Incidence of aflatoxins in Iran pistachio nuts

Article *in* Food and Chemical Toxicology · June 2007

DOI: 10.1016/j.fct.2006.10.026 · Source: PubMed

22 authors, including:

Majid Cheraghali
Baqiyatallah University of Medical Sciences
70 PUBLICATIONS **1,039** CITATIONS

SEE PROFILE

Hassa Yazdanpanah
Shahid Beheshti University of Medical Sciences
52 PUBLICATIONS **973** CITATIONS

SEE PROFILE

Amirhossein Askarian
Polytechnique Montréal
9 PUBLICATIONS **148** CITATIONS

SEE PROFILE

Available online at www.sciencedirect.com
ScienceDirect
Food and Chemical Toxicology 45 (2007) 812–816
www.elsevier.com/locate/foodchemtox

Incidence of aflatoxins in Iran pistachio nuts

A.M. Cheraghali [a], H. Yazdanpanah [b,c,d,*], N. Doraki [d], G. Abouhossain [d], M. Hassibi [d], S. Ali-abadi [d], M. Aliakbarpoor [d], M. Amirahmadi [d], A. Askarian [d], N. Fallah [d], T. Hashemi [d], M. Jalali [d], N. Kalantari [d], E. Khodadadi [d], B. Maddah [d], R. Mohit [d], M. Mohseny [d], Z. Phaghihy [d], A. Rahmani [d], L. Setoodeh [d], E. Soleimany [d], F. Zamanian [d]

[a] *Department of Pharmacology, University of Baqiyatallah Medical Sciences, Tehran, Islamic Republic of Iran*
[b] *Department of Pharmacology and Toxicology, School of Pharmacy, Shaheed Beheshti Medical University, Tehran 14155-6153, Islamic Republic of Iran*
[c] *Pharmaceutical Sciences Research Center, Shaheed Beheshti Medical University, Tehran, Islamic Republic of Iran*
[d] *Food (and Drug) Control Laboratories, Department of Food and Drug, Ministry of Health and Medical Education, Tehran, Islamic Republic of Iran*

Received 30 September 2005; accepted 31 October 2006

Abstract

Aflatoxins (AF) are highly toxic and carcinogenic secondary fungal metabolites and have been detected in various food commodities including pistachio nuts. Pistachio nuts were produced in Iran during March 2002–February 2003 analyzed for aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2) using immunoaffinity column and quantitated by HPLC and/or TLC-scanner. In this regard, 3356 pistachio nut samples were collected. After dividing samples to sub-samples, 10,068 AF analyses were done. Among 10,068 samples analyzed, AFB1 was detected in 3699 samples (36.7% of the total) with the mean and median of 5.9 (±41.7) ng/g and 0.1 ng/g, respectively. Total AF (AFT) was detected in 2852 samples (28.3% of the total) with the mean and median of 7.3 (±53.2) ng/g and 0.4 ng/g, respectively. AFB1 level in 1191 samples (11.8%) was above the maximum tolerated level (MTL) of AFB1 in pistachio nut in Iran (5 ng/g). Regarding AFT, the mean contamination level (7.3 ng/g) was lower than MTL of AFT in pistachio nut in Iran as well as lower than the proposed draft maximum level of Codex Committee on Food Additives and Contaminants for AFT (15 ng/g), and only 7.5% of samples had levels above the MTL.

Keywords: Aflatoxins; Pistachio nuts; Iran; HPLC; Incidence

1. Introduction

The aflatoxins (AF) are a group of toxic and carcinogenic polyketide secondary metabolites, which are produced by strains of *Aspergillus flavus, Aspergillus parasiticus, Aspergillus nomius* and *Aspergillus pseudotamarii* (Ito et al., 2001; Kurtzman et al., 1987; Payne, 1998).

