given that a positive change was observed in the parameters describing the goodness of the fit, the distribution chosen were considered to be acceptable.

Descriptive statistics for AFM1 test results and the fitted distribution are summarized below.

*Table 3. Descriptive statistics of AFM1 test results (ng/kg) used for the calculations*

| | DE | NÉBIH | Total | LogLOQ5 |
|---|---|---|---|---|
| Number | 1,177 | 1,176 | 2,465 | 2,465 |
| Minimum | 2.9 | 3 | 2.9 | 0.50 |
| P0.05 | 2.9 | 3 | 2.9 | 1.60 |
| Median | 3.17 | 7 | 4.19 | 3.80 |
| Mean | 6.96 | 15.3 | 10.9 | 9.66 |
| P0.975 | 42 | 68.5 | 56 | 55.4 |
| Maximum | 71.0 | 860 | 860 | 860 |

The relative frequency distributions of NÉBIH and DE test results are shown in ***Figure 5***.

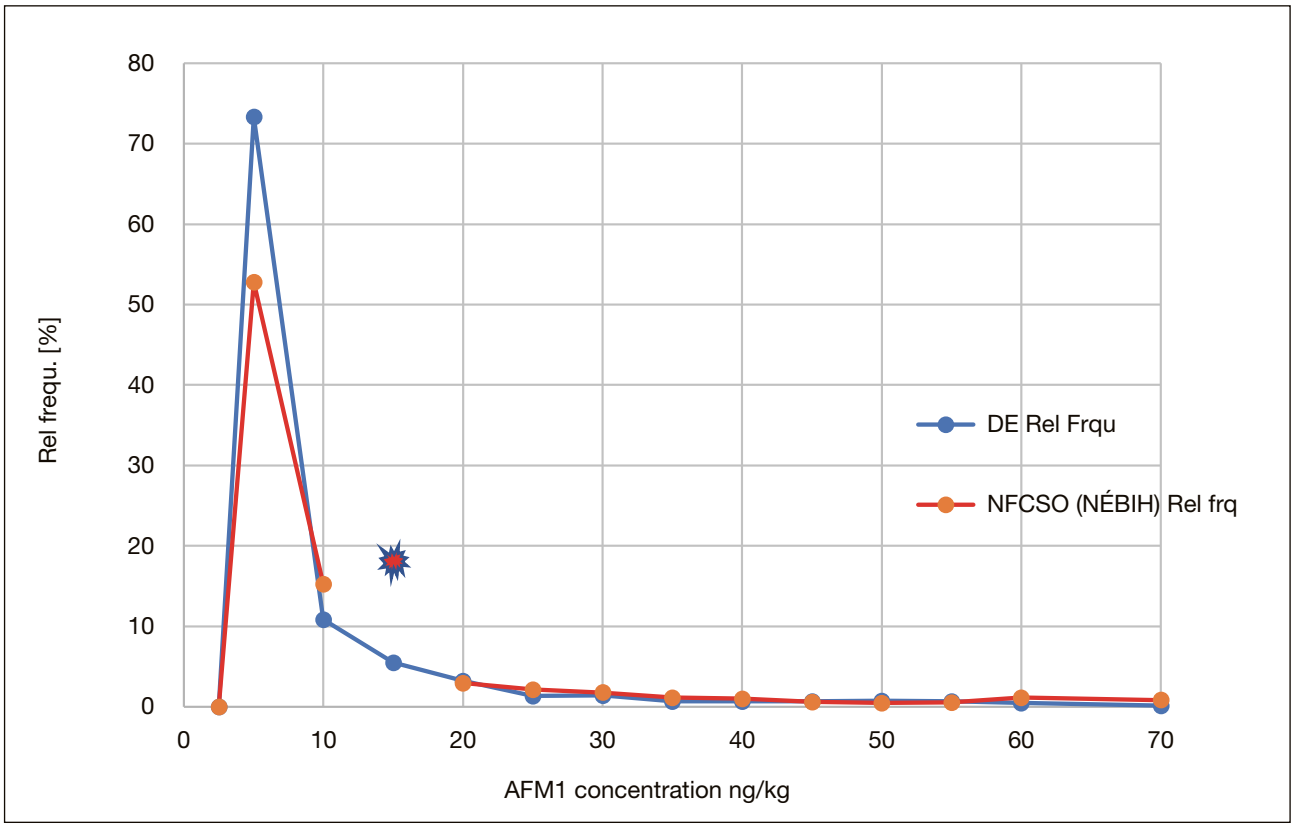


*Figure 5. Relative frequency distribution of the AFM1 contamination of milk samples taken in the NÉBIH monitoring program and in the framework of the DE-NÉBIH cooperation*

With the exception of the outstanding NÉBIH measurement result values (indicated with blue-red asterisk) in the 10-15 ng/kg range, the frequency of AFM1 concentrations in the LOQ-70 ng/kg range was very similar in the two series of measurements and this justifies the joint evaluation of the measurement results. The relative frequency of samples containing AFM1 in concentrations above 70 ng/kg was <0.5% in the NÉBIH study.

A limiting factor in the risk assessment of aflatoxins was the lack of contamination data. According to the recommendation of EFSA **[29]**, food categories for which the number of positive samples does not exceed 25 or for which the proportion of samples below the limit of quantification is greater than 80% should be excluded. In terms of AFM1 results, only the testing of milk met this criterion (***Table 4***), the number of tests for processed dairy products proved to be very small.

*Table 4. Number of samples tested and % of >LOQ values*

| | **Cow's milk** | **Other milk** | **Formula[1]** | **Cheese, cottage cheese** | **Yogurt, kefir** | **Other[2]** |
|---|---|---|---|---|---|---|
| No. of samples | 1288 | 61 | 193 | 30 | 28 | 12 |
| >LOQ [%] | 25 | 16.4 | 0 | 6.7 | 0 | 0 |

