FOOD CHEMICAL CONTAMINANTS

Surveillance and Control of Aflatoxins B_1, B_2, G_1, G_2, and M_1 in Foodstuffs in the Republic of Cyprus: 1992–1996

ELENI IOANNOU-KAKOURI, MARIA ALETRARI, EFTYCHIA CHRISTOU, ARTEMISIA HADJIOANNOU-RALLI, ATHENA KOLIOU, and DINA AKKELIDOU
Ministry of Health, State General Laboratory, Food Additives and Contaminants Section, 44 Kimonos St, 1451 Nicosia, Cyprus

Aflatoxins (AFs) B_1, B_2, G_1, and G_2 in locally produced and imported foodstuffs (nuts, cereals, oily seeds, pulses, etc.) were monitored and controlled systematically and effectively from 1992–1996. Samples (peanuts, pistachios, etc.) with total AFs above the Cyprus maximum level (ML) of 10 μg/kg fluctuated between 0.7 and 6.9%. The results indicate the effectiveness of monitoring, as well as the need for constant surveillance and control, especially at critical control points (sites of import, primary storage, etc.), to prevent unfit products from entering the Cyprus market. The control included sampling, retainment, analysis, and destruction of foodstuff lots with AF levels above MLs. The highest incidence of aflatoxin contamination was observed in peanut butter (56.7%) and the highest level of AF B_1 was found in peanuts (700 μg/kg). Levels of AF M_1 in raw and pasteurized milk analyzed in 1993, 1995, and 1996 were within both the Cyprus ML (0.5 μg/L) and the lower ML (0.05 μg/L) of some European countries. Only 12% of samples had detectable levels of AF M_1. Analyses were performed by immunochemical methods. When recoveries were lower than 80%, the AF levels were corrected for recovery.

Aflatoxins (AFs) B_1, B_2, G_1, and G_2 are potent teratogenic, mutagenic, and carcinogenic mycotoxins (1). They are produced by *Aspergillus flavus* and *A. parasiticus*, which grow on foods (e.g., nuts, cereals, oily seeds, and beans) under favorable temperature and humidity conditions, before or during harvest or improper storage. AF M_1, a metabolite of AF B_1 in mammals, may be found in the milk of animals ingesting feed contaminated with AF B_1. AFs B_1, B_2, G_1, and G_2 are classified as Group 1 human carcinogens, whereas AF M_1 is classified as a Group 2B probable human carcinogen (2). The primary target organ of AFs in humans and animals is the liver (2, 3).

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Many countries have adopted legal maximum levels (MLs) for AFs in various foodstuffs. In Cyprus, the MLs for total AFs (B_1, B_2, G_1, and G_2) is 10 μg/kg, of which the concentration due to AF B_1 should not be higher than 5 μg/kg; the ML for AF M_1 in milk is 0.5 μg/L (4). Similar, lower, or higher MLs have been adopted by European Union (EU) countries, the United States, Canada, and Australia (5) to reduce the health risk due to dietary intake of AFs.

Many studies on AF contamination of food are short-term and undertaken on limited food items. Long-term studies covering a wide variety of food items are limited (6).

In Cyprus since 1989, local and imported foodstuffs have been systematically and effectively tested for AFs B_1, B_2, G_1, and G_2. Cyprus legislation requires the control of AFs for all lots of specified imported food items (i.e., nuts, nut products, pulses, oily seeds, and cereals). Control includes representative sampling, retainment, analysis, and destruction of foodstuffs lots with AF levels above MLs, or return to the country of origin.

In this paper, we present results from the several years of monitoring (1992–1996) of AFs in 6362 samples of a wide variety of foodstuffs. Results from similar studies for the years 1989–1991 have been published (7). Samples were collected, according to the National Monitoring Program for the Prevention and Control of Aflatoxins in Foodstuffs in Cyprus (8), at critical control points (9), such as the sites of import, primary storage, processing, and sale. The aims of the program are (*1*) to prevent products with AF levels above MLs from entering the Cyprus market, (*2*) to keep the levels of AFs at the lowest possible values, and (*3*) to reduce the potential risk to public health due to intake of AFs.

AF M_1 in Cyprus raw or pasteurized milk and other types of milk was also monitored during 1993, 1995, and 1996. Samples of raw milk were collected from several farms according to the Monitoring and Research Project on Environmental Contamination of Food of Cyprus, based on the European Global Environmental Monitoring System, GEMS-Food/EURO, project (10).

