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Original Research

Assessment of the Contamination Level by Fumonisins B₁ and B₂ of the Corn Food Products Available on Polish Consumer Market

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Abstract

The aim of the study was to determine the level of contamination by fumonisins of selected corn food products that are available on the market in Silesia. The material covered by the study were samples of corn flour, corn grits, corn flakes, products for infants and young children, popcorn and samples of corn grain. In these samples the total amount of fumonisins as well as separately fumonisin B_1 and B_2 were detected by high performance liquid chromatography (HPLC) using a fluorescence detector prior to the derivatization. Of the 106 surveyed corn foods samples, more than 1/3 (34.9%) were found contaminated by fumonisins B_1 and B_2 ; Fumonisin B_1 was present in 45.3% of samples, and Fumonisin B_2 in 17.0% of the samples. One sample of corn flour was found contaminated by fumonisins on the level of 6342 mg/kg, what makes that the maximum permissible level has been exceeded of more than three times. In other food products, the maximum permissible level of fumonisin was not exceeded. Exposure to mycotoxins in the analyzed corn food products is not a significant health risk to consumers.

Keywords: mycotoxins, corn products, fumonisins

Introduction

Fumonisins are a group of toxic metabolites produced primarily by the filamentous fungi *Fusarium verticillioides* and *Fusarium proliferatum*, which most often attack maize crops worldwide. Taking into consideration the toxicity of fumonisins, the most prominent ones are fumonisin B₁ (FB₁), fumonisin B₂ (FB₂), and fumonisin B₃

(FB₃) [1]. Among 15 forms of fumonisins, which have so far been isolated from the filamentous fungi or food based on corn, fumonisin B₁ is considered the most widespread and most toxic [2]. Fumonisin B₁ was classified by the International Agency of Research on Cancer (IARC) in group 2b as a potential carcinogen in humans [3]. Moreover, fumonisins belong to neurotoxins due to the fact that they can damage the biosynthesis pathway of sphingosine, which is a component of brain and nervous tissue [4]. They cause damage in DNA and induce cell apoptosis [5-6]. Fumonisins are also a cause of kidney

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and intestine damage [7-8] and demonstrate carcinogenic activity [7].

Fumonisins occur worldwide mainly in a grain of maize and products of its processing intended for food and feeding purposes [9]. The contamination of crops with fumonisins depends on the geographical location, in which the plants are cultivated and season or conditions in which the growth and harvest of maize take place, as well as storage [2]. The type of maize grain and such characteristics as types of endosperm, chemical composition, and stage of development may also influence fungal infections and then the production of fumonisins. However, the varieties of maize characterized by vertical corncobs, tight corn shells, thin pericarp of grains, and its increased tendency for dividing may be more susceptible to infections [10]. Furthermore, the cereals grown in tropical and subtropical regions are more susceptible to mycotoxin contamination due to a long and warm growing season [2].

Consumption of forage contaminated with fumonisins by horses causes softening of the brain (leukoenephalomalacia, or LEM), which eventually may lead to death [11-12]. Moreover, liver damage occurs in horses fed with maize contaminated with mould metabolites [12]. Pigs fed with forage that contains the fungus *F. verticillioides* were diagnosed with porcine pulmonary edema [13].

For people in South Africa, Asia, and Central America, where maize is the basis of the diet, exposure to fumonisin is linked to a more frequent prevalence of primary liver cancer and oesophagal cancer [3, 14]. In some countries of Central America exposure to fumonisin is in turn linked to a bigger number of cases of neural tube defect in children [3], since fumonisin B_1 inhibits the absorption of folic acid in various cell lines [14].

Contamination of food and forage with fumonisins poses a serious problem for the health of humans and animals as well as being a significant obstacle in national and international trade. In order to decrease exposure to potential contamination of food and forage with fumonisins, the limits of fumonisin content in food products have been set, which are included in Regulation (EU) No. 1881/2006 of the European Parliament and of the Council of 19 December 2006 setting the maximum allowed levels of some contaminants in food and its subsequent amendments [15]. The Food and Agriculture Organization of the United Nations (FAO) together with the World Health Organization (WHO) have set the maximum tolerable daily consumption limit of fumonisins at 2 µg/kg of body weight daily for FB1, FB2, and FB3 occurring separately, or for the total amount of fumonisins [16].