*[1]: Formula: infant formula and other baby food*
*[2]: Milk-based food*

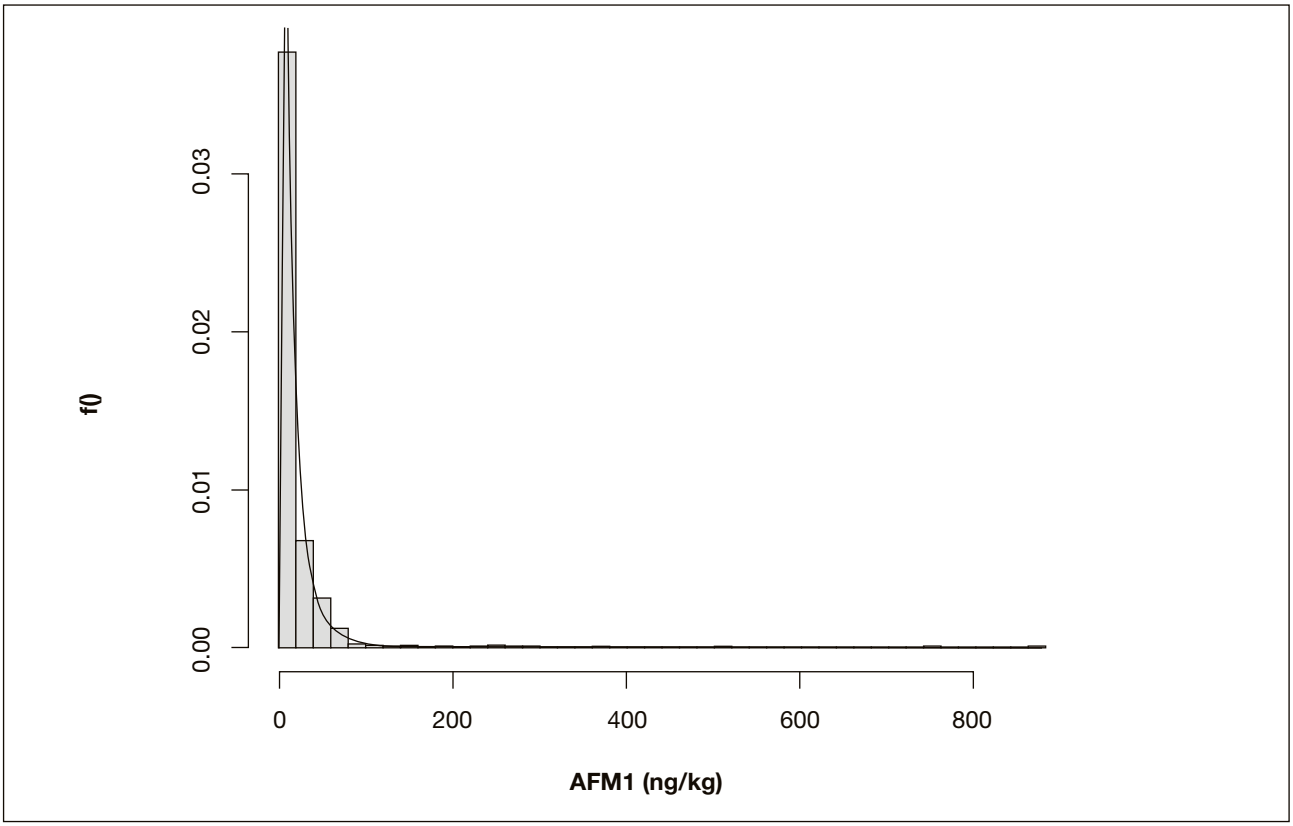


*Figure 6. Lognormal distribution fitted to AFM1 concentration results measured in milk*

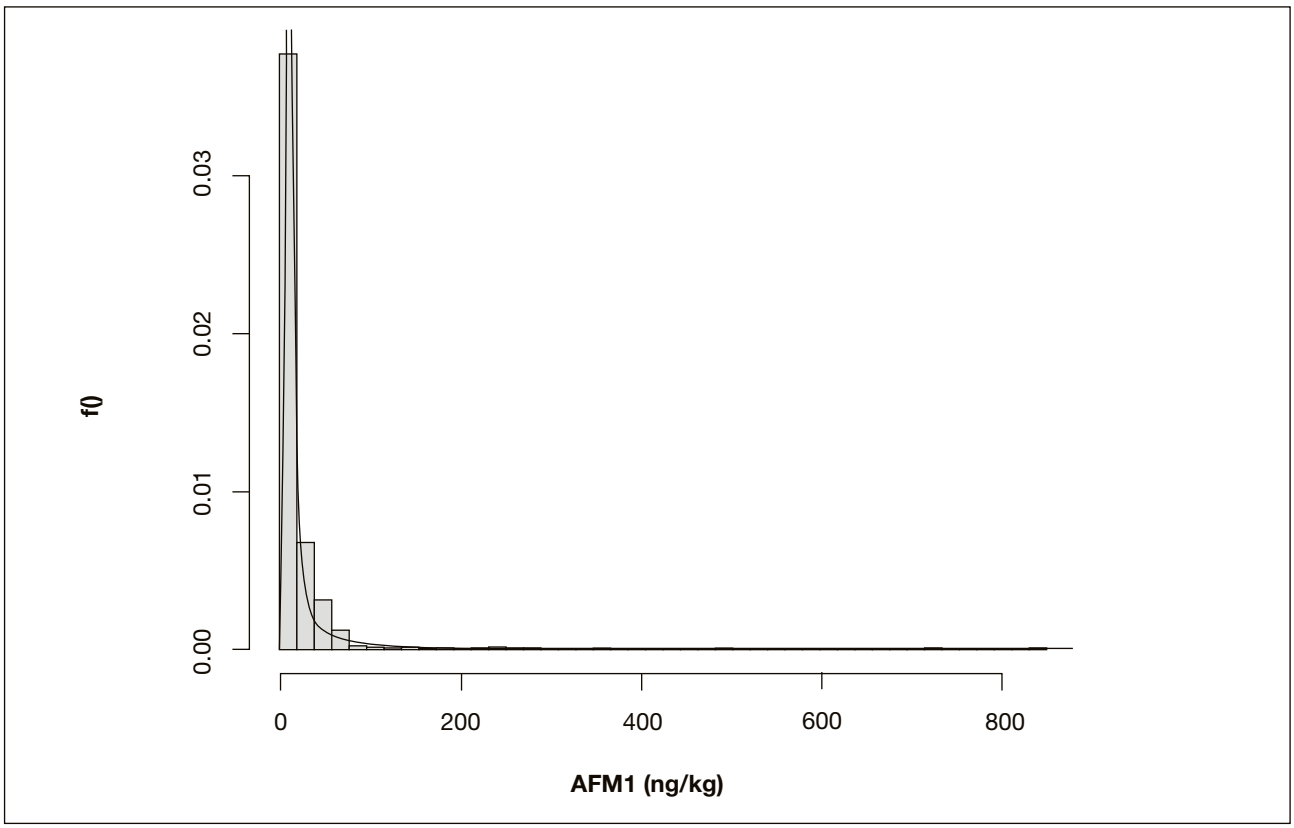


*Figure 7. Box-Cox t-distribution fitted to AFM1 concentration results measured in milk*

**4. Consumer exposure**

In the case of food consumption data, the Observed Individual Means (OIM) method recommended for long-term estimation was used. First, all milk and dairy product consumption data were converted to milk equivalent, using the enrichment and processing factors specific to the given food category (***Equations 2 and 3***).

The intake of foods $e_1$, ..., $e_j$ expressed in g/kg body weight *(B)* on a given consumption day *(n)*, expressed in milk equivalent is

$$B_n = \frac{\sum_{e=1}^{j}(m_e \times F_e)}{ttkg_n} \quad (2),$$

where

$m_e$ is the mass (*g*) of food *e* on consumption day $n_i$,

*F* is the processing (e.g., enrichment) factor characteristic of food *e*,

*kg body weight* is the body weight of the person belonging to the given consumption day,

$$F = \frac{C_{AFM1_e}}{C_{AFM1_{tej}}} \quad (3),$$

where  is the AFM1 concentration in the milk used for the preparation of food *e* and  is the AFM1 concentration in the processed food.

$F_e$ is the value calculated from the  min., med. and max. results obtained in the experiments.