The International Agency for Research on Cancer (IARC) has classified aflatoxin B1 (AFB1) as a group I carcinogen, primarily affecting liver (IARC, 1993). AF are found as contaminants in various agricultural commodities. The commodities with the highest risk of AF contamination include corn, peanut, cottonseed, Brazil nut, pistachio nut, fig, spice and copra (Pittet, 1998). The most

Abbreviations: AF, aflatoxins; AFB1, aflatoxin B1; AFB2, aflatoxin B2; AFG1, aflatoxin G1; AFG2, aflatoxin G2; AFT, total aflatoxins; EU, European Union; FAO, Food and Agriculture Organization of the United Nations; (FAPAS®), Food Analysis Performance Assessment Scheme; FDCL, Food And Drug Control Laboratories; FSA, Food Standards Agency; HPLC, high performance liquid chromatography; IAC, immunoaffinity column; IARC, International Agency for Research on Cancer; ISIRI, Institute of Standard and Industrial Research of I.R. Iran; JECFA, Joint FAO/WHO Expert Committee on Food Additives and Contaminants; LOD, limit of detection; LOQ, limit of quantification; MTL, maximum tolerated level; PCD, post-column derivatization; RSD_r, relative standard deviation for repeatability; STD, standard deviation; TLC, thin-layer chromatography.

* Corresponding author. Address: Department of Pharmacology and Toxicology, School of Pharmacy, Shaheed Beheshti Medical University, Tehran 14155-6153, Islamic Republic of Iran. Tel.: +98 21 88 77 42 83; fax: +98 21 88 79 50 08.
E-mail addresses: yazdanpanah@sbmu.ac.ir, hasyazdan@yahoo.com (H. Yazdanpanah).

0278-6915/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fct.2006.10.026

important dietary sources of AF are maize and groundnut and their products (JECFA, 1998).

The knowledge that mycotoxins can have serious effects on humans and animals has led many countries to establish maximum tolerated level (MTL) on mycotoxins in foodstuffs and feedstuffs in the last decades to safeguard the health of humans, as well as the economical interests of producers and traders. Currently, worldwide range of limits for AFB1 and total AF (AFT) are 1–20 ng/g and 0–35 ng/g, respectively (FAO, 2004).

Molds in the genus *Aspergillus* frequently decay the kernel of pistachio nuts (Mojtahedi et al., 1979). On the other hand, pistachio nuts are among the commodities with the highest risk of AF contamination (Pittet, 1998). The incidence of AF contamination in tree nuts is low, but their levels are quite variable and high levels develop in a small percentage of nuts (Schatzki, 1995).

Natural occurrence of AF in pistachio nuts has been studied in various countries. According to a report from Mexico, 2.2% of pistachio nut samples analyzed contained AF higher than 20 ng/g (JECFA, 1998). In Sweden, 9.5% pistachio nut samples contained AFB1 higher than 2 ng/g (Thuvander et al., 2001). According to the report of Japanese Ministry of Health, among pistachio nut samples analyzed during 1972–1989, only 2% of samples were contained AFB1 higher than 10 ng/g (JECFA, 1998). Analysis of 523 pistachio nut samples in Turkey revealed the mean of AFB1 ranged 1–3.78 ng/g and the maximum level detected was 113 ng/g (Ministry of Agriculture and Rural Affairs, Republic of Turkey, 2002). According to the Food Standards Agency (FSA) survey in UK, among 52 pistachio nut samples analyzed using HPLC, 44 samples were not contaminated (lower than limit of quantification [LOQ]), two samples contained AFT between LOQ-4 ng/g, two samples contained AFT between 4 ng/g and 10 ng/g and four samples contained AFT higher than 10 ng/g (Food Standards Agency, 2002). In the Netherlands, among 29 pistachio nut samples, AFB1 was found in 17 samples ranging from 0.8 ng/g to 165 ng/g (Scholten and Spanjer, 1996). In Qatar, 27.7% analyzed pistachio nut samples were contaminated with AFT above the 20 ng/g (Abdulkadar et al., 2000).