Aim

The aim of the study was to assess the fumonisin contamination level of maize grain and corn-processed products marketed on the area of the Silesian Region.

Materials and Method

Preparing Samples for FB, and FB, Assay

The material for the study was constituted by food product samples that were collected in trade points in Silesian Voivodship in 2010-14. The analysis comprised 46 samples of maize flour, 13 samples of corn grits, 15 samples of cornflakes, 10 samples of products for babies and small children, 13 samples of popcorn, and nine samples of maize grain. The samples were analyzed in an accredited laboratory of the Voivodship Sanitary and Epidemiological Station in Katowice in the Liquid Chromatography Department.

All samples used in the study were homogenized, which ensured homogeneity. The samples that required crumbling were ground with a laboratory mill, while the homogenous samples underwent dry homogenization by careful mixing up to the moment of obtaining a homogenous aggregate sample.

In order to assay the content of B_1 and B_2 fumonisins in the above-mentioned food products, the methods described in the norm PN-EN 14352:2005 were applied for assaying B_1 and B_2 fumonisins in corn food products using the HPLC method with immune affinity purification and our own modifications.

One-hundred ml of extraction solvent was added to 20 g of homogenized sample (acetonitrile/methanol/water, 25:25:50. v/v/v) shaken for 20 minutes in a shaker, then centrifuged for 10 minutes and filtered through a fluted filter paper. 10 ml of the extract was measured and then 40 ml of PBS was added and then mixed and filtered through a glass fiber filter. 10 ml of the filtered extract was poured into the immunoaffinity column (Fumonitest WB, VICAM), which was then washed with 10 ml of PBS. Fumonisins B_1 and B_2 were eluted with 1.5 ml of methanol. The obtained eluate was evaporated to dryness in a stream of nitrogen at 50°C. The dry residue was dissolved in 200 μ l of acetonitrile-water (50:50, v/v). All samples were analyzed in duplicate.

High-performance Liquid Chromatography (HPLC)

The obtained extracts were analyzed using high-performance liquid chromatography (HPLC) with fluorescent detection after prior derivatization. In order to create derivatives of fumonisins with the automatic control program system, 10 µl of each calibration solution of the fumonisin mixture was mixed with 10 µl of the reagent in order to obtain the OPA/MCE (phthaldialdehyde/2-mercaptoethanol) derivatives, and was poured into the chromatographic column in the volume of 20 µl in the repeatable time of one minute directly before analysis. The FB₁ and FB₂ derivatives were analyzed on the Alliance 2695 liquid chromatograph produced by Waters with a Waters 2474 fluorescent detector with excitation at wavelength 335 and emission at wavelength 44 nm. The conditions of chromatographic analysis were as follows:

Waters Xterra MS C18 4, 6x150 nm chromatographic column with analogical pre-column, mobile phase; mixture of methanol and 1 mol/lof sodium dihydrogenphosphate solution at a ratio of 77:23 by volume brought to pH 3.35 with o-phosphoric acid (V); and flow rate of mobile phase was 1 ml/min, temperature of column thermostat 25°C, temperature of autosampler thermostat 4°C, and dosing volume 20 µl, time of analysis was 15 minutes.

The applied analytical method was checked and validated. The limit of detection, quantification, operating range, linearity, sensitivity, accuracy, repeatability, recovery, and uncertainty were set. The obtained values fulfill the analytical performance criteria determined for analytical methods required in official controls of the level of mycotoxins in food products [17].

Specification of the Analytical Method

In order to determine the linearity of the method a triple calibration curve consisting of four points was prepared: 0.025, 0.063, 0.250, and 0.500 ng/ml. Linearity was determined by the analysis of linear regression and was expressed as linear correlation coefficient r, which for FB₁ amounted to 0.99980 and for FB₂ 0.99989. On the other hand, the coefficient of calibration variation for FB₁ was 2.51, while for FB₂ it amounted to 1.87. The detection limit of the method was 12.5 μ g/kg, while the quantification limit was 25 μ g/kg. The content of fumonisin B₁ and B₂ in samples was calculated from the calibration curve prepared prior to each analysis.

