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Risk assessment of aflatoxins in food products consumed in South Korea

Hyang Sook Chun[1]*, Hyun Ee Ok[1], Hyun Jung Kim[1], Jin-Bong Hwang[1], Duck-Hwa Chung[2]

[1]Korea Food Research Institute, Sungnam 463-746, Korea, [2]Gyeongsang National University, Chinju 660-701, Korea

Correspondence: Hyang Sook Chun, E-mail: hschun@kfri.re.kr

Tel.: +82-31-780-9273; fax: +82-31-709-9876.

Abstract

A total of 694 food samples for aflatoxin B_1 (AFB_1) were assessed using a monitoring scheme consisting of enzyme linked immunosorbent assay (ELISA) for rapid screening and high performance liquid chromatography (HPLC) for quantification. One hundred-four out of 694 samples were found to give positive ELISA readings and were further determined by HPLC analysis. Thirty-two samples including 2 maizes, 3 soybean products, 20 nuts and nut products and 7 spices were found to be contaminated with AFB_1 (4.6% of incidence), ranging in various levels up to 48.6 μg kg^{-1}. The contamination levels of AFB_1 in 28 out of 32 contaminated samples were below 10 μg kg^{-1} of AFB_1, which is the legal tolerance limit in Korea. Based on the daily food consumption data, estimated exposure dose of AFB_1 was 6.42×10^{-7} mg kg^{-1} bw day^{-1}. Furthermore, estimated excess cancer risk values to liver cancer incidence by ingestion of these foods

for AFB_1 were calculated to be 5.78×10^{-6} for individuals negative for hepatitis B and 1.48×10^{-4} mg kg^{-1} bw day^{-1} for individuals positive for hepatitis B.

Key words: aflatoxin B_1 (AFB_1), risk assessment, dietary exposure, Korean, food consumption

Introduction

Aflatoxins are a group of structurally related toxic metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus* (Eaton and Groopman, 1994). Among aflatoxins, AFB_1 is the most frequent metabolite present in contaminated foods and is classified as a human carcinogen. Generally, aflatoxin B_2 (AFB_2), G_1 (AFG_1) and G_2 (AFG_2) are not reported in the absence of AFB_1 and are classified as possible carcinogens to humans (IARC 1993, JECFA 1998). Epidemiological studies have also suggested that aflatoxins might be associated with human liver cancer and acute hepatitis (Li et al. 2001).

Because of potential health hazards to humans, regulatory levels have been recently documented. The ranges of worldwide regulations for AFB_1 and total aflatoxins were from 0 to 30 μg kg^{-1} and from 0 to 50 μg kg^{-1}, respectively (FAO 1997). In the European Union, AFB_1 and aflatoxins levels in human commodities are regulated with maximum residue levels (MRLs) that cannot be greater than 2 and 4 μg kg^{-1}, respectively (EEC 1998). Recently, the Codex Alimentarius Commission, Joint FAO/WHO Food Standards Program adopted a limit of 15 μg kg^{-1} for aflatoxins (Codex 2001). In Korea, a residue limit of 10 μg kg^{-1} AFB_1 for foodstuffs has been established since 1989 (KFDA 2000).

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A review of monitoring studies on the aflatoxins occurrence in food products has demonstrated that aflatoxins are still being found frequently in food products at levels that are of significant concern for consumer protection (Scott and Lawrence 1997, Stroka and Anklam 2002). Recently, a study on the daily exposure of Koreans to AFB_1 through food consumption revealed that the calculated probable daily intake (PDI) of AFB_1 for Koreans ranged from 1.19 to 5.79 ng kg^{-1} bw day^{-1} (Park et al. 2004). This exceeds the estimated provisional maximum tolerable daily intakes, 0.4 ng kg^{-1} bw day^{-1} for adults with hepatitis B or 1.0 ng kg^{-1} bw day^{-1} for adults and children without hepatitis B (Kuiper-Goodman 1998). However, the PDI of AFB_1 for Koreans was estimated from the AFB_1 levels in barley, barley-based foods, corn, corn-based foods, fermented soybean products and rice. Levels of aflatoxins in nuts, spices and their products were not reflected even though they are the most important dietary sources of aflatoxins.

ELISA analysis is convenient for simultaneous determination of contaminants in a large number of samples with relatively low cost and short time. However, it is not suitable for quantification of contaminants since it can be influenced by matrix effect of samples and has the possibility to overestimate the contaminants at very low concentration. Therefore, a monitoring scheme, which consists of ELISA for screening of possible contaminated samples, HPLC for quantification of contaminated levels is considered to be useful to assess the existence and level of aflatoxins.