Experimental

Samples

(a) *For analysis of AFs B_1, B_2, G_1, and G_2.*—Representative samples were collected in 1992–1996 from sites of pri-

Table 1. Incidence and level of aflatoxins in nondairy foodstuffs, other than nut and nut products, 1992–1996

Sample	No. of samples	No. of positives[a]	B_1, μg/kg	Range (avg of positives), μg/kg		
				B_2	G_1	G_2
Cereals and cereal products						
Corn[b]	170	2	1	—	—	—
Barley	127	—	—	—	—	—
Wheat	55	—	—	—	—	—
Rice	56	—	—	—	—	—
Breakfast cereals and others	78	—	—	—	—	—
Coffee and cocoa products						
Coffee beans	171	—	—	—	—	—
Cocoa products	10	—	—	—	—	—
Dried fruits and dried fruit products						
Raisins	22	—	—	—	—	—
Figs and figpie[b]	24	4	1.4–6 (3.7)	0.9–1.5 (1.2)	0.8–2.1 (1.4)	4.2
Dates	5	—	—	—	—	—
Pulses						
Bean	69	—	—	—	—	—
Broad bean	16	—	—	—	—	—
Chick pea	284	—	—	—	—	—
Lentils	21	—	—	—	—	—
Seeds and seed products						
Sunflower and pine seeds	8	—	—	—	—	—
Pumpkin seed	190	—	—	—	—	—
Sesame products[b]	130	5	1	—	—	—
Sesame[b]	211	4	2	—	<0.4[c]	<0.3[c]
Spices and tea						
Pepper, turmeric, and others	6	1	<0.4[c]	—	—	—
Total	1653	16	—	—	—	—

[a] Positive samples were those with detectable aflatoxins >0.1 μg/kg, by SPE or IAC cleanup followed by HPTLC with scanner fluorodensitometric detection.

[b] Data for these products were corrected for recovery when analyzed by IAC cleanup method, which gave recoveries of <80% as follows: corn, 40.7% B_1; figs and figpie 38.0% B_1, 35.0% B_2, 31.0% G_1, and 13.0% G_2; sesame and sesame products, 36.0% B_1.

[c] Lower than the limit of determination.

Table 2. Incidence and level of aflatoxins in nut and nut products, 1992–1996

Sample	No. of samples	No. of positives[a]	B_1, µg/kg	Range (avg of positives), µg/kg		
				B_2	G_1	G_2
Peanut butter[b]	74	21	1.2–73 (40.6)	0.3–9 (6.4)	<0.4[c]–0.9 (0.6)	0.3
Desiccated coconut	71	—	—	—	—	—
Almond	615	—	—	—	—	—
Walnut	560	6	<0.4[c]–0.2 (0.1)	<0.3[c]	<0.4[c]	—
Chestnut	118	10	<0.4[c]	—	—	—
Cashew nut	310	—	—	—	—	—
Brazil nut[b]	51	10	8.3–20 (14.1)	1.1 (1.1)	2.3–9.4 (5.8)	—
Hazelnut	182	—	—	—	—	—
Pistachio[b]	856	53	1.4–206 (54.9)	<0.3[c]–2.3 (1.2)	—	—
Peanut[b]	1860	179	<0.4[c]–700 (25.6)	<0.3[c]–12.5 (1.5)	<0.4[c]–72.2 (8.9)	<0.3[c]–3 (0.6)
Nut products	12	—	—	—	—	—
Total	4709	279	—	—	—	—

[a] Positive samples were those with detectable aflatoxins >0.1 µg/kg, by SPE or IAC cleanup followed by HPTLC with scanner fluorodensitometric detection (*see* text).

[b] Data for these products were corrected for recovery when analyzed by IAC cleanup method, which gave recoveries of <80% as follows: peanut butter, 57.4% B_1, 36.8% B_2, 35.8% G_1, 20.0% G_2; Brazil nut, 43.0% B_1, 41.3% B_2, 48.6% G_1, and 16.6% G_2; pistachio, 46.3% B_1, 37.0% B_2, 39.1% G_1, and 17.0% G_2; peanut, 54.2% B_1, 34.8% B_2, 38.1% G_1, and 15.4% G_2.

[c] Lower than the limit of determination.

mary storage or processing (domestic products) and sites of importation, according to a sampling plan based on the UK plan for peanuts (11). A 10 kg sample of each item (nuts, cereals, beans, dried fruit, etc.), or a 20 kg sample of items in shells, was taken as randomly as possible from a lot containing up to 25 000 kg. The whole large sample (the 10 kg sample or the 20 kg sample of items in shells) was sufficiently mixed, ground, and homogenized. After suitable subsampling by quartering, the material was analyzed for AFs. Samples of imported and domestic products were also taken from the market. A total of 6362 samples (4709 samples of nuts and nut products; 486 samples of cereals; 390 samples of pulses; 539 samples of seeds and related products; and 238 samples of coffee beans, dried fruit, and other foods) were tested for the presence of AFs B_1, B_2, G_1, and G_2.

(**b**) *For analysis of AF M_1.*—Samples of raw milk (1 L each) were collected from several farms in Cyprus in 1993, 1995, and 1996. Pasteurized milk, evaporated milk, and baby milk powder were collected from the market. A total of 112 samples (71 samples of raw milk and 41 samples of other types of milk) were analyzed for AF M_1.