The recovery for corn grits, cornflakes, corn flour, and products for babies and small children (for the level of $100 \mu g/kg$) was performed for 50% of the maximum allowed level (MAL) for a given matrix (Table 1). The recovery for maize grain was checked in the upper range of the method, while products for babies and small children (level $25 \mu g/kg$) were in the lower range of the method.

The sum of fumonisin B_1 and B_2 is the sum of the concentration of those that exceeded the limit of quantification. When the concentration of B_1 or B_2 is between the detection and quantification levels, and

another one is above the limit of quantification, the sum of fumonisin B_1 and B_2 is synonymous with that concentration of fumonisin that exceeds the limit of quantification (Table 2).

Estimating Tolerable Weekly Intake (TWI)

The calculations were based on the average levels of the sum of fumonisins FB₁ and FB₂ found in samples and the maximum concentration (95th percentile), which were multiplied by the daily consumption of corn-based food per capita in Eastern Europe (FAO, 2013) and calculated for average body weight of an adult.

Results and Discussion

The conducted study indicated the presence of toxic metabolites produced by filamentous fungi in products marketed on the Polish consumer market.

Among all 106 analyzed corn food products, 37 samples (34.9%) were contaminated with the total amount of fumonisins B_1 and B_2 . Fumonisin B_1 was assayed in 48 samples (45.3) while B_2 was present in 18 samples (17.0%). In one of the samples the maximum allowed level (MAL) was exceeded (317% MAL). In the other samples the average content of fumonisins B_1 and B_2 , and the sum of fumonisins FB_1 and FB_2 was, respectively: 21-479 μ g/kg, 20-243 μ g/kg, and 39-740 μ g/kg.

In 11 samples the concentration of fumonisin B_1 and in three samples the concentration of fumonisins B_2 were assayed at levels between the detection and quantification limits (i.e., 12.5-25.0 µg/kg). The highest average concentration of total fumonisins B_1 and B_2 amounting to 740 µg/kg, was assayed in corn flour. The average level of contamination of maize grain for total amount of fumonisins B_1 and B_2 amounted to 614 µg/kg. Moreover, for the samples of popcorn the average content of total amount of fumonisins amounted to 222 µg/kg. However, the samples of cornflakes and corn grits samples showed

Tab	le 1	. F	Recovery	and	repeat	abı	lity	for se	lected	l matrices.	
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Matrix	I1 (/1)	Recovery (%)	RSD* (%)	Recovery (%)	RSD (%)
Matrix	Level (μg/kg)	FE	31	FB	2
Corn flour	600	-	-	86.2	13
Com nour	1,400	97.3	6.38	-	-
Corn, grain	4,000	92.2	4.4	81.6	4.8
Corn grits	700	104.0	12.6	93.6	17.5
Cornflakes	400	95.6	13.1	86.0	13.6
Products for infants and	25	92.7	5.0	96.0	6.5
young children	100	98.5	5.2	88.3	12.0