By multiplying the amounts consumed in g/kg body weight/day by the average AFM1 concentration (ng/kg) calculated from the values of the fitted distribution functions, the exposure values for each consumption day were obtained (ng/kg body weight/day). The intake values of the 2 (2018-2020 survey) or 3 (2009 survey) consumption days of the participating persons were averaged. The results were aggregated by consumer age group and consumer exposure was calculated using the data from both food consumption surveys.

First, the effects of the minimum ($F_{min}$), median ($F_{med}$) and maximum ($F_{max}$) values of the processing factors on the result of the exposure estimation were examined. The calculation was performed with the fitted lognormal AFM1 mean data, as well as with the mean ($EDI_{átl}$) and 97.5 percentile ($EDI_{0,975}$) milk consumption values of the 2018-2020 survey. The results are summarized in Table 5. The table illustrates the differences between the mean calculated using the deterministic method and the 97.5 percentile results, based on the 2018-2020 (EU MENU) survey.

*Table 5. Estimated combined daily milk and milk product consumption of the various age groups as a function of dairy product processing factors*

| | Toddlers | Children | Adolescents | Adults | Elderly |
|---|---|---|---|---|---|
| $EDI_{átlag}$ ($F_{min}$) | 0.16 | 0.12 | 0.05 | 0.03 | 0.03 |
| $EDI_{átlag}$ ($F_{med}$) | 0.19 | 0.14 | 0.06 | 0.04 | 0.03 |
| $EDI_{átlag}$ ($F_{max}$) | 0.21 | 0.15 | 0.07 | 0.04 | 0.03 |
| $EDI_{0,975}$ ($F_{min}$) | 0.54 | 0.36 | 0.14 | 0.10 | 0.08 |
| $EDI_{0,975}$ ($F_{med}$) | 0.55 | 0.39 | 0.15 | 0.11 | 0.09 |
| $EDI_{0,975}$ ($F_{max}$) | 0.56 | 0.40 | 0.16 | 0.12 | 0.10 |

Taking into account the minimum-median-maximum values of the processing factors did not notably affect the results. There were significant differences in the mean values of the toddler age group when considering the minimum and median factors, therefore, the values calculated with the median of the processing and enrichment factors are used below to present the different exposure estimation results.

The exposure of the various age groups was calculated based on the P0.05, mean, median P0.975 percentile estimated daily intake values (EDI) of the 2009 food consumption survey, median processing factors and mean AFM1 concentration data. The exposures of the different consumer age groups were compared on the basis of the calculated EDI.

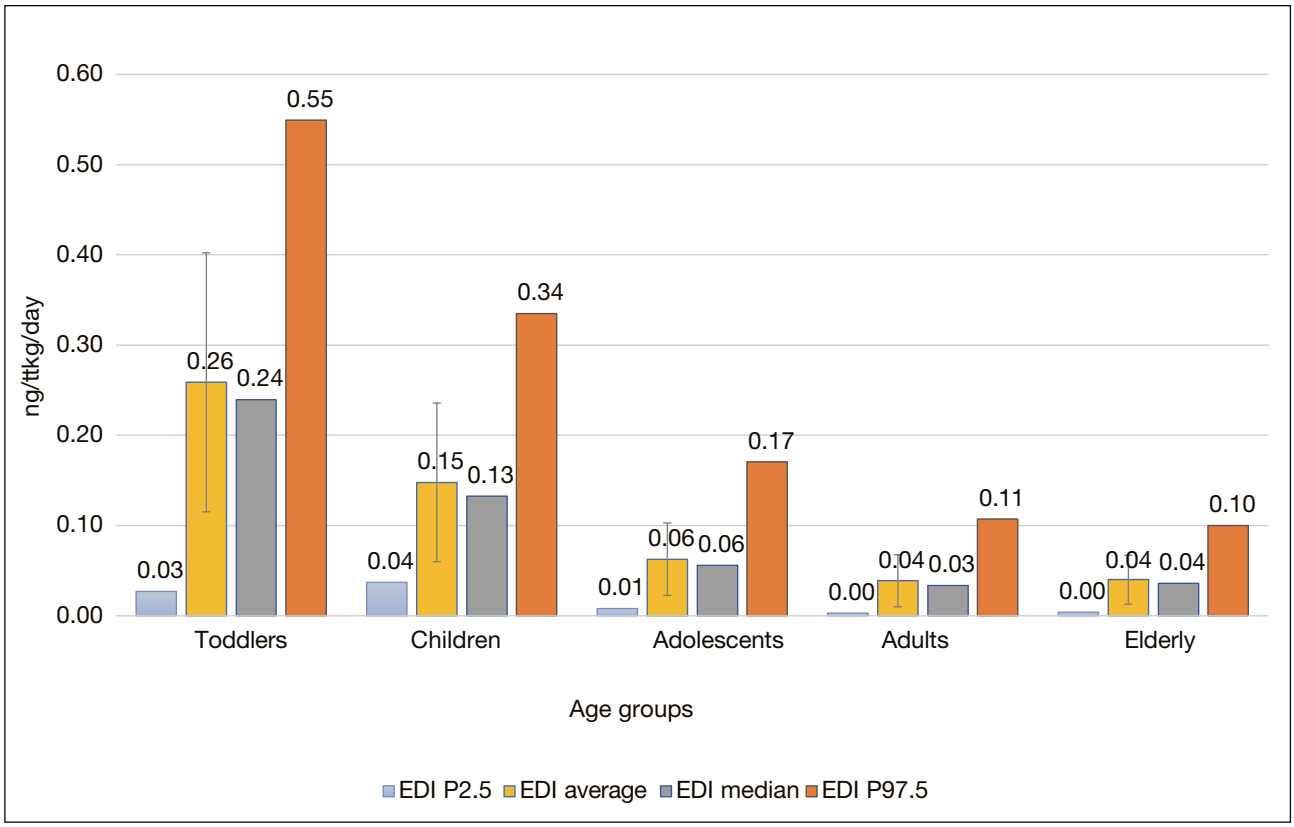


*Figure 8. Estimated daily intakes (ng/kg/day) deterministic estimation; Comparison of the P0.025, mean, median and P0.95 EDI values of the different age groups, based on the 2009 consumption data*

The estimated daily intake values of the age groups of the 2018-2020 survey show a trend similar to that of the 2009 data (***Figure 9***).