Therefore, in the present study, the natural occurrence of aflatoxins in foodstuffs from South Korea was determined using a monitoring scheme consisting of enzyme linked immunosorbent assay (ELISA) for rapid screening and high performance liquid chromatography (HPLC) for quantification. Furthermore, we attempted to perform a risk analysis on AFB_1, based on dietary exposure to AFB_1 and its potency in induction of liver

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cancer.

Materials and methods

Samples

Samples were randomly selected from a variety of grocery markets in six different cities in Korea, between May 2004 and June 2005. Six cities including Seoul, Daejeon, Gwangju, Gangneung, Daegu and Busan were chosen considering demographic and regional viewpoints. In consideration of consumption frequency, twenty food products were selected in six food groups, major dietary components of human consumption in South Korea, based on data available at the Ministry of Health and Welfare (KMOHW 2002). Aflatoxin B_1 was determined in 694 samples, which were made up of 431 samples of cereals and their products, 123 samples of soybean and their products, 119 samples of nuts and their products, and 21 samples of spices. A minimum sample size of 2.5 kg or 2 L was purchased, and delivered to the laboratory within 24 h of collection. The samples were stored under cool conditions until analysis. All the samples were finely grinded with a blender till it passes through No. 20 sieve or become paste, and were kept at -18°C in zipper bags to be subsampled before analysis. Among collected samples, pistachios were dehulled prior to sample preparation.

Chemicals and reagents

Acetone, ethanol, diethyl ether, hexane, methanol and chloroform were supplied by Junsei (Tokyo, Japan). Acetonitrile, HPLC-grade water was obtained from Burdick & Jackson (Muskegon, MI). The AFB_1-horseradish peroxidase conjugate, hydrogen peroxide and 2,2-azinobis (3-ethylbenzthiazoline-6-sulfonate) (ABTS) were purchased from Sigma (St. Louis, MO). Stock standard solutions of AFB_1 with concentrations of

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500 μg ml^{-1} were prepared in benzene-acetonitrile (98+2, v/v), wrapped in aluminum foil to prevent gradual break down of aflatoxins under UV light and kept under protected conditions at –20 °C. All other inorganic chemicals and organic solvents were of the reagent grade or higher.

Aflatoxin B_1 analysis

For ELISA analysis of AFB_1, the powder-type samples were extracted as follows: a 20 g sample was extracted with 100 ml of 60% methanol and 5 g of NaCl for 20 min with shaking. After extraction, the sample was centrifuged at 3,000 rpm for 5 min and the supernatant was filtered with filter paper (Whatman No. 1) and glass microfibre filter pretreated by 1 ml of 100 % methanol. For butter-type samples, a 10 g sample was extracted with 50 ml of 50 % acetonitrile for 3 min with shaking. After extraction, the sample was centrifuged at 3,000 rpm for 15 min and the supernatant was filtered with filter paper (Whatman No. 4). An aliquot of sample was diluted with PBST and analyzed for AFB_1. Direct competitive (DC)-ELISA procedure was performed as described by Kang et al. (2001).

HPLC was performed to confirm positive samples from ELISA. Aflatoxins in the samples were quantified by HPLC subsequent to extraction, partitioning, and derivatization based on the Korean Food Code (KFDA 2000) and the AOAC method 990.33 (AOAC 2000) with minor modifications. Among food groups, analyses of soy sauce, soybean paste and seasonings were performed by Immunoaffinity column (IAC) clean-up method using Aflatest P columns (Vicam, Watertown, USA) and were carried out based on the previously reported methods using IAC (AOAC 991.31 2000) with minor modifications. The purified sample extracts were derivatized by adding 0.1 ml of

trifluoroacetic acid, allowed to stand for 15 min and diluted with 2 ml of acetonitrile-water (1+1, v/v). Injection volumn was 20 μl. All the procedures were carried out in subdued light and kept from direct UV light. HPLC analysis was carried out by using a Jasco HPLC system (Tokyo, Japan) equipped with a FP-920 fluorescence detector. The chromatographic separation was performed on a μ Bondapak C_{18} column (3.9 × 300 mm I.D., Waters, Ireland) using a water-acetonitrile (3+1, v/v) mobile phase at a flow-rate of 1.0 ml/min. Detection of aflatoxin was carried out using 365 and 418 nm as wavelengths for excitation and emission, respectively. In method of HPLC, the recoveries of AFB_1 on spiked all samples were 76 – 114%. Limit of detection (LOD) and limit of quantification (LOQ) were determined on signal to noise ratio of 3:1 for LOD and 10:1 for LOQ. The estimated LOD and LOQ of AFB_1 were 0.05 and 0.15 μg kg^{-1}.

Risk assessment

Risk assessment of AFB_1 was conducted based on dietary exposure to AFB_1 and its potency in induction of liver cancer. Dietary exposure to AFB_1 was estimated using the occurrence data, food consumption data of 2001 National Health and Nutrition Survey, and mean body weight from Korea Food and Drug Administration, 2002. Dietary exposure to AFB_1 based on these survey results was estimated by Monte Carlo simulation using a @Risk program.