^{*}RSD: relative standard deviation

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	Number	Num	unber of contar nated samples	Number of contaminated samples		<lod** (µg="" kg)<="" th=""><th>D** kg)</th><th>T</th><th>OD – LC (µg/kg)</th><th>LOD – LOQ (µg/kg)</th><th></th><th>> LOQ*** (µg/kg)</th><th>*** (g)</th><th>>MAL* (µg/kg)</th><th></th><th>X_a**** (μg/kg)</th><th>*** kg)</th><th>2</th><th>Min. content (μg/kg)</th><th>ntent :g)</th><th>M</th><th>Max. content (µg/kg)</th><th>ent (</th><th>MAL* (µg/kg)</th></lod**>	D** kg)	T	OD – LC (µg/kg)	LOD – LOQ (µg/kg)		> LOQ*** (µg/kg)	*** (g)	>MAL* (µg/kg)		X _a **** (μg/kg)	*** kg)	2	Min. content (μg/kg)	ntent :g)	M	Max. content (µg/kg)	ent (MAL* (µg/kg)
Matrix	of test samples	FB_1	FB_2	Sum of FB ₁ and FB ₂	$\overline{\mathrm{FB}_{_{1}}}$	FB_2	of test samples FB ₁ FB ₂ FB ₃ and FB ₁ FB ₂ FB ₂ and FB ₃ FB ₂ FB ₂	FB	FB_2	Sum of FB, and FB2	FB	FB_2	Sum of FB ₁ and FB ₂	FB ₂ FB ₃ and FB ₁ FB ₂ FB ₂ and FB ₃ FB ₂ FB ₂ FB ₃	$\mathbf{FB}_{_{1}}$	FB_2	Sum of FB ₁ and FB ₂	FB_1	FB2		\mathbf{FB}_{1}	FB_2	Sum of Sum of FB ₁ and FB ₂ FB ₂	Sum of FB ₁ and FB ₂
Corn	6	9	3	5	3	9	4	-	0	1	5	3	5	0	334	172	614	23	61	29	1,400	392	1,792	4,000
Popcorn	13	8	2	8	5	11	5	0	0	0	8	2	8	0	185	148	222	25	121	25	588	175	763	1,000
Cornflakes	15	5	2	4	10	13	111	-	2	1	4	0	4	0	64	20	75	23	16	28	132	23	132	800
Products for infants and young children	10	0	0	0	0	0	0	0	0	0	0	0	0	0	ı	1	ı	1	1	1	ı	ı	ı	200
Corn grits	13	5	0	1	8	13	12	4	0	4	-	0	1	0	21	ı	39	13		39	39	0	39	1,400
Corn flour	46	24	11	19	22	35	27	5	1	5	19	10	61	1	479	479 243	740	13	16	29	5,098 1,244	1,244	6,342	2,000
*MAL by Commission Regulation (EC) No. 1881/2006 of 19 December 2006 as amended setting maximum levels for certain contaminants in foodstuffs.	nmission	Regui	lation (EC) No.	1881/	2006	of 19 Dece	mber	2006	as amende	d sett	ing m.	aximum le	evels for ce	rtain	conta	minants ir) food	stuffs.					

Table 2. Contents of fumonisin B, and B, and their sums in the tested foods.

****x..: The average concentration value taking into account the results above LOD **LOD: Limit of detection; ***LOQ: Limit of quantitation;

the average content of total amount of FB_1 and FB_2 , respectively, at the levels of 75 μ g/kg and 39 μ g/kg.

Current EU regulations allow for the concentration of total B₁ and B₂ fumonisins in unprocessed maize intended for the first degree of processing at the level of 4,000 µg/kg [15]. In five out of nine samples of maize grain (55,6%) the average content of total fumonisins ranged between 29-1,792 µg/kg, and in none of these trials was the maximum allowed level exceeded (Table 2). B, was found in six out of nine analyzed products (66.7%) while B₂ was assayed in three samples (33.3%). The maximum concentrations of B₁ and B₂ assayed in maize grains amounted to 1,400 µg/kg and 392 µg/kg, respectively, while the average content of assayed mycotoxins was 334 µg/kg for FB, and 172 µg/kg for FB, Table 2. Shepard et al. assayed FB₁, FB₂, and FB₃ in 11 samples of maize intended for animal feed and in eight samples of corn cobs intended for human consumption. All samples analyzed by Shephard were contaminated with mycotoxins. In corn intended for forage the concentration of FB, was assayed at the level of 1,270-3,980 µg/kg, the concentration of FB, at the level of 190-1,180 FB₂µg/kg, and the concentration of FB₂ at the level of 150-960 µg/kg. The total amount of fumonisins ranged from 1,625 to 6,115 µg/kg with a recommendation in horse forages of maximum allowed level of fumonisins lower than 5,000 µg/kg [18]. The study conducted by Klaric et al. indicated on a high level the sum of fumonisins B₁, B₂, and B₃ in maize samples. The highest levels of fumonisins were found in the range 200 µg/kg to 20,700 µg/kg [19]. Corn cobs intended for human consumption were infected with fumonisin B₁ at the level of 10-590 µg/kg, while fumonisin B, and B, were at the level of 5 to 80 μ g/kg [18].