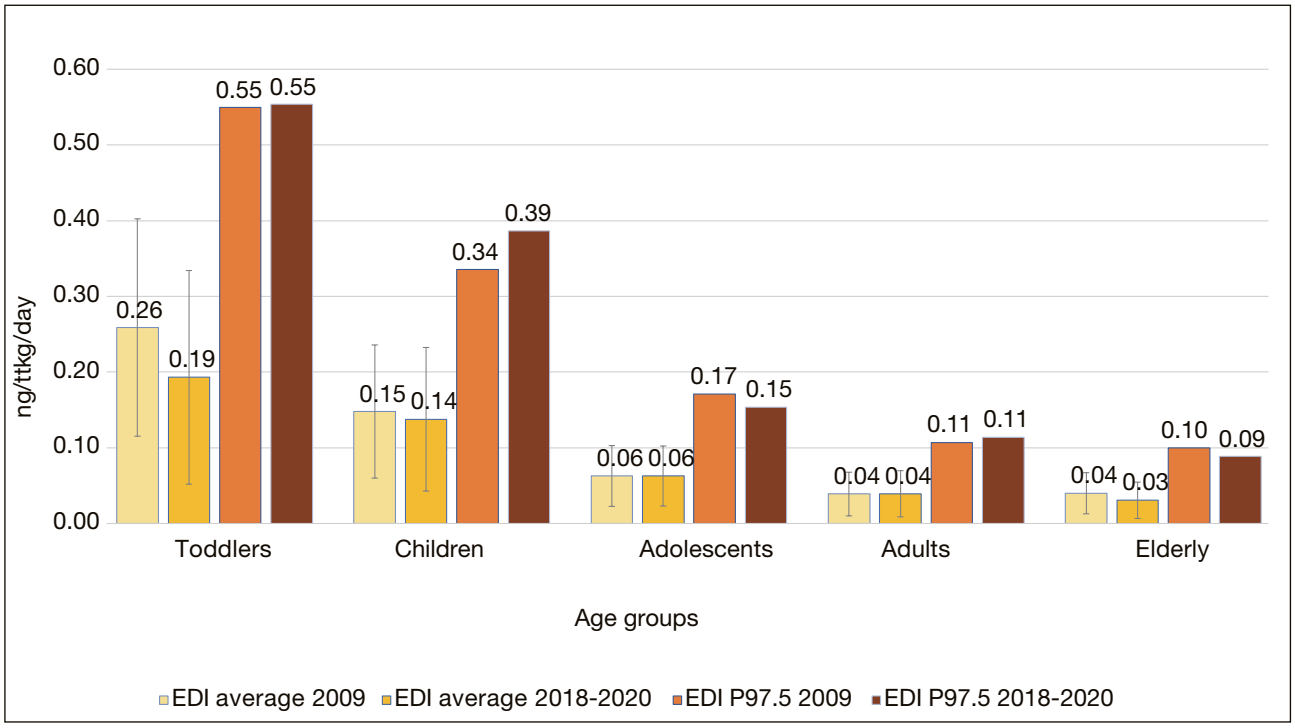


*Figure 9. Estimated daily intakes, deterministic estimation; Comparison of the P0.025, mean, median and P0.95 EDI values of the different age groups, based on the 2009 and 2018-2020 consumption data*

Comparing both the mean and the 97.5 percentile estimated daily intake values, it is clear that the exposure of each age group has been found mostly constant over the past 10 years. The only noticeable differences are in the mean values of the toddler age group and the 97.5 percentile values of the children age group, but the differences are not significant. The number of items in the toddler age group in the 2009 survey is very low (90 people) compared to the 2018-2020 survey (482 people). Values calculated with a smaller number of elements are burdened with a greater uncertainty.

### *4.1. Assessment of consumer exposure*

Based on the obtained exposure values, the Margin of Exposure (MoE) approach (Equation 3), the hazard index (HI) (Equation 4) and the probability increase of liver cancer attributable to AFM1 intake were used to assess the risk of the Hungarian population. For the MoE method, the $BMDL_{10}$ value for AFM1 of 4 µg/kg body weight/day was taken into consideration:

$$MoE = \frac{BMDL_{10}}{EDI} \quad (4).$$

Consumer exposure is considered to be risky if the value of MoE is <10,000. The MoE values of exposure calculated by the deterministic estimation from the consumption data of the 2018-2020 food consumption survey are shown in ***Table 6***.

*Table 6. MoE values of average and large consumers (97.5 percentile) by age group*

| | Toddlers | Children | Adolescents | Adults | Elderly |
|---|---|---|---|---|---|
| MoE EDI mean | 20722 | 29056 | 63779 | 101949 | 130605 |
| MoE EDI P0.975 | 7179 | 10353 | 26059 | 35082 | 45182 |

The health risk threshold (10,000) is reached or approached only by the “large consumers” (97.5 percentile) of the toddler and children age groups. In the case of the other age groups, no significant risk can be identified using this risk characterization methodology.

For the calculation of the hazard index, the safe dose recommended by Kuiper-Goodmann **[59]** was used (0.2 ng/kg body weight):

$$HI = \frac{EDI\ (ng\ ttkg^{-1})}{0{,}2\ ng\ ttkg^{-1}} \quad (5).$$

When calculating the hazard index (HI), the degree of risk is directly proportional to the EDI values and is considered to be of concern when the value is 1 or higher. As an example, the results of deterministic estimates using the consumption data of the 2018-2020 food consumption survey by age group are shown in ***Table 7***.

*Table 7. Hazard indices (HI) calculated from the EDI values of the different age groups*

| | Toddlers | Children | Adolescents | Adults | Elderly |
|---|---|---|---|---|---|
| HI Mean | **1.0** | 0.7 | 0.3 | 0.2 | 0.2 |
| HI P0.975 | **2.8** | **1.9** | 0.8 | 0.6 | 0.4 |

*Note: HI values that pose a health risk are indicated by bold numbers*

HI values calculated from the mean and 97.5 percentile values of the estimated daily intakes of the age groups indicate that the risk from exposure of the adolescent, adult and elderly age groups is not considered to be of concern. However, for toddlers and children, in the case of the 97.5 percentile values (large consumers), exposure is well above levels considered to be safe. One of the most important of the above results is the HI value of 1, characterizing the average intake of toddlers, as it suggests that a significant proportion of this age group is exposed to large amounts of AFM1, which is of great health concern.

Assuming a Hungarian hepatitis B prevalence of 0,7% **[70]**, the incidence of liver cancer associated with aflatoxin was calculated using Equation 1. Calculations were also performed with the mean and upper 95% confidence values for the likelihood of developing liver cancer:

$$Mean\ R_{Mo} = [(0.0269 \times 0.007) + (0.0017 \times 0.993)] \times EDI,$$

$$CI,.95\ R_{Mo} = [(0.0562 \times 0.007) + (0.0049 \times 0.993)] \times EDI.$$

The values of hepatocellular carcinoma (HCCi) attributable to aflatoxin exposure derived from the mean and 97.5 percentile results of AFM1 exposure values calculated from the consumption data of the 2018-2020 food consumption survey by deterministic estimation (DET) (illness/100,000 persons/year) are summarized by age group in ***Table 8***.

*Table 8. Incidence of liver cancer as a function of EDI by age group*

| | Toddlers | Children | Adolescents | Adults | Elderly |
|---|---|---|---|---|---|
| R (EDI mean) | 0.00036 | 0.00026 | 0.00012 | 0.000074 | 0.000057 |
| R (EDI mean, CI0.95) | 0.0010 | 0.00072 | 0.00033 | 0.00021 | 0.00016 |
| R (EDI 0.975) | 0.00083 | 0.00062 | 0.00025 | 0.00018 | 0.00014 |
| R (EDI 0.975, CI0.95) | 0.0023 | 0.0017 | 0.00069 | 0.00050 | 0.00039 |

The risk of developing HCC is increased many times by aflatoxin exposure in the presence of chromic hepatitis B. As the prevalence of hepatitis B is low in Hungary (and in Europe in general), the increase in HCCi induced by aflatoxin is not high either. Although the numerical value of the estimated incidence of liver cancer proved to be very low, their relative values show in this case as well the high risk of “large consumers” of toddlers and children compared to the other age groups.