Reagents

(**a**) *Solvents and reagents.*—All solvents used were high performance liquid chromatographic (HPLC) grade, and the reagents were analytical grade.

(**b**) *Standards.*—Standards for AFs B_1, B_2, G_1, G_2, and M_1 were purchased from Sigma Chemical Co., St Louis, MO. Standard and stock solutions were prepared and assayed according to AOAC Method **970.44** (12).

(**c**) *Water.*—(*1*) *Distilled.*—For preparation of samples. (*2*) *Further purified with Milli-Q Plus purification system.*—For chromatographic use.

(**d**) *Certified reference materials (CRMs).*—The following CRMs were purchased from the Community Bureau of Reference, Brussels, Belgium: (*1*) *Peanut butter CRM No. 385.*—Certified values for AF B_1, 7.0 ± 0.8 µg/kg; AF B_2, 1.1 ± 0.2 µg/kg; AF G_1, 1.7 ± 0.3 µg/kg; AF G_2, 0.3 ± 0.2 µg/kg; total AF, 10.1 ± 1.5 µg/kg. (*2*) *Whole milk powder CRM No. 285.*—Certified value, 0.76 ± 0.05 µg AF M_1/kg, which corresponds to 0.076 µg/L after reconstitution.

(**e**) *Quality control samples.*—FAPAS (Food Analysis Performance Assessement Scheme; CSL Food Science Laboratory, Norwich, UK) nut powder test material No. T0410. Assigned values: AF B_1, 18 µg/kg; AF B_2, 3 µg/kg; AF G_1, 10 µg/kg; AF G_2, 1 µg/kg; total AF, 32 µg/kg.

Apparatus

(**a**) *Grinding and mixing devices.*—(*1*) *Food cutter.*—Cuisine Systeme 5100, automatic (Magimix, Bourgogne, France). (*2*) *Waring blender.*—Vicam, Watertown, MA.

(**b**) *Immunoaffinity columns (IAC).*—Aflatest-P and all other relevant accessories from Vicam.

(**c**) *Fluorimeter.*—Torbex Model FX-100 (Vicam).

(**d**) *High performance thin-layer chromatography (HPTLC) plates.*—Silica gel 60 without fluorescence indicator, 10 × 20 cm (Merck, Darmstadt, Germany).

(**e**) *Solid-phase extraction (SPE) columns.*—Supelclean Lc-Si, 3 mL (Supelco, Deisenhofen, Germany).

Table 3. Incidence and level of aflatoxin M_1 in milk, 1993, 1995, and 1996

Sample	No. of samples	No. of positives[a]	Range (avg of positives), μg/L
Milk raw (cow)[b]	71	3	0.03–0.04 (0.035)
Pasteurized milk, full[b]	19	5	0.01[c]–0.02 (0.015)
Pasteurized milk, light[b]	4	1	0.01[c]
Pasteurized milk, skimmed[b]	8	3	0.01[c]–0.04 (0.02)
Baby milk (imported)	6	—	—
Evaporated milk (imported)	4	—	—
Total	112	12	—

[a] Positive samples were those with AF M_1 >0.005 μg/L, by the IAC/HPLC method (13).
[b] Data were corrected for 42.8% average recovery.
[c] Limit of determination, with recovery of 23.6%.

(f) *Fluorodensitometer and scanner.*—Shimadzu CS-9000 (Shimadzu, Kyoto, Japan).

(g) *HPLC system.*—Pump, Waters 600; sample injector, U6K; fluorescence detector, Waters 470 (excitation at 355 nm, emission at 433 nm); reversed-phase column, Waters Nova Pak C_{18} (Millipore, Milford, MA); recorder and integrator, Varian 4400 (Varian Associates, Walnut Creek, CA); LC Software Millenium from Waters; mobile phase, H_2O–CH_3OH–CH_3CN (80 + 10 + 10); flow rate, 2 mL/min (13).

(h) *Water purification system.*—Milli-Q Plus (Millipore).

Determination of AFs B_1, B_2, G_1, and G_2

(a) *Screening by Aflatest.*—Cleanup of samples and isolation and purification of AFs were performed with IACs followed by fluorometric detection (14; detection limit range, 1–2 μg total AFs/kg).

(b) *Determination.*—Samples that gave an Aflatest reading >2 μg total AFs/kg were analyzed either by (*1*) SPE cleanup followed by HPTLC analysis with scanner fluorodensitometric detection (15) or (*2*) IAC cleanup as mentioned above (14) with the following modifications: 30 mL of the filtered diluted sample extract (instead of 15 mL) was passed through the IAC, the aflatoxins were eluted with 1 mL CH_3OH, the eluant was evaporated to dryness on a rotary evaporator at 35°C, and the residue was reconstituted with 200 μL $CHCl_3$ and analyzed by HPTLC with scanner fluorodensitometric detection (15). In cases of inadequate separations of AFs B_1, B_2, G_1, and G_2, the alternative developing solvents proposed by AOAC Method **975.35** (12) were used. $CHCl_3$–C_2HCl_3–*n*-amyl alcohol–HCOOH (80 + 15 + 4 + 1) was the most successful alternative to $CHCl_3$–acetone (9 + 1; 15).