Results and discussion

Natural occurrence of AFB_1

AFB_1 levels in 32 samples showing positive readings from ELISA analysis were determined by HPLC. As shown in Tables I-II, the contamination levels varied from

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below the LOQ (0.15 μg kg^{-1}) to 48.6 μg kg^{-1} in AFB_1. AFB_1 levels of 1 maize sample, 2 peanut and 1 pistachio sample were higher than the permitted level (10 μg kg^{-1} AFB_1) established in Korea as a guideline (KFDA 2000), but the incidence level was very low (4.6%). The aflatoxin contamination in cereals was negligible except maize. In agreement with our result, Tabata et al. (1993) reported that 6 of 359 rice, barley and maize samples were contaminated with aflatoxins.

Measurable levels of aflatoxins were found in soybean and their products (Table I). Of 3 aflatoxin-positive samples,1 sample was soy sauce, 2 samples were soybean paste, but levels were low. No aflatoxin was detected in soybean, soybean oil and hot soy paste.

Eight samples out of 27 peanut samples and five out of 19 peanut butter samples were contaminated with AFB_1, indicating that peanut and peanut products were the most common commodities contaminated with AFB_1 (Table II). Our result was supported by previous reports that the peanut is one of the most susceptible foodstuffs to be contaminated by toxicogenic fungi producing aflatoxins (Mphande 2004). Particularly, AFB_1 levels of peanut and pistachio were ranged from 0.11 to 88.04 μg kg^{-1} and from 3.36 to 38.66 μg kg^{-1}, respectively. Abdulkadar et al. (2004) reported high levels of aflatoxin contamination in pistachio and peanut butter, up to 81.60 and 13.26 μg kg^{-1}, respectively. Bnakole et al. (2005) also reported that AFB_1 was found in 64% of peanut samples with a mean of 25.5 μg kg^{-1}.

The incidences and levels of AFB_1 in spices are given in Table II. Of 21 samples, 7 samples (2 curry powder, 1 black pepper powder and 4 hot pepper powder) contained AFB_1 below 5 μg kg^{-1}. Among spices, the incidence levels of curry powder and hot pepper powder samples were 29% and 57%, respectively. It was reported that aflatoxins were found in 3 hot pepper powders and range of aflatoxins in detected samples was 1.8−16.4

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μg kg^{-1}.

Estimation of the dietary exposure

To reduce uncertainty and improve reliability, a probabilistic approach was applied to the actual survey data. Based on the occurrence level obtained by probability distributions, the estimated Korean mean dietary exposure to AFB_1 was 0.642 ng kg^{-1} bw day^{-1} and 95th percentile exposure was 2.51 ng kg^{-1} bw day^{-1}. The major contributing foods for AFB_1 were soybean paste, soy sauce and peanut, whereas the highest contaminated level was found in pistachio, followed by peanut butter and curry powder. Dietary intakes of AFB_1 due to soybean paste, soy sauce and peanut were 0.397, 0.185 and 0.025 ng kg^{-1} bw day^{-1}, respectively. The estimated high exposures to AFB_1 by consumption of soybean paste and soy sauce, which comprise 91% of total exposure to AFB_1, suggested that risk management on these foods would be required (Table III).

The mean dietary intakes of aflatoxin are 0.15 ng kg^{-1} bw day^{-1} for Australians, 0.8 ng kg^{-1} bw day^{-1} for Swedes (Thuvander et al. 2001), 0.26 ng kg^{-1} bw day^{-1} for Americans (Joint FAO/WHO Expert Committee on Food Additives 1998), and 0.1 ng kg^{-1} bw day^{-1} for adults (>15 ages), 0.3 ng kg^{-1} bw day^{-1} for children (3-14 ages) in French, respectively. Thus, considering Korean probable daily intakes (0.642 ng kg^{-1} bw day^{-1}), Koreans probably consume higher amounts of AFB_1 than other countries. However, from Chinese survey data (Li et al. 2001), the average daily intake of AFB_1 from maize in the high-risk area was 184.1 μg, and the probable daily intake is estimated to be 3.68 μg kg^{-1} bw day^{-1}.

Risk assessment

Among various factors influencing the risk of primary liver cancer, carriage of

hepatitis B virus as determined by the presence in serum of the hepatitis B surface antigen (presence denoted $HBsAg^+$ and absence denoted $HBsAg^-$) was considered in risk assessment of AFB_1. For estimation of excess cancer risk for HBsAg positive and negative individuals, cancer potencies of 230 $mg^{-1}kg\ bw^{-1}day$ and 9 $mg^{-1}kg\ bw^{-1}day$ were applied, respectively. The excess cancer risk for HBsAg positive individuals was calculated as 1.5×10^{-4} that is 25-fold higher than that for HBsAg negative individuals having excess cancer risk of 5.89×10^{-6} (Table IV). These excess cancer risks are estimated on the basis of the mean dietary exposure to AFB_1. Therefore, excess cancer risk could be increased reflecting the consumers for highly contaminated foodstuffs.