## 5. Situation assessment, recommendations

Chronic exposure to AFM1 calculated by the deterministic method and compared to various reference values consistently indicates that the exposure of children aged 1<3 is the highest in the studied age groups. The lowest exposure values are observed for the oldest age groups. Due to a lack of data, exposure in infants aged <1 could not be studied. However, the correlation is not directly between age and intake amounts, but between the change (typically increase) in body weight of increasingly older age groups and the intake amounts.

Given that the toxicity of aflatoxins primarily poses a health risk to developing organisms, special attention should be paid to reducing their exposure and keeping it to a minimum. However, it should be emphasized that the presence of carcinogenic compounds should be kept to a minimum in all age groups.

The body is burdened not only with the AFM1 contamination of breast milk and other milks or milk-based products, but also with the AFB1 taken with other foods and which is 10 times more toxic than AFM1. Since their mechanism of action is the same, the effects of aflatoxins and AFM1 add up. Therefore, we need to pay attention to the quality of our food and the storage conditions of products with open packaging. Products with a musty smell and traces of mold should not be consumed, even after cooking or baking. Milks, analyzed during the annual monitoring inspections and exhibiting contamination levels that are 10 to 15 times higher than the maximum tolerable level allowed by the law are also marketed. Particularly at risk are those individuals who regularly consume milk from the same source where the animals are fed aflatoxin-contaminated feed.

To date, there is no routine and industry-wide large scale process that can reliably and completely eliminate the aflatoxin content of foods, so the focus remains on preventing contamination. This is a complex task that requires the involvement of all stakeholders in the food chain, starting with the application of good agricultural practices, and the proper preparation and management of arable land. This is followed by the selection of hybrids resistant to mold, and then a series of measures taken during the harvesting, transport and storage of crops that can prevent the growth of molds (setting appropriate temperature and humidity levels, sorting, peeling and physical treatment of the crops). Last but not least, the appropriate storage and treatment cereals, silage or other processed feed products intended for animal feed, checking their aflatoxin levels and their physical, chemical or biological detoxification, if necessary **[4]**.

The success of prevention and the adequacy of milk shipments can also be checked at the level of dairy farms and dairy plants. With a sampling plan developed for the detection of the aflatoxin M1 content of raw milk and an early warning system, and by applying the 20 ng/kg action threshold already proven to be useful in Italy, an increase in the level of contamination can be predicted effectively. Based on the warning, the dairy farm can prevent the maximum tolerable AFM1 concentration (50 ng/kg) from being reached in accordance with local conditions, for example, by modifying the composition of the feed or by using toxin binders. This can reduce the use of contaminated milk batches in primary and secondary milk processing and, consequently, reduce consumer exposure **[1, 10, 71]**.

It should also be noted that, due to the uncertainty of the detection, ELISA kits set to indicate an AFM1 concentration of 50 ng/kg may still classify batches of milk with contaminations of ≤ 65-70 ng/kg as adequate in 50% of the cases.

In order to protect infants and young children who are most exposed to AFM1 and who are also the most vulnerable, but also to protect the health of the entire population, it is recommended that the regulation of dairy plant inspections is amended in a way that ensures that if the AFM1 contamination of the milk delivered from a farm is ≥20 ng/kg, the plant is obligated to notify the dairy farm and NÉBIH, and thereafter to monitor the effectiveness of the dairy farm measures taken to reduce the contamination by daily monitoring of the contamination of the milk delivered from the farm.

It is also recommended that the warning level used in the self-inspection of dairy farms is set to 20 ng/kg instead of the current 50 ng/kg. ELISA kits for the detection of AFM1 at a concentration of 5-10 ng/kg are available for both dairy plant and producer monitoring, so there is no methodological obstacle to the establishment of a new warning threshold.

## 6. Acknowledgment

The authors would like to thank to Judit Sali and Katalin Csizmadia for providing the relevant data of the 2018-2020 NÉBIH consumption survey, the staff of the DE Instrument Center and NÉBIH for the AFM1 analysis of the milk samples, Attila Nagy and Gabriella Miklós of NÉBIH for their useful suggestions, Béla Béri, who participated in the DE-NÉBIH project, and to the managers and employees of Alföldi Tej and the dairy farms for their cooperation.

Our research program was supported by the National Research, Development and Innovation Fund in the 2018-1.2.1-NKP foundation system with the designation 2018-1.2.1-NKP-2018-00002 (AA, KK).

## 7. References


**[1]** Ambrus Á., Szenczi-Cseh, J., Griff, T., Kerekes K., Miklós G., Szigeti, T., Vásárhelyi, A. (2020): Élelmiszereink mikotoxin és növényvédőszer-maradék szennyezettségének élelmiszerbiztonsági megítélése 2. rész Mikotoxinok; Food safety assessment of the mycotoxin and pesticide residue contamination of our foods, Part 2. Mycotoxins. *Journal of food Investigation*, **LXVI** (2) pp. 2923-2949.

**[2]** Pfliegler, W. P., Pócsi, I., Győri, Z., & Pusztahelyi, T. (2020): The Aspergilli and Their Mycotoxins: Metabolic Interactions With Plants and the Soil Biota. *Frontiers in Microbiology, Vol.* **10** 2908. doi:10.3389/fmicb.2019.02921

**[3]** Ráduly, Z., Szabó, L., Madar, A., Pócsi, I., & Csernoch, L. (2020): Toxicological and Medical Aspects of Aspergillus-Derived Mycotoxins Entering the Feed and Food Chain. *Frontiers in Microbiology* **10**. doi:10.3389/fmicb.2019.02908

**[4]** Peles, F., Sipos, P., Kovács, S., Győri, Z., Pócsi, I., Pusztahelyi, T. (2021): Biological Control and Mitigation of Aflatoxin Contamination in Commodities. *Toxins* **13** (104). https://doi.org/10.3390/toxins13020104

**[5]** Mahato, D.K., Lee, K.E., Kamle, M., Devi, S., Dewangan, K.N., Kumar, P, Kang, S.G. (2019): Aflatoxins in Food and Feed: An Overview on Prevalence, Detection and Control Strategies. *Front. Microbiol. Vol.* **10** (2266) doi: 10.3389/fmicb.2019.02266

**[6]** Filazi, A., Tansel, U. (2019): Occurrence of Aflatoxins in Food (2013): in Mehdi Razzaghi, Abyaneh (Szerk.), *Aflatoxins - Recent Advances and Future Prospects.* doi:10.5772/51031

**[7]** Mikló, G., Angeli, C., Ambrus, Á., Nagy, A., Kardos, V., Zentai, A., Kerekes, K., Farkas, Z., Józwiak, Á., Bartók, T. (2020): Detection of Aflatoxins in Different matrices and Food-Chain Positions. *Front. Microbiol* **11** (1916) doi: 10.3389/fmicb.2020.01916