Mycotoxins contamination of foodstuffs and feedstuffs have been studied in Iran (Yazdanpanah et al., 2006a; Yazdanpanah, 2006b; Yazdanpanah et al., 2005; Kamkar, 2005; Cheraghali et al., 2005; Hadiani et al., 2003; Shephard et al., 2002; Yazdanpanah et al., 2001; Shephard et al., 2000). Iran is the largest pistachio nut producer in the world and most of its crops exported to other countries. In this study, incidence of AF in Iran pistachio nuts intended for exportation into European Union (EU) was investigated.

2. Materials and methods

AF standards for the experiments were purchased from Sigma Chemical Company, USA. All solvents used for the experiments were of either HPLC or analytical grade. Aflatest immunoaffinity columns (IAC) were purchased from Vicam Company, Watertown, MA, USA. HPLC column (Partisil 5 ODS3) was purchased from Hichrom Limited, Berkshire, England.

2.1. Sampling

Kerman and Rafsanjan, in center of Iran, are the major pistachio nut producer regions in Iran. Therefore, most of the samples were collected from these regions. Inspectors of Food Control Offices in Tehran, Kerman and Rafsanjan, Ministry of Health of Iran, collected 3356 pistachio nut samples during March 2002–February 2003. Samples were taken exactly according to the sampling procedure of EU for sampling of nuts (European Commission, 1998). The pistachio nut consignments, intended for export to EU, are usually about 25 tons. Following taking one 30-kg sample (100 incremental samples, each 300 g), it was mixed and divided to three 10-kg sub-samples. Then, the sub-samples were transferred to Toxicology Labs in Food and Drug Control Laboratories (FDCL) of Iran Ministry of Health and AF were analyzed in 10,068 samples.

2.2. Sample preparation

For minimizing the sub-sampling error in AF analysis, a water slurry of pistachio nut samples was prepared. In this regard, 1.5 l of water was added for each 1 kg of pistachio nut (15 l for each 10-kg sub-sample). The mixture then was used to make slurry using a slurry machine, grinding mixture for 15 min. Finally, 125 g of test portion from the slurry was taken for analysis.

2.3. Extraction and clean up

Samples were analyzed either using a high performance liquid chromatography (HPLC) method (the AOAC official method 999.07) with some minor modification (Stroka et al., 2000a) or using a thin-layer chromatography (TLC) method with some minor modification (Stroka et al., 2000b). Regarding HPLC analysis, pistachio nut slurries were extracted with methanol/water/hexane (300 ml/75 ml/100 ml). After filtration, the extract was diluted with water and filtered through glass microfiber filter. For clean up of samples, Aflatest IACs were used. First, 10 ml phosphate buffer saline (PBS) was passed through the IAC. Then, 75 ml of the filtrate was passed through the IAC at flow rate of ca. 1 drop/s. The column was washed with 15 ml water and dried by applying little vacuum. Finally, AF were eluted with methanol by the following procedure. First, 0.5 ml methanol was applied on the column which passed through by gravity. After 1 min, the second portion of 0.75 ml methanol was applied and collected. The eluate was diluted with water and analyzed by HPLC.

Regarding TLC analysis, pistachio nut slurries were extracted with methanol/water/hexane (300 ml/75 ml/100 ml). After filtration, the extract was diluted with PBS and filtered through glass microfiber filter. For clean up, first, 10 ml PBS was passed through the IAC. Then, 75 ml of the filtrate was passed through the IAC at flow rate of ca. 1 drop/s. The column was washed with 15 ml water and dried by applying little vacuum. Finally, AF were eluted with methanol by the following procedure. First, 0.5 ml methanol was applied on the column which passed through by gravity. After 1 min, the second portion of 1 ml methanol was applied and collected. Prior to elution, approximately 50 μl of a methanol–formic acid solution was passed into the glass vial. The eluate was gently mixed and taken to dryness at 40 °C under a gentle stream of N_2. The aflatoxins were re-dissolved in 150 μl of a hexane–acetone–methanol solution (90 + 5 + 5, v/v/v) and analyzed using TLC.