The results presented by Queiroz et al. indicate contamination of all analyzed samples (40/40) with the total amount of fumonisins in the concentration range between 230-6,450 μ g/kg, which in turn implies that the exceedance of the maximum allowed level was noted. Although in most samples concentration of fumonisins were below 4,000 μ g/kg, the acceptable European Communities limit for unprocessed, 33 of the 40 maize samples analysed (82.5%) were contaminated at levels above 1,000 μ g/kg [20].

In all the maize grain samples (12/12) analyzed by Krnjaja et al., B₁ ranged from 880 to 2,950 μg/kg [21]. Another study reported that 39.7% of all maize samples were contaminated with fumonisins, both FB₁ and FB₂. The contamination levels of fumonisins in all maize samples ranged as follows: the maximum levels were 15,221 μg/kg, 7,141 μg/kg, and 22,362 μg/kg, and the mean levels were 340 μg/kg, 157 μg/kg, and 497 μg/kg for FB₁, FB₂ and total fumonisins, respectively [22]. The results obtained by Chilaka et al. showed that fumonisins were the most dominant mycotoxins occurring at high levels and incidence rates in all food types, especially in maize samples. From the total of 136 samples, B₁ was detected in 88 samples (65%) and B₂ was detected in 73 samples (54%). The highest assayed concentration

of B_1 amounted to 8,222 µg/kg, while fumonisin B_2 was 2,885 µg/kg [23].

 B_1 and B_2 were detected in 61.5% (8/13) and 15.4% (2/13) of the popcorn samples analysed. A similar level of concentration is observed in other studies, where 88.2% of popcorn samples were contaminated by fumonisins, ranging from 89 to 1,170 μ g/kg (B_1) and 57 to 211 μ g/kg (B_2), respectively [24].

The maximum allowed level of total fumonisins for cornflakes, which equals 800 µg/kg, was not exceeded in any of the 15 analyzed samples, while the content of fumonisin was assayed in four samples (26.7%) in the range between 28 and 132 µg/kg. In 5 samples FB, was assayed at an average level of 64 µg/kg, while FB, was found in two samples at an average level of 20 μg/ kg (Table 2). The analysis conducted by the Cano-Sancho et al. showed contamination sum of fumonisins in 28 of 72 samples (39%). The average level of fumonisin contamination in samples of cornflakes was 78.9 ug/kg [25]. In other studies fumonisins were present in 50% of cornflake samples (2/4), and the mean value was 30.5 µg/kg [26]. In Martin's et al. studies, cornflakes showed significantly higher contamination by fumonisins, where the average values of B₁ and B₂ were 341 µg/kg and 87 μg/kg, respectively [24]. Bryła et al. analyzed 19 samples of cornflakes, from which nine were contaminated by B_1 and B_2 in the range of 13 to 248 μ g/kg [27].

In none of the samples from the products intended for consumption for babies and small children were fumonisins detected (Table 2). However, other studies have shown contamination by fumonisins 7 with 30 samples of baby food (23.3%), where the average concentration of fumonisin was 36.4 µg/kg [25]. Girolamo et al. showed the presence of fumonisins in six out of 19 maize-based baby foods at levels up to 53 µg/kg [28]. In the case of maize milling fractions and the products of maize milling of 500 microns in size (not intended for direct consumption), the maximum concentration was set at the level of 1,400 μg/kg. Only in one out of 13 samples of corn grits (7.7%) was the total amount of fumonisin assayed at the level of 39 μg/kg. B, was assayed in five products at an average level amounting to 21 µg/kg. In four out of five samples in which B, was assayed, the concentration level ranged between the detection and quantification limits. The analyzed samples of corn grits were free from contamination with B₂ (Table 2). Martins et al. observed fumonisins in 19 samples of corn grits (67.9%), where high levels of B₁ and B₂ were found in concentrations ranging from 91 to 3,462 μg/ kg and from 45 to 886 μg/kg, respectively [24].