**[8]** Pócsi, I., Giacometti, F., Ambrus, Á. and Logrieco, A.F. (2020): Editorial: Aspergillus-Derived Mycotoxins in the Feed and Food Chain. *Front. Microbiol* **11** (606108). doi: 10.3389/fmicb.2020.606108

**[9]** Martinez-Miranda, M. M., Rosero-Moreano, M., and Taborda-Ocampo, G. (2019): Occurrence, dietary exposure and risk assessment of aflatoxins in arepa, bread and rice. *Food Control* **98** pp. 359–366. doi: 10.1016/j.foodcont.2018.11.046

**[10]** Serraino, A., Bonilauri, P., Kerekes, K., Farkas, Z., Giacometti, F., Canever, A., Zambrini, A.V., Ambrus, Á. (2019): Occurrence of Aflatoxin M1 in raw milk marketed in Italy: Exposure Assessment and Risk Characterization. *Front. Microbiol*. **10** (2516) doi: 10.3389/fmicb.2019.02516

**[11]** Udovicki, B., Ilija Djekic, I., Eleni P., Kalogianni, E.P., Rajkovic, A. (2019): Exposure assessment and risk characterization of aflatoxin m1 intake through consumption of milk and yoghurt by student population in Serbia and Greece. *Toxins* **11** (4) pp. 205-216. https://doi.org/10.3390/toxins11040205

**[12]** Peles, F., Sipos, P., Győri, Z., Pfliegler, W.P., Giacometti, F., Serraino, A., Pagliuca, G., Gazzotti, T., Pócsi, I. (2019): Adverse Effects, Transformation and Channeling of Aflatoxins Into Food Raw Materials in Livestock. *Front. Microbiol.* **10** (2861) doi: 10.3389/fmicb.2019.02861

**[13]** Campagnollo, F. B., Ganev, K. C., Khaneghah, A. M., Portela, J. B., Cruz, A. G., Granato, D., Corassin, C. H., Oliveira, C. A. F., Sant'Ana, A. S. (2016): The occurrence and effect of unit operations for dairy products processing on the fate of aflatoxin M1: A review. *Food Control* ***68*** pp. 310-329. doi:10.1016/j.foodcont.2016.04.007

**[14]** Wolzak, A., Pearson, A. M., Coleman, T. H. (1986): Aflatoxin carry-over and clearance from tissues of laying hens. *Food and Chemical Toxicology* **24** pp. 37-41. doi: 10.1016/0278-6915(**86**)90262-0

**[15]** Hussain, Z., Khan, M. Z., Khan, A., Javed, I., Saleemi, M. K., Mahmood, S., Asi, M. R. (2010): Residues of aflatoxin B1 in broiler meat: Effect of age and dietary aflatoxin B1 levels. *Food and Chemical Toxicology* **48** pp. 3304-3307. doi: 10.1016/j.fct.2010.08.016

**[16]** Bintvihok, A., Thiengnin, S., Doi, K., & Kumagai, S. (2002): Residues of Aflatoxins in the Liver, Muscle and Eggs of Domestic Fowls. *Journal of Veterinary Medical Science* **64** (11) pp. 1037–1039. doi:10.1292/jvms.64.1037

**[17]** Moran, C. A., Kettunen, H., Yiannikouris, A., Ojanperä, S., Pennala, E., Helander, I. M., & Apajalahti, J. (2013): A dairy cow model to assess aflatoxin transmission from feed into milk – Evaluating efficacy of the mycotoxin binder Mycosorb®. *Journal of Applied Animal Nutrition, 2.* doi:10.1017/jan.2013.12

**[18]** Britzi, M., Friedman, S., Miron, J., Solomon, R., Cuneah, O., Shimshoni, J., Shlosberg, A. (2013): Carry-Over of Aflatoxin B1 to Aflatoxin M1 in High Yielding Israeli Cows in Mid- and Late-Lactation*. Toxins* ***5****(1),* pp. *173–183.* doi:10.3390/toxins5010173

**[19]** Battacone, G., Nudda, A., Palomba, M., Pascale, M., Nicolussi, P., Pulina,G. (2005): Transfer of Aflatoxin B1 from Feed to Milk and from Milk to Curd and Whey in Dairy Sheep Fed Artificially Contaminated Concentrates. *J. Dairy Sci*. **88** (9) pp. 3063–3069. doi: 10.3168/jds.S0022-0302(05)72987-8

**[20]** JECFA (2018): Aflatoxins. In: Safety evaluation of certain contaminants in food: prepared by the eighty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). *FAO JECFA Monographs,* pp. 3-280. SBN (PDF) 978-92-4-069847-5

**[21]** Fakhri, Y., Ghorbani, R., Taghavi, M., Keramati, H., Amanidaz, N., Moradi, B., Nazari, S.H., Shariatifar, N., Khaneghah, A.M. (2019): Concentration and Prevalence of Aflatoxin M1 in Human Breast Milk in Iran: Systematic Review, Meta-Analysis, and Carcinogenic Risk Assessment: A Review. *Journal of Food Protection* **82** (5) pp. 785–795. doi: 10.4315/0362-028X.JFP-18-367

**[22]** Fakhri, Y., Rahmani, J., Oliveira, C.A.F., Franco, L.T., Corassin, C.H., Saba, S., Rafique, J., Khaneghah, A.M. (2019): Aflatoxin M1 in human breast milk: a global systematic review, metaanalysis, and risk assessment study (Monte Carlo simulation). *Trends in Food Science & Technology* **88** (5) pp. 333-342. doi:/10.1016/j.tifs.2019.03.013

**[23]** Radonić, J. R., Kocić Tanackov, S. D., Mihajlović, I. J., Grujić, Z. S., Vojinović Miloradov, M. B., Škrinjar, M. M., & Turk Sekulić, M. M. (2017): Occurrence of aflatoxin M1 in human milk samples in Vojvodina, Serbia: Estimation of average daily intake by babies. *Journal of Environmental Science and Health, Part B* **52** (1) pp. 59-63. doi: 10.1080/03601234.2016.1229454

**[24]** Kunter, I., Hürer, N., Gülcan, H. O., Öztürk, B., Dogan, I., Sahin, G. (2017): Assessment of Aflatoxin M1 and Heavy Metal Levels in Mothers Breast Milk in Famagusta, Cyprus. *Biol Trace Elem Res.* **175** pp. 42-49. doi:10.1007/s12011-016-0750-z

**[25]** Valitutti, F., De Santis, B., Trovato, C.M., Montuori, M., Gatti, S., Oliva, S., Brera, C., Catassi, C. (2018): Assessment of Mycotoxin Exposure in Breastfeeding Mothers with Celiac Disease. *Nutrients* **10** (3) doi:10.3390/nu10030336.