2.4. AF standards

After preparation of standard solutions of individual AF, the concentration of each one was determined using UV spectrophotometer. These standards were used to prepare mixed working standards for HPLC and/or TLC analysis (Stroka et al., 2000a,b).

2.5. Analysis of AF using HPLC and/or TLC-scanner

AF were mostly (66.5% of samples) quantitated by reverse-phase HPLC and fluorescence detector with post-column derivatization (PCD) involving bromination (Stroka et al., 2000a). PCD was achieved with a kobra cell and addition of bromide to the mobile phase. After dilution of AF eluate with water, 100 μl was injected into HPLC. The analytical column used was C_{18}, 5 μm, 250 mm × 4.6 mm i.d. Mobile phase was water:methanol:acetonitrile (54:29:17, v/v/v) with a flow rate of 1 ml/min. The fluorescence detector was operated at an excitation wavelength of 365 nm and emission wavelength of 435 nm. Each working day, a five-point calibration curve was built for each individual AF including AFB1, aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2), checked for the linearity and used for quantification of AF in pistachio nut samples. AFG2 was eluted first followed by AFG1, AFB2 and AFB1. The LOD for AFB1 and AFT were 0.1 ng/g and 0.4 ng/g, respectively.

In 33.5% of samples, after extraction and IAC clean up, AF were quantitated using TLC-scanner (Stroka et al., 2000b). Each working day, a five-point calibration curve was built for AFB1, AFB2, AFG1, and AFG2, checked for the linearity and used for quantification of AF in pistachio nut samples. The LOD for AFB1 and AFT were 0.2 ng/g and 1 ng/g, respectively.

2.6. Quality assurance

For evaluation of the reliability of the results of AF analysis, in addition to using validated methods, internal and external quality control experiments were performed. Regarding internal quality control, the accuracy and precision of the methods were verified. In this regard, recoveries of AFB1, AFB2, AFG1 and AFG2 were recorded by analyzing a blank pistachio nut sample spiked at 2 ng/g for each AFB1 and AFG1 and 0.4 ng/g for each AFB2 and AFG2. According to the recovery values, AF levels were corrected for recoveries. Regarding external quality control, Iran National Food Control Labs participated regularly in proficiency testing of Food Analysis Performance Assessment Scheme (FAPAS®) in UK.

3. Results

The average recoveries and relative standard deviation for repeatability (RSD_r) of the analytical methods applied for AFB1, AFB2, AFG1 and AFG2 in pistachio nuts were investigated. The results are shown in Table 1. Both recoveries and RSD_r of AF were in the acceptable range. The results of participation of labs in the proficiency testing of FAPAS were satisfactory, since almost all of Z-scores of individual AF as well as AFT were in the acceptable range ($-2 \leqslant Z$ score $\leqslant +2$). These results indicated good accuracy and precision of the used analytical methods.

Table 1
Average recoveries and coefficient of variations for aflatoxins spiked into blank pistachio nut samples and analyzed using HPLC and fluorescence detector

Aflatoxins	No.[a]	Spike level (ng/g)	Average recovery (%)	RSD_r[b] (%)
AFB1	94	2.0	97.3	10.2
AFB2	94	0.4	95.1	9.9
AFG1	94	2.0	97.8	10.1
AFG2	94	0.4	61.8	21.6

[a] Number of spiked pistachio nut samples analyzed using HPLC.
[b] The relative standard deviation for repeatability.

Results of analysis of AFB1 and AFT in pistachio nut samples during March 2002–February 2003 are summarized in Table 2.

Among 10,068 samples analyzed, 6369 samples (63.3%) were not contaminated with AFB1 (<LOD) (Fig. 1).