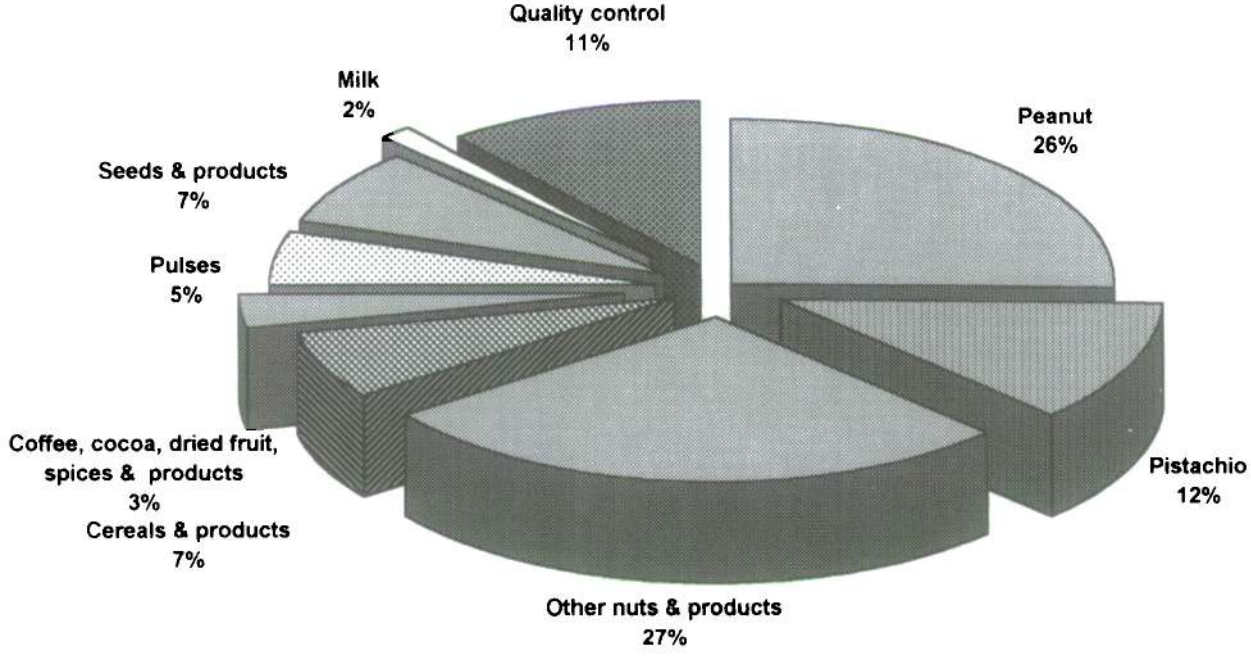

Figure 1. Mean distribution of samples analyzed for aflatoxins, 1992–1996. Total number of samples (*N*) is 7251, including quality control samples.