**[26]** Bogalho, F., Duarte, S., Cardoso, M., Almeida, A., Cabeças, R., Lino, C., Pena, A. (2018): Exposure assessment of Portuguese infants to Aflatoxin M1 in breast milk and maternal social-demographical and food consumption determinants, *Food Control* doi: 10.1016/j.foodcont.2018.02.043

**[27]** Csapó, J., Albert, C., Sipos, P. (2020): The aflatoxin content of milk and dairy products as well as breast milk and the possibilities of detoxification. *Acta Universitatis Sapientiae, Alimentaria,* **13** pp. 99-117. doi:10.2478/ausal-2020-0006

**[28]** Trevisani, M., Farkas, Z., Serraino, A., Zambrini, A. V., Pizzamiglio, V., Giacometti, F. Ambrus, A. (2014): Analysis of industry-generated data. Part 1: a baseline for the development of a tool to assist the milk industry in designing sampling plans for controlling aflatoxin M1 in milk. *Food Additives & Contaminants: Part A: Chemistry, Analysis, Control, Exposure & Risk Assessment* **31** (7) pp. 1246-1256. doi: 10.1080/19440049.2014.925587

**[29]** EFSA Panel on Contaminants in the Food Chain (2020): Scientific opinion - Risk assessment of aflatoxins in food. *EFSA Journal* **18** (e06040) pp. 1-112. doi: 10.2903/j.efsa.2020.6040

**[30]** IARC (2012): Aflatoxins. Chemical Agents and Related Occupations. A review of Human Carcinogens. *IARC monographs on the evaluation of carcinogenic risks to humans.*

**[31]** Pascal, A., Risco-Castillo, V., Jouvion, G., Le Barzic, C. and Guillot, J. (2021): Aspergillosis in Wild Birds. *J. Fungi* **7** (3) p. 241; doi:10.3390/jof7030241

**[32]** JECFA, (2001): Aflatoxin M1. In: Safety evaluation of certain mycotoxins in food. *FAO Food and Nutrition Paper* **74** pp. 1-102.

**[33]** WHO (2002): Evaluation of certain mycotoxins in food TRS 906-JECFA 56/8 *WHO technical report series* 906 https://apps.who.int/iris/bitstream/handle/10665/42448/WHO_TRS_906.pdf?sequence=1 (Hozzáférés: 2021.01.28.)

**[34]** Bedard, L. L., Massey, T. E. (2006): Aflatoxin B1-induced DNA damage and its repair. *Cancer Letters,* **241** (2) pp. 174-83. doi: 10.1016/j.canlet.2005.11.018

**[35]** EFSA (2007): Opinion of the scientific panel on contaminants in the food chain (CONTAM) related to the potential increase of consumer health risk by a possible increase of the existing maximum levels for aflatoxins in almonds, hazelnuts and pistachios and derived products. *EFSA Journal* **5** 446. doi: 10.2903/j.efsa.2007.446

**[36]** Williams, J. H., Phillips, T. D., Jolly, P. E., Stiles, J. K., Jolly, C. M. & Aggarwal, D. (2004): Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. *The American Journal of Clinical Nutrition* **80** pp. 1106-1122. doi:10.1093/ajcn/80.5.1106

**[37]** Wang, J. S. & Groopman, J. D. (1999): DNA damage by mycotoxins. *Mutation Research* **424** (1-2) pp. 167-181. doi: 10.1016/s0027-5107(99)00017-2

**[38]** Denning, D. W., Allen, R., Wilkinson, A. P. & Morgan, M. R. (1990): Transplacental transfer of aflatoxin in humans. *Carcinogenesis* **11** (6) pp. 1033-1035. doi: 10.1093/carcin/11.6.1033

**[39]** Serrano-Niño, J. C., Cavazos-Garduño, A., Hernandez-Mendoza, A., Applegate, B., Ferruzzi, M. G., San Martin-González, M. F., García, H. S. (2013): Assessment of probiotic strains ability to reduce the bioaccessibility of aflatoxin M1 in artificially contaminated milk using an in vitro digestive model. *Food Control* **31** (1) pp. 202-207. doi:10.1016/j.foodcont.2012.09.023

**[40]** Bullerman, L. B., Bianchini, A. (2014): Good Food-Processing Techniques: Stability of Mycotoxins in Processed Maize-Based Foods. In*:* LESLIE, J. F. (Szerk.) *Mycotoxin Reduction in Grain Chains.* Ames, Iowa, USA: Wiley Blackwell, John Wiley & Sons, Inc. p. 92-97 ISBN 978-0-8138-2083-5

**[41]** Ali, N. (2019): *Aflatoxins in rice: worldwide occurrence and public health perspectives. Toxicology Reports.* doi:10.1016/j.toxrep.2019.11.007

**[42]** Prandini, A., Tansini, G., Sigolo, S., Filippi, L., Laporta, M., Piva, G. (2009): On the occurrence of aflatoxin M1 in milk and dairy products. *Food and Chemical Toxicology,* **47** (5) pp. 984-991. doi: 10.1016/j.fct.2007.10.005.

**[43]** Yosef, T. A., Al-Julaifi, M. Z., Salah-El-Dein, W. M., Al-Rizqi, A. M. (2013): Assessment of Aflatoxin M1 Residues in Raw Cow Milk at Al- Riyadh Area with Reference to Some Detoxification Applications. *Life Science Journal - Acta Zhengzhou University Overseas Edition,* **10** pp. 3365-3369.

**[44]** Iqbal, S. Z., Jinap, S., Pirouz, A. A. & Faizal, A. R. A. (2015): Aflatoxin M-1 in milk and dairy products, occurrence and recent challenges: A review. *Trends in Food Science and Technology,* **46** pp. 110-119. doi: 10.1016/j.tifs.2015.08.005

**[45]** Kuharic, Z., Jakopovic, Z., Canak, I., Frece, J., Bosnir, J., Pavlek, Z., Ivesic, M., Markov, K. (2018): Removing aflatoxin M1 from milk with native lactic acid bacteria, centrifugation, and filtration. *Archives of Industrial Hygiene and Toxicology* **69** (4) pp. 334-339. doi: 10.2478/aiht-2018-69-3160

**[46]** Foroughi, M., Jamab, M. S., Keramat, J. & Foroughi, M. (2018): Immobilization of Saccharomyces cerevisiae on Perlite Beads for the Decontamination of Aflatoxin M1 in Milk. *Journal of Food Science* **83** (7) pp. 2008-2013. doi:10.1111/1750-3841.14100

**[47]** Mohammadi, H., Mazloomi, S. M., Eskandari, M. H., Aminlari, M., Niakousari, M. (2017): The Effect of Ozone on Aflatoxin M1, Oxidative Stability, Carotenoid Content and the Microbial Count of Milk. *Ozone: Science & Engineering **39**(6)* pp. 447-453. doi:10.1080/01919512.2017.1329647

**[48]** Assaf, J. C., El Khoury, A., Atoui, A., Louka, N., Chokr, A. (2018): A novel technique for aflatoxin M1 detoxification using chitin or treated shrimp shells: in vitro effect of physical and kinetic parameters on the binding stability. *Applied Microbiology & Biotechnology* **102** pp. 6687-6697. doi:10.1007/s00253-018-9124-0

**[49]** Womack, E. D., Sparks, D. L., Brown, A. E. (2016): Aflatoxin M-1 in milk and milk products: a short review. *World Mycotoxin Journal* **9** (2) pp. 305-315. doi:10.3920/WMJ2014.1867