AFB1 was detected in 3699 samples (36.7%), and its level in 1191 samples (11.8%) was above the MTL of AFB1 in pistachio nut in Iran (5 ng/g), which has been set by Institute of Standard and Industrial Research of I.R. Iran (ISIRI) in 2002 (Fig. 1) (ISIRI, 2002). The mean and median of AFB1 in the samples were 5.9 (±41.7) ng/g and 0.1 ng/g, respectively (Table 2).

Of total samples were analyzed, 7216 samples (71.7%) did not show any AFT contamination (Fig. 2).

However, AFT was detected in 2852 samples (28.3% of the total) with the mean and median of 7.3 (±53.2) ng/g and 0.4 ng/g, respectively (Table 2). The mean contamination level (7.3 ng/g) was lower than both MTL of AFT in pistachio nut in Iran (15 ng/g) (ISIRI, 2002) and the proposed draft maximum level of Codex Committee on Food Additives and Contaminants for AFT (15 ng/g) (Codex Committee on Food Additives and Contaminants, 2005).

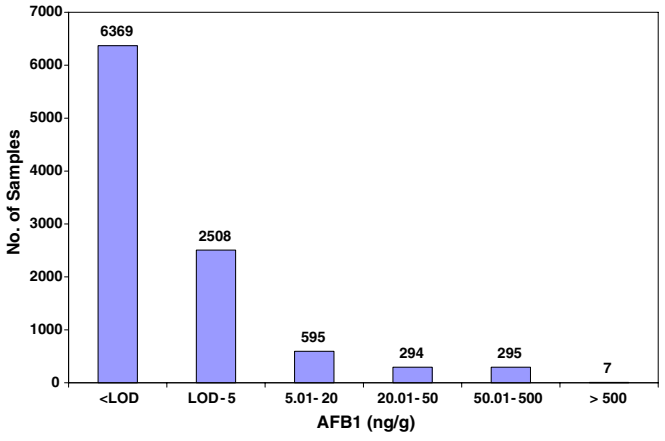

Fig. 1. Incidence of AFB1 in Iran pistachio nut samples analyzed by Food Control Labs during March 2002–February 2003.

Table 2
Mean, standard deviation, median and percentiles 25% and 75% of aflatoxin B1 and total aflatoxins (ng/g) in Iran pistachio nut samples analyzed by Food Control Labs during March 2002–February 2003

	No. of samples analyzed	Samples without AF (<LOD)[a]	Mean	STD	Median	Percentile, 25%	Percentile, 75%
AFB1	10,068	6369 (63.3%)[b]	5.9	41.7	0.1	0.1	0.5
AFT	10,068	7216 (71.7%)[b]	7.3	53.2	0.4	0.4	0.6

[a] LOD of HPLC method for AFB1: 0.1 ng/g and AFT: 0.4 ng/g; LOD of TLC method for AFB1: 0.2 ng/g and AFT: 1 ng/g.
[b] Percentage of non-contaminated samples.

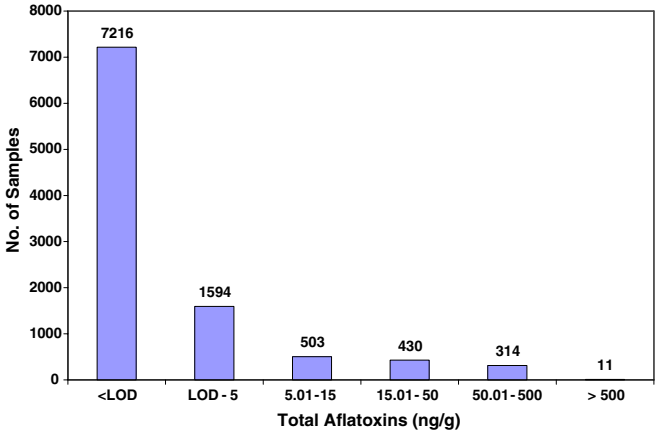

Fig. 2. Incidence of AFT in Iran pistachio nut samples analyzed by Food Control Labs during March 2002–February 2003.