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Table I. Natural occurrence of aflatoxin B_1 in cereals, soybean and their products during 2004-2005.

Food commodity	Incidence		Range of AFB_1 concentration	Means of AFB_1 concentration ($\mu g\ kg^{-1}$)[b]
	ELISA	HPLC[a]		
Rice	8/134	0/8	–	0
Barley	6/134	0/6	–	0
Sweet corn	5/7	0/5	–	0
Corn flake	8/92	0/8	–	0
Maize, dry	3/50	2/3	0.97 – 48.61	24.79
Wheat flour	1/7	0/1	–	0
Beer	1/7	0/1	–	0
Soybean	0/7	-	–	0
Soy sauce	1/7	1/1	1.81	1.81
Soybean paste	13/56	2/13	0.05 – 0.17	0.11
Hot soy paste	0/46	-	–	0
Soybean oil	3/7	0/3	–	0
Total	49/554	5/49		

[a]Number of detected sample by HPLC in ELISA-positive samples. [b]Mean of AFB_1 were calculated on the basis of each AFB_1 level detected in samples. The mean of AFB_1 was calculated by establishing that values<LOD are set to 0 and between LOD and LOQ are set to LOQ/2 in each sample.

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Table II. Natural occurrence of aflatoxin in nuts, spices and their products during 2004 - 2005.

Food commodity	Incidence		Range of AFB_1 concentration	Means of AFB_1 concentration ($\mu g\ kg^{-1}$)[b]
	ELISA	HPLC[a]		
Peanut	15/27	8/15	0.11 – 18.04	4.07
Peanut butter	5/19	5/5	1.30 – 6.44	3.60
Walnut	16/19	0/16	–	0
Almond	5/15	3/5	0.08 – 0.55	0.02
Pistachio	6/15	3/6	3.36 – 38.66	16.22
Pine nut	0/12	–	–	0
Assorted nuts	1/12	1/1	6.68	6.68
Curry powder	2/7	2/2	3.64 – 4.12	3.88
Pepper powder	1/7	1/1	0.08	0.08
Hot pepper powder	4/7	4/4	0.08 – 0.63	0.40
Total	55/140	27/55		

[a]Number of detected sample by HPLC in ELISA-positive samples. [b]Mean of AFB_1 were calculated on the basis of each AFB_1 level detected in samples. The mean of AFB_1 was calculated by establishing that values<LOD are set to 0 and between LOD and LOQ are set to LOQ/2 in each sample.

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Table III. Daily intake of AFB_1 from various food commodities using monitoring data.

Commodity	Occurrence (μg kg^{-1})	Consumption[a] (g $^{-1}$person^{-1}day)	Daily intake[b] (ng^{-1}kg bw^{-1}day)	Percentage of total daily intake
Rice	0	216.9	0	0
Barley	0	4.3	0	0
Sweet corn	0	0.3	0	0
Corn flake	0	0.3	0	0
Maize, dry	0	0.3	0	0
Wheat flour	0	5	0	0
Beer	0	37.1	0	0
Soybean	0	3.1	0	0
Soya sauce	2.210	5.2	0.185	28.8
Soybean paste	2.302	10.7	0.397	61.8
Korean hot pepper paste	0	5.1	0	0
Soybean oil	0	3.8	0	0
Peanut	2.621	0.6	0.025	3.9
Peanut butter	4.219	0	0	0
Walnut	0	0	0	0
Almond	0.132	0.03	0[d]	0
Pistachio	13.473	0.03	0.007	1.1
Pine nut	0	0	0	0
Assorted nut	7.89	-[c]	0	0
Curry powder	3.882	0.2	0.013	2.0
Black pepper powder	0.028	0.1	0[e]	0
Hot pepper powder	0.397	2.3	0.015	2.3
Total			0.642	100

[a]Average of adult consumption per day (20-64 ages). [b]Average body weight of adults in Korean population was applied to 62 kg. [c]Consumption data was absent in national nutrition survey report. [d]6.39×10^{-5}. [e] 4.52×10^{-5}.

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Table IV. Excess cancer risk of dietary AFB_1 exposure based on monitoring data from this study.

Chronic daily intake ($mg^{-1}kg\ bw^{-1}day$)	Excess cancer risk ($mg^{-1}kg\ bw^{-1}day$)	
	$HbsAg^-$	$HbsAg^+$
6.42×10^{-7}	5.78×10^{-6}	1.48×10^{-4}