**[50]** Corassin, C. H., Bovo, F., Rosim, R. E., Oliveira, C. A. F. (2013): Efficiency of Saccharomyces cerevisiae and lactic acid bacteria strains to bind aflatoxin M-1 in UHT skim milk. *Food Control,* **31** (1) pp. 80-83. doi:10.1016/j.foodcont.2012.09.033

**[51]** Kamyar, S., Movassaghghazani, M. (2017): Reduction of Aflatoxin M1 in Milk Using Kefir Starter. *Iranian Journal of Toxicology* **11** (6) pp. 27-31. doi:10.29252/arakmu.11.6.27

**[52]** Rad, M. N., Razavilar, V., Anvar, S. A. A., Akbari-Adergani, B. (2018): Selected bio-physical factors affecting the efficiency of Bifidobacterium animalis lactis and Lactobacillus delbrueckii bulgaricus to degrade aflatoxin M-1 in artificially contaminated milk. *Journal of Food Safety,* **38** (4) (e12463) doi:10.1111/jfs.12463

**[53]** Elsanhoty, R. M., Salam, S. A., Ramadan, M. F., Badr, F. H. (2014): Detoxification of aflatoxin M1 in yoghurt using probiotics and lactic acid bacteria. *Food Control* **43** pp. 129-134. doi:10.1016/j.foodcont.2014.03.002

**[54]** Hamilton, D., Murray, B., Ambrus, Á., Baptista, G., Ohlin, B., Kovacicova, J. (1997): Optimum use of available residue data in the estimation of dietary intake of pesticides. *Pure & Applied Chemistry* **69** (6) pp. 1373-1410. doi:/10.1351/pac199769061373

**[55]** Zentai, A., Szeitzné Szabó, M., Mihucz, G., Szeli, N., Szabó, A., Kovács, M. (2019): Occurrence and risk assessment of fumonisin B1 and B2 mycotoxins in maize-based food products in Hungary. *Toxins* **11** (12) p. 709. doi: 10.3390/toxins11120709

**[56]** Zentai, A., Sali, J. , Szabó, I.J., Szeitzné-Szabó, M., Ambrus, Á., Vásárhelyi, A. (2013): Factors affecting the estimated probabilistic acute exposure to captan from apple consumption. *Food Additives & Contaminants: Part A* **30** (5) pp. 833-842. doi:10.1080/19440049.2013.794977

**[57]** Zentai, A., Kerekes, K, Szabó, I., Ambrus, Á. (2015): A fogyasztók növényvédőszermaradékokból származó expozíciójának finomítása, 1. rész. *Élelmiszervizsgálati Közlemények* **LXI** (3) pp. 681-719.

**[58]** EFSA (2010): Management of left-censored data in dietary exposure assessment of chemical substances. *EFSA Journal* **8** (3) p. 1557 doi:10.2903/j.efsa.2010.1557

**[59]** Szenczi-Cseh, J. & Ambrus, A. (2017): Uncertainty of exposure assessment of consumers to pesticide residues derived from food consumed. *Journal of Environmental Science and Health B,* **52** (9) pp. 658-670. doi: 10.1080/03601234.2017.1331671

**[60]** EFSA (2006): Opinion of the Scientific Committee related to Uncertainties in Dietary Exposure Assessment. *EFSA Journal* **438** pp. 1-54. doi:https://doi.org/10.2903/j.efsa.2007.438

**[61]** Delmaar, C., Heinemeyer, G., Jantunen, M., Schneide, K., Schümann, M. (2020): General Aspects of Exposure Evaluation. In: Heinemeyer, G. (Szerk.) *The Practice of Consumer Exposure Assessment.* Gewerbestrasse 11, 6330 Cham, Switzerland: Springer Nature Switzerland AG., pp. 55-155. ISBN 978-3-319-96148-4

**[62]** Gürtler, R. (2020): Hazard Assessment and Derivation of Health-Based Guidance Values. In: Heinemeyer, G. (Szerk.) *The Practice of Consumer Exposure Assessment.* Gewerbestrasse 11, 6330 Cham, Switzerland: Springer Nature Switzerland AG. pp. 253-254. ISBN 978-3-319-96148-4

**[63]** Sieke, C. (2020): Principles of Consumer Exposure Assessment for Pesticide Residues. In: Heinemeyer, G. (Szerk.) *The Practice of Consumer Exposure Assessment.* Gewerbestrasse 11, 6330 Cham, Switzerland: Springer Nature Switzerland AG. pp. 315-322. ISBN 978-3-319-96148-4

**[64]** Kuiper-Goodman, T. (1990): Uncertainties in the risk assessment of three mycotoxins: aflatoxin, ochratoxin, and zearalenone. *Canadian Journal of Physiology and Pharmacology* **68** pp. 1017-1024. doi: 10.1139/y90-155

**[65]** Szeitzne Szabo, M., Bíró, L., Bíró, Gy., Sali, J. (2011): Dietary survey in Hungary, 2009. Part I. Macronutrients, alcohol, caffeine, fibre. *Acta Alimentaria* **40** (1) pp. 142-152. doi: 10.1556/AAlim.40.2011.1.16

**[66]** Csizmadia, K., Larnsak, L., Pfaff, N., Sali, J., (2020): Hungarian national food consumption survey on adults. *EFSA supporting publication* **17** (12)EN-1981. p. 26.

**[67]** Csizmadia, K., Larnsak, L., Pfaff, N., Sali, J. (2020): Hungarian national food consumption survey on toddlers and other children. *EFSA supporting publication* **17** (12)EN-1982. p. 26. doi:10.2903/sp.efsa.2020.EN-1982

**[68]** Ferrari, S.L.P., Fumes, G. (2017): Box–Cox symmetric distributions and applications to nutritional data. *AStA Adv. Stat. Anal.* **101** pp. 321–344. doi:10.1007/s10182-017-0291-6

**[69]** Rigby, R.A., Gillian, M. D. S., Heller, Z., De Bastiani, F. (2019): Continous three parameter distribution on (0,∞). *Distributions for Modelling Location, Scale and Shape: Using GAMLSS in R.* 1 ed.: Chapman and Hall/CRC. ISBN 9780367278847

**[70]** Horváth, G., Gerlei, Zs., Gervain, J., Lengyel, G., Makara, M., Pár, A., Rókusz, L., Szalay, F., Tornai, I., Werling, K., Hunyady, B. (2018): Diagnosis and treatment of chronic hepatitis B and D. National consensus guideline in Hungary from 22 September 2017. *Orvosi Hetilap* **159** (1) pp. 24-37. doi: 10.1556/650.2018.31004

**[71]** Kerekes, K., Bonilauri, P., Serraino, A., Giacometti, F., Piva, S., Zambrini, V., Canever, A., Farkas, Z., Ambrus, A. (2016): An effective self-control strategy for the reduction of aflatoxin M1 content in milk and to decrease the exposure of consumers. *Food Additives and Contaminants Part A Chemistry, Analysis, Control, Exposure & Risk Assessment* **33** (12) pp. 1840-1849. doi: 10.1080/19440049.2016.1241895