Only 755 samples (7.5%) had levels above this MTL (Fig. 2).

It should be mentioned that due to the heterogeneity of AF in pistachio nuts, it is expected that the level of contamination in sub-samples might be different. However, the mean of the contamination in sub-samples was used in this study.

4. Discussion

Pistachio nut as an important tree nut is cultivated in several countries including Iran, USA, Turkey, Greece, Syria and Italy. Pistachio nuts are a rich source of fat and contain linoleic and linolenic fatty acids, essential for the human diet (International Nut Council, 2005). However, *Aspergillus* molds frequently decay the kernel of pistachio nuts (Mojtahedi et al., 1979). Pistachio nuts are among the commodities with the highest risk of AF contamination (Pittet, 1998). In a survey in UK, 11.5% of pistachio nut samples were contaminated with AFT above MTL of EU (4 ng/g) (Food Standards Agency, 2002). This is in agreement with our results that showed 13.6% of the analyzed pistachio nuts contained AFT higher than EU MTL. In another study in Sweden, 9.5% of pistachio nut samples were contaminated with AFB1 above MTL of EU (2 ng/g) (Thuvander et al., 2001). However, we found that 15.9% of samples contained AFB1 higher than EU regulation.

Approximately 100 countries including Iran have developed MTL for mycotoxins in foodstuffs and feedstuffs (FAO, 2004). The MTL of AFT in pistachio nuts in Iran (15 ng/g) is close to figure in USA (20 ng/g), but quite different when compared with those proposed by EU to the Codex Committee (2 ng/g for AFB1 and 4 ng/g for AFT) (FAO, 2004). Considering the Iran limit for AFB1 in pistachio nut (5 ng/g), 1191 samples (11.8%) had levels above the MTL. However, this figure will increase to 1600 samples (15.9%) according to EU limit. Concerning AFT, considering the Iran limit (15 ng/g), 755 samples (7.5%) had levels above the MTL, while considering USA and EU limits, this figure is 656 samples (6.5%) for USA and 1365 samples (13.6%) for EU.

Joint FAO/WHO Expert Committee on Food Additives and Contaminants (JECFA) has considered estimates of the carcinogenic potency of AF and the potential risks associated with their intake (JECFA, 1998). JECFA has estimated that in a western consumer society (Europe), where food contamination levels by aflatoxins are low and the prevalence of Hepatitis B runs only at about 1%, a tolerance level of 20 ng/g for peanuts present an estimated population risk of 0.0041 cancers/year per 100,000 people. By reducing the tolerance level by half to 10 ng/g, the estimated risk to the European population falls to 0.0039 cancers/year per 100,000 people. This reduction from 20 ng/g to 10 ng/g amounts to a drop in estimated risk of two additional cancers/year, per 1000,000,000 people (JECFA, 1998).

Since pistachio nuts are among the commodities with the highest risk of AF contamination (Pittet, 1998), a prevention program should be established considering various steps from cultivation through harvesting, post-harvesting, processing, storage, transportation and marketing. In recent years, Iranian health and agriculture authorities have implemented strict regulations in order to control AF contamination. Efforts have been made to manage AF contamination by promoting Good Agricultural Practice (GAP) principles in the orchards and Hazard Analysis and Critical Control Point (HACCP) principles in storage and processing plants. These activities were supported by research projects funded through FAO and national organizations (FAO TCP Project in Iran, Pineiro, 2003). On the other hand, a code of practice for the prevention and reduction of AF contamination in tree nuts including pistachio nut is being elaborated by Codex Committee on Food Additives and Contaminants (Codex Committee on Food Additives and Contaminants, 2005).