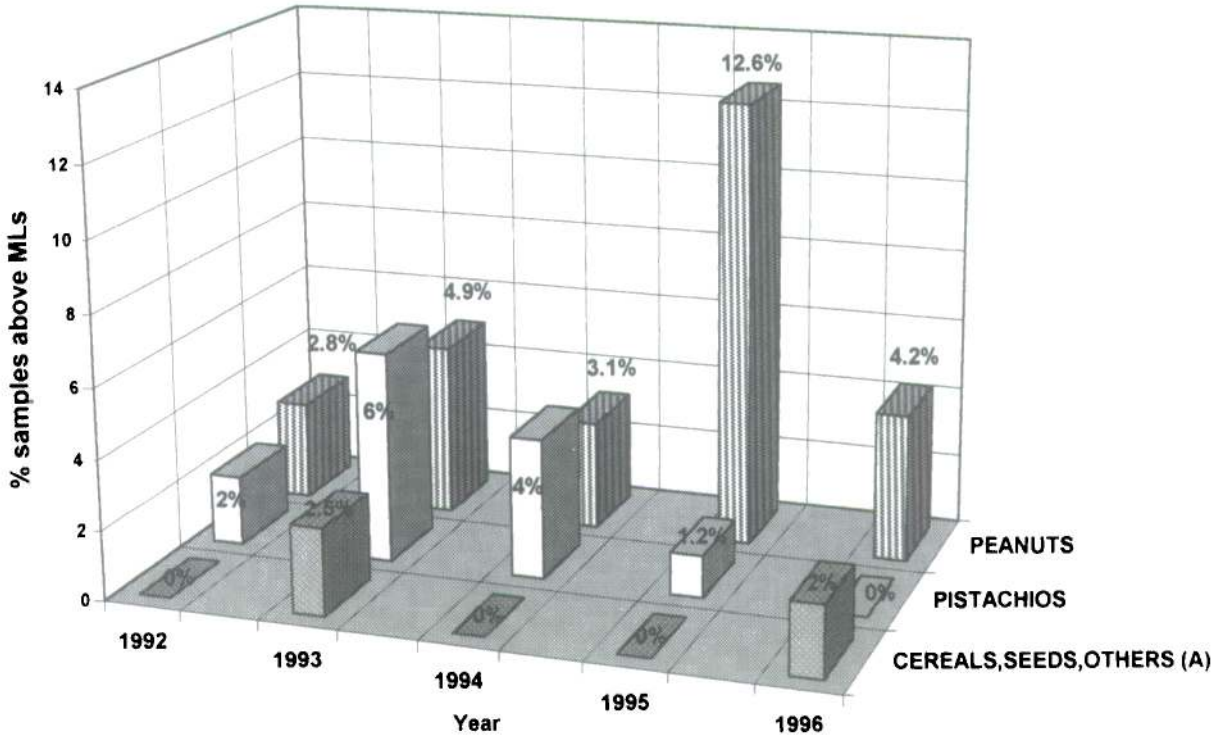

Figure 2. Aflatoxin control per food item and group violation rates, 1992–1996: (A) includes pulses, coffee, cocoa and cocoa products, dried fruit and dried fruit products, spices, and other nuts and nut products (Brazil nuts, chestnuts, walnuts, peanut butter, etc.).

(c) *Confirmation of identities of AFs B_1, B_2, G_1, and G_2.*—Confirmation of AF identity was performed as prescribed in AOAC Methods **978.15F**, **968.22F(e)**, and **975.37** (12).

Determination of AF M_1 in Milk

(a) *Determination.*—AF M_1 in milk was analyzed by an IAC cleanup followed by HPLC with fluorescence detection, a method that had been reported earlier (13).

(b) *Confirmation of identify of AF M_1.*—Confirmation of identity of AF M_1 was performed as prescribed in AOAC Method **980.21F** (12) at spiking levels of 0.050 μg/L and higher.

Results and Discussion

Results are presented in Tables 1–3 and in Figures 1–6. Figure 1 shows the mean distribution of the various types of foods analyzed for AFs, including quality control samples (11%) for analytical quality assurance (intra- and interlaboratory controls). Nuts constituted the highest percentage of samples analyzed (65%), followed by seeds, cereals, pulses, and other foods.

Analytical Quality Assurance

(a) *Internal quality control.*—For internal quality control, the following procedures were performed: (*1*) replicate analysis of spiked samples of several food matrixes for recovery checks, which was performed for each batch of IAC and SPE column and at least 4 times a year; (*2*) CRMs and quality control samples analyzed every 30 samples; (*3*) control charts for mean recovery checks and quality control samples; (*4*) confirmation of results and identity of AF, especially in samples exceeding MLs by a different method and by another analyst.

Recoveries of AFs B_1, B_2, G_1, and G_2 after SPE cleanup and analysis by HPTLC were 71–99%. Average recoveries from pistachios spiked at 5 μg/kg were AF B_1, 85.5%; AF B_2, 80.0%; AF G_1, 83.0%; and AF G_2, 92.0%. Average recoveries from peanuts spiked at 15 μg total AFs/kg (AF B_1, 5 μg/kg; AFs B_2 and G_1, 4 μg/kg; and AF G_2, 2 μg/kg) were AF B_1, 85.0%; AF B_2, 71.0%; AF G_1, 85.0%; and AF G_2, 81.8%. Recoveries after IAC cleanup and analysis by HPTLC were 15–87%. Average recoveries from samples spiked at 10 μg total AFs/kg (AF B_1, 5 μg/kg; AFs B_2 and G_2, 1 μg/kg; AF G_1, 3 μg/kg) were 54.2% AF B_1, 34.8% AF B_2, 38.1% AF G_1, and 15.4% AF G_2 for peanuts and 46.3% AF B_1, 37.0% AF B_2, 39.1% AF G_1, 17.0% AF G_2 for pistachios. Repeatabilities varied between 4–18% for both methods. Similar low recoveries, especially for AF G_2, have been reported (16) at this low spiking level. Nevertheless, the incidence and levels of AF G_2 in the samples analyzed were very low, and the results of the IAC method were confirmed in many cases by the SPE method. Recoveries from other food matrixes (peanut butter and maize) spiked at 1–20 μg/kg for each AF were also checked (data to be published).