For maize milling fraction and the products of maize milling of a size below 500 microns not intended for direct consumption, the maximum allowed level amounts to 2,000 μ g/kg. Among 46 samples of corn flour, 19 were found with total B_1 and B_2 and 24 samples contained B_1 , while 11 contained B_2 . In one sample of corn flour the concentration of the analyzed mycotoxin was assayed at 6,342 μ g/kg, which indicates a threefold exceedance of the maximum allowed level. In the other samples of corn flour, the sum of fumonisin was assayed in the range of

29-1,302 μg/kg. The average content of fumonisin amounted to 479 µg/kg for FB₁ and 243 µg/kg for FB₂, respectively (Table 2). The results obtained by Esposito et al. showed a high percentage of positive samples for FB₁, FB₂, and FB₃ (85%). The highest concentration was recorded for the total of fumonisins for cornmeal and savory snacks, amounting to 961 µg/kg and 439 µg/kg, respectively [29]. Similarly, the study conducted by Brera et al. also showed the highest contamination in snacks (761 µg/kg) and flour (544 μg/kg) [30]. Martins et al. found 73.3% of corn flour samples where the levels of positive samples FB, and FB, ranged from 128 to 555 µg/kg and from 56 to 235 µg/kg, respectively [24]. However, in other studies samples of maize flour were contaminated by a sum of fumonisins in the range 13 to 1,688 µg/kg [27].

The studies also showed that 64% of samples of maize snacks (nine out of 14) were highly contaminated with fumonisins B₁, B₂, and B₃ (13-3,297 µg/kg). However, in 12 out of 14 samples of noodles (86%) we detected a sum of fumonisin at an average level of 175 µg/kg [27]. In the study conducted by Mruczyk et al. we found the presence of fumunisin in four out of seven analyzed samples. In four samples of cereal, preparations did not exceed the maximum allowed level and fell within the range of 25% of MAL. However, in the other three samples the content of mycotoxins did not exceed the limit of quantification of the method [31]. The low levels of fumonisins were detected in rice and sweet snacks, where concentrations of fumonisins were 31 µg/kg and 77 µg/kg, respectively [29]. Rubert et al. analyzed a total of 1,250 samples including 716 and 534 organic and non-organic products, respectively. B, was detected in 11.4% of organic and 3.6% of conventional samples. The obtained concentration for FB₁ ranged from 20.2 to 1,201.7 μg/kg for both organic and conventional cereals products. B2 was identified in 11.3% and 3.4% of the organic and conventional samples, respectively, at levels ranging from 21.7 to 1,010.5 µg/kg. The highest concentration of fumonisins was found in gofio organic samples of corn and wheat, amounting to 1,201.7 µg/kg and 1,001.4 µg/kg, respectively [32].

For the assessment of health risk from exposure to mycotoxins in corn food products available in the Polish consumer market the amount of mycotoxins taken in with these products was estimated and compared with the tolerable weekly intake (TWI) [16]. It was assumed that the average body weight of an adult consumer is 60 kg, and the average consumption of maize products in Eastern Europe is 18.05 g/capita/day (FAO, 2013) [33]. According to our study the average level of the sum of fumonisins FB₁ and FB₂ and the maximum concentration (95th percentile) in the analyzed samples were 176.2 and 890.0 µg/kg, respectively, representing 2.6% and 13.3% of TWI.

The most recent comprehensive risk assessment on health effects related to the presence of fumonisins in food was presented by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2014. The evaluation was

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considering the exceptional situation of high contamination of maize and maize-based products. The dataset used for the exposure assessment of fumonisins contained 3,654 analytical results obtained on food samples that were reported by 11 European countries and collected between 2000 and 2010. The calculated exposure to fumonisins showed that mean lower bound and upper bound exposure across age class and surveys was lower than the provisional maximum tolerable daily intake (PMTDI), whereas the 95th percentile exposure in toddlers and other children was above the PMTDI in several surveys [34].

In conclusion, the analyzed food products, except for the products for infants and small children, were characterized by contamination with fumonisins at different levels. The obtained results together with many bibliographical references indicate that there is a risk of exposure of general population to fumonisins by consumption of food products prepared on the basis of maize. Therefore, it is necessary to constantly monitor the corn products being marketed on the consumer market for contamination with mycotoxins.

Conclusions

- The analyzed food products, excluding products for babies and small children, show contamination by fumonisins
- Threefold exceedance of the maximum allowed level was observed in the sample of corn flour; in the other samples no exceedance of MAL was found.
- Due to contamination of food products with fumonisins it is recommended to constantly monitor the presence of mycotoxins in food.
- Exposure to mycotoxins in the analyzed corn food products is not a significant health risk to consumers.

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