When preventive action cannot be achieved, corrective action needs to be done. Removal of AF-contaminated nuts by means of physical segregation is the most effective control measure for reducing levels of AF in a lot to an acceptable level (FAO, 2001). This is reported that roasting pistachio nuts could reduce AF contamination (Yazdanpanah et al., 2005). Meanwhile in order to protect health of consumers, respecting national MTL for AFB1 and AFT is mandatory and Iran Ministry of Health do not allow distribution or exportation of pistachio nuts contaminated with AF above the limits. At the international level, Iranian Codex Committee on Food Contaminants in cooperation with USA, EU and other countries is working on the elaboration of maximum level for AF in pistachio nuts (Codex Committee on Food Additives and Contaminants, 2005).

5. Safety

AF are carcinogens and care should be exercised to avoid personal exposure and potential risk of contamination. All

handling of pure compounds were done in the fume hood with protective gear such as safety glasses, gloves, laboratory coat and a disposable face mask. The glasswares were washed with hypochlorite and dilute acid before re-using and the waste materials treated with hypochlorite before disposal.

Conflict of interest statement

There is no conflict of interest.

References

Abdulkadar, A.H.W., Al-Ali, A., Al-Jedah, J., 2000. Aflatoxin contamination in edible nuts imported in Qatar. Food Cont. 11, 157–160.

Cheraghali, A.M., Mohammadi, H.R., Amirahmadi, M., Yazdanpanah, H., Abouhossain, G., Zamanian, F., Ghazi Khansari, M., Afshar, M., 2005. Incidence of patulin contamination in apple juice produced in Iran. Food Cont. 16, 165–167.

Codex Committee on Food Additives and Contaminants, Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission, 2005. Report of the 37th session of the Codex Committee on Food Additives and Contaminants, pp. 18–20.

European Commission, 1998. Commission Directive 98/53/EC of 16 July, 1998, laying down the sampling methods and the methods for analysis for the official control of the levels for certain contaminants in foodstuffs. Off. J. Eur. Commun. L201, 93–101.

Food and Agriculture Organization of the United Nations, 2001. Manual on the application of the HACCP system in mycotoxin prevention and control. FAO Food and Nutrition Paper, No. 73. FAO, Rome.

Food and Agriculture Organization of the United Nations TCP Project in Iran (TCP/IRA/2905), Pineiro, 2003. Analysis, control and management of mycotoxins in foodstuffs. FAO, Rome.

Food and Agriculture Organization of the United Nations, 2004. Worldwide regulations for mycotoxins in food and feed in 2003. FAO Food and Nutrition Paper, No. 81. FAO, Rome.

Food Standards Agency, 2002. Food Survey information sheet no. 21/02. http://www.foodstandards.gov.uk/multimedia/pdfs/21nuts.pdf (accessed 23.08.2005).

Hadiani, M.R., Yazdanpanah, H., Ghazi Khansari, M., Cheraghali, A.M., Goodarzi, M., 2003. Survey of the natural occurrence of zearalenone in maize from northern Iran by thin-layer chromatography densitometry. Food Addit. Contam. 20, 380–385.

Institute of Standard and Industrial Research of I.R. Iran., 2002. Maximum tolerated limits of mycotoxins in foods and feeds. National Standard No. 5925.

International Agency for Research on Cancer, 1993. IARC monograph on the Evaluation of Carcinogenic Risk to Humans, vol. 56. IARC, Lyon, France.

International Nut Council. http://www.nuthealth.org/nutrition/nutrient100g.html (accessed 23.08.2005).

Ito, Y., Peterson, S.W., Wicklow, D.T., Goto, T., 2001. *Aspergillus pseudotamarii*, a new aflatoxin producing species in *Aspergillus* section *flavi*. Mycol. Res. 105, 233–239.

JECFA, 1998. Forty-ninth meeting of the joint FAO/WHO expert committee on food additives. In: Safety Evaluation of Certain Food Additives and Contaminants: AflatoxinsWHO Food Additives Series 40. WHO, Geneva, pp. 359–469.

Kamkar, A., 2005. A study on the occurrence of aflatoxin M1 in raw milk produced in Sarab city of Iran. Food Cont. 16, 593–599.