When recoveries were lower than 80%, a correction for recovery was made. This correction was applied mainly to the IAC method. The detection limit was 0.1 μg/kg for each AF. Limits of determination were 4 μg/kg for AF B_1 and AF G_1 and 0.3 μg/kg for AF B_2 and AF G_2 for both methods. Recov-

eries from pistachios at the limit of determination by the SPE method were 67.5% AF B_1, 45.0% AF B_2, 65.7% AF G_1, and 27.0% AF G_2. Recoveries from peanuts by the IAC method were 26.3% AF B_1, 20.9% AF B_2, 31.0% AF G_1, and 11.0% AF G_2. Repeatabilities at the limit of determination varied between 8 and 40% for both methods.

In the period 1993–1996, the IAC cleanup method was preferred over the SPE method for routine purposes, because the large number of samples required a simple and specific procedure. The whole sample pretreatment was reduced to a one-step extraction, and the repeatabilities were adequate to allow implementation of the method, with results corrected for recovery (when recovery was lower than 80%).

Typical average recoveries of AF M_1 in milk by IAC cleanup followed by analysis by HPLC with fluorescence detection (13) were 42.8, 57.2, 89.0, and 94.9% for spiking levels of 0.050, 0.100, 0.3, and 0.5 μg/L, respectively. The limit of determination for AF M_1 was 0.010 μg/L, with a recovery of 23.6%. Repeatabilities varied between 3 and 15%. The limit of detection for AF M_1 was 0.005 μg/L.

The methods for AF determination were also subjected to quality control by analysis of CRMs and quality control samples. Results for CRM No. 385 peanut butter by the IAC method corrected for recovery were 5.8 μg AF B_1/kg, 0.8 μg AF B_2/kg, 1.5 μg AF G_1/kg, 0.3 μg AF G_2/kg, and 8.4 μg total AFs/kg. Results for the analysis of CRM No. 285 whole milk powder corrected for recovery were 0.60 μg AF M_1/kg powder. Results of IAC analysis of the quality control sample FAPAS No. T0410 nut powder were 14 μg AF B_1/kg, 3 μg AF B_2/kg, 11 μg AF G_1/kg, 1 μg AF G_2/kg, and 29 μg total AFs/kg. The value for total AFs by the Aflatest solution fluorometry method (14) was 30 μg/kg. Results were in good agreement with certified or assigned values.

(**b**) *External quality control.*—External quality control for both SPE and IAC cleanup methods was performed by participation in proficiency testings for AF analysis in nut powder test materials (17). Results were satisfactory, with *z*-scores between –2 and +2 for each AF. External quality control also was achieved by participation in the AF B_1 check sample (maize meal) survey program performed in 1994 by the World Health Organization and the International Agency for Research on Cancer in Lyon, France. Results were satisfactory: AF B_1 found, 19.0 μg/kg; reported median value (from statistical analysis), 20.75 μg/kg; and mean value, 21.95 μg/kg.

AFs B_1, B_2, G_1, and G_2

The cumulative results in Table 1 show that AFs B_1, B_2, G_1, and G_2 were detected in only 16 of 1653 samples (1% positive) of imported or locally produced cereals, pulses, coffee beans, cocoa products, seeds, dried fruit, and spices. The relatively low number of contaminated samples (9 samples of imported sesame seeds and sesame seed products, 2 imported

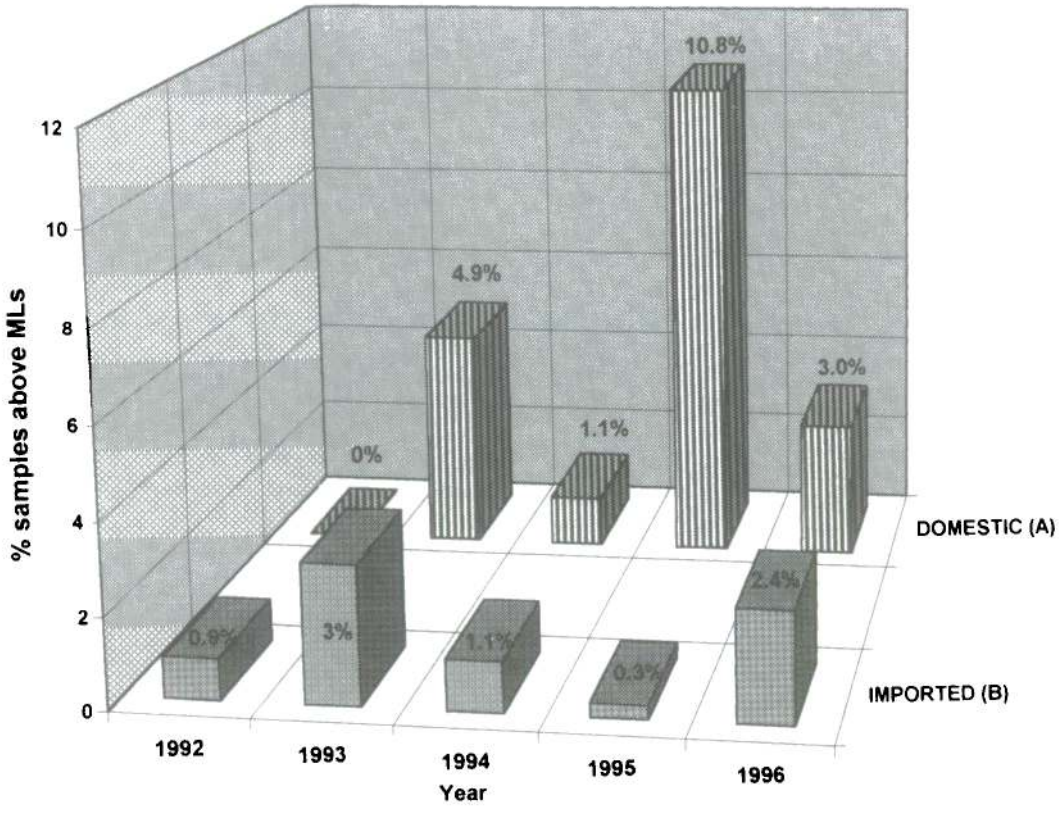

Figure 3. Aflatoxin control for nondairy foodstuffs: domestic and import violation rates, 1992–1996: (A) includes wheat, pulses, peanuts, almonds, walnuts, hazelnuts, raisins, and fig and fig products; (B) includes cereals and cereal products, coffee, cocoa and cocoa products, dried fruit, seeds, pulses, spices, nuts and nut products (peanuts, peanut butter, pistachios, walnuts, almonds, hazelnuts, chestnuts, Brazil nuts, cashew nuts, etc.).

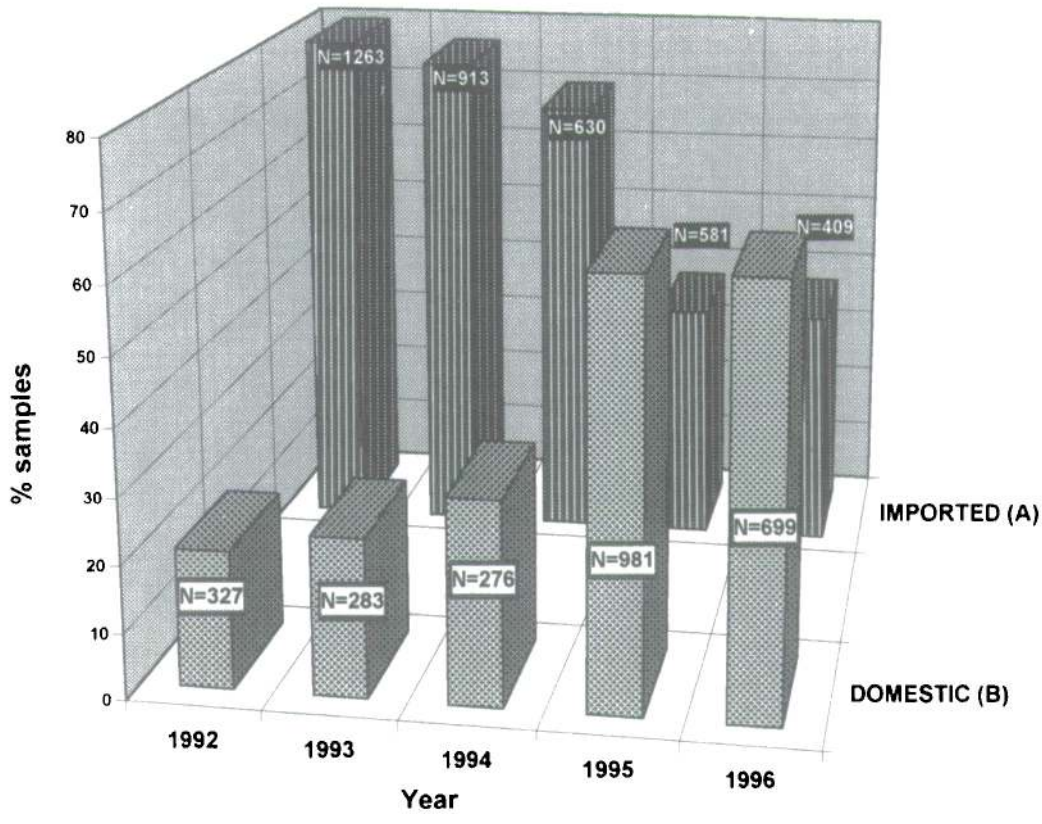

Figure 4. Aflatoxin control for domestic and imported nondairy foodstuffs, 1992–1996. *N* is the number of samples analyzed per year. (A) Includes cereals and cereal products, coffee, cocoa and cocoa products, dried fruit, seeds, pulses, spices, and nuts and nut products (peanuts, peanut butter, pistachios, Brazil nuts, walnuts, almonds, hazelnuts, cashew nuts, chestnuts, etc). (B) Includes wheat, peanuts, almonds, walnuts, hazelnuts, raisins, figs and fig products, and pulses.

corn samples, 4 local fig products taken from the market, and 1 imported turmeric sample) indicates that the products sampled can resist the fungal infection when preventive measures are taken (e.g., proper drying and storage conditions). Results per year are shown in Figure 2.

Measurable levels, sometimes above the MLs, of AFs in peanuts (local and imported from Egypt), peanut butter (imported), pistachios (imported from Iran), Brazil nuts (imported from The Netherlands), chestnuts (imported from Italy), and walnuts (imported from China) were found (Table 2 and Figure 2), indicating that the nuts and nut products examined were sensitive to AF contamination. Violation rates of locally produced foodstuffs (wheat, pulses, peanuts, almonds, walnuts, figs, raisins, etc.) were comparable with those of imported foodstuffs (cereals, pulses, seeds, coffee, cocoa, peanuts, pistachios, Brazil nuts, etc.) in all years except 1995 (Figure 3).

The highest level of AF B_1 (700 μg/kg) and the highest percentage of samples with levels above MLs (12.6%) were detected in 1995 in stored local peanuts produced in 1994 (Table 2 and Figures 2 and 3). This finding strongly indicated a need for more intensive control (proper drying of peanuts, inspection of storage conditions such as temperature and humidity, sampling, retainment, analysis, destruction of lots with levels of AFs above MLs) of locally produced peanuts at primary storage. This finding also led to an increase in the number of domestic peanut and other samples tested for AFs during 1995 and 1996, compared with the period 1992–1994 (Figure 4). Moreover, the number of samples of imported foodstuffs analyzed between 1992–1994 was higher than the number of domestic products, but not in subsequent years (1995 and 1996).

The highest incidence of AF contamination was observed in 1993, with the major contaminated food item being imported peanut butter (56.7% positive samples, which were also violative; Table 2 and Figures 2 and 3). Identities of AFs B_1, B_2, G_1, and G_2, especially in the cases of violative samples, were confirmed as prescribed in AOAC Methods **978.15**, **968.22**, and **975.37** (12).

Generally, the percentage of samples with AF levels above MLs varied between 0.7 and 6.9% for 1992–1996 and between 0.7 and 12% for 1990–1996 (Figure 5). These results show fluctuations, but the overall declining trend indicates the effectiveness of the monitoring program, as well as the need for constant surveillance and control of AFs, especially at critical control points.

In general, samples with AF levels above MLs were detected before being released to the market through control (inspection, sampling, retainment, analysis, etc.) at critical control points and were retained for further action. More specifically, imported lots were sampled and retained in cus-

toms until analysis was completed. If the AF level in samples was above MLs, the respective lots were returned to the country of origin or destroyed. Locally produced foodstuffs were sampled and retained at the stage of primary storage or processing site until the end of analysis. If AF levels were above MLs, the respective lots were rejected and destroyed. The same procedure was applied for samples with AF levels above MLs obtained from the market and for exported food items before their export.

The aims of the program are to prevent entry of contaminated samples in the market, to keep levels of AFs at the lowest possible level, and to reduce the probable health risk due to the consumption of dietary AFs.

The results (Tables 1 and 2) show that these goals were attained to a great extent. The AF levels and the percentage of samples with AF levels above MLs (Figures 2 and 3) are lower than or comparable with those of other developed countries such as the United Kingdom (18), the United States (19), and Japan (6).

Monitoring of AFs must be continued, methods must be improved for better recoveries (14), and control must be extended to other food items such as spices, herbs, and soybeans, which have not been systematically monitored, and are widely used in prepared foods consumed in Cyprus and in the Mediterranean region.

AF M_1

The AF M_1 levels in local raw or pasteurized milk samples in 1993, 1995, and 1996 (Table 3) were not only below the ML specified by Cyprus law (0.5 μg/L; 4) but also below the lower ML (0.05 μg/L) adopted by EU countries such as Germany and The Netherlands (5). Only a small percentage of raw and pasteurized milk samples (12% average for 1993, 1995, and 1996) contained detectable levels of AF M_1 (0.010–0.040 μg/L; Table 3 and Figure 6). The results (Figure 6) show a general decline, with samples analyzed in 1996 showing no detectable levels of AF M_1 (<0.005 μg/L). Similar results have been reported by researchers in Italy (20), Germany (21), and Greece (22). This finding indicates that the Cyprus ML for AF M_1 can be lowered to 0.050 μg/L to harmonize with the ML of the EU. Average recovery of AF M_1 spiked at 0.050 μg/L was 42.8% after IAC cleanup and HPLC analysis with fluorescence detection (13). The identity of AF M_1 in milk at 0.050 μg/L was confirmed as prescribed in AOAC Method **980.21F** (12). Moreover