Kurtzman, C.P., Horn, B.W., Hesseltine, C.W., 1987. *Aspergillus nomius*, a new aflatoxin-producing species related to *Aspergillus flavus* and *Aspergillus tamarii*. Antonie van Leeuwenhoek 53, 147–158.

Ministry of Agriculture and Rural Affairs, Republic of Turkey, 2002. Data on aflatoxins in hazelnuts, pistachios, walnuts and almonds during 1998–2002.

Mojtahedi, H., Rabie, C.J., Lubben, A., Steyn, M., Danesh, D., 1979. Toxic aspergilli from pistachio nuts. Mycopathol. 67, 123–127.

Payne, G.A., 1998. Process of contamination by aflatoxin-producing fungi and their impact on crops. In: Sinha, K.K.S., Bhatnagar, D. (Eds.), Mycotoxins in Agriculture and Food Safety. Marcel Dekker, Inc., New York, pp. 279–306.

Pittet, A., 1998. Natural occurrence of mycotoxins in foods and feeds – an updated review. Revue. Med. Vet. 149, 479–492.

Schatzki, T.F., 1995. Distribution of Aflatoxin in pistachios. 2. Distribution in freshly harvested pistachios. J. Agric. Food. Chem. 43, 1566–1569.

Scholten, J.M., Spanjer, M.C., 1996. Determination of aflatoxin B1 in pistachio kernels and shells. J. AOAC Int. 79, 1360–1364.

Shephard, G.S., Marasas, W.F.O., Leggott, N.L., Yazdanpanah, H., Rahimian, H., Safavi, N., 2000. Natural occurrence of fumonisins in corn from Iran. J. Agric. Food Chem. 48, 1860–1864.

Shephard, G.S., Marasas, W.F.O., Yazdanpanah, H., Rahimian, H., Safavi, N., Zarghi, A., Shafaati, A., Rasekh, H.R., 2002. Fumonisin B1 in maize harvested in Iran during 1999. Food Addit. Contam. 19, 676–679.

Stroka, J., Ankle, E., Jorissen, U., Gilbert, J., 2000a. Immunoaffinity column clean up with liquid chromatography using post-column bromination for determination of aflatoxins in peanut butter, pistachio paste, fig paste, and paprika powder: collaborative study. J. AOAC Int. 83, 320–340.

Stroka, J., van Otterdijk, R., Anklam, E., 2000b. Immunoaffinity column clean up prior to thin-layer chromatography for the determination of aflatoxins in various food matrices. J. Chromatgr. A. 904, 251–256.

Thuvander, A., Moller, T., Barbieri, H.E., Jansson, A., Salomonsson, A.C., Olsen, M., 2001. Dietary intake of some important mycotoxins by the Swedish population. Food Addit. Contam. 18, 696–706.

Yazdanpanah, H., Miraglia, M., Calfapietra, F.P., Brera, C., 2001. Natural occurrence of aflatoxins and ochratoxin A in corn and barley from Mazandaran and Golestan provinces in north of I.R. Iran. Mycotoxin Res. 17, 21–30.

Yazdanpanah, H., Mohammadi, T., Abouhossain, G., Cheraghali, A.M., 2005. Effect of roasting on degradation of aflatoxins in contaminated pistachio nuts. Food Chem. Toxicol. 43, 1135–1139.

Yazdanpanah, H., Shephard, G.S., Marasas, W.F.O., van der Westhuizen, L., Rahimian, H., Safavi, S.N., Eskandari, P., Ghiasian, S.A., 2006a. Human dietary exposure to fumonisin B1 from Iranian maize harvested during 1998–2000. Mycopathol. 161, 395–401.

Yazdanpanah, H., 2006b. Mycotoxin contamination of foodstuffs and feedstuffs in I. R. Iran. Iran. J. Pharmaceut. Res. 5, 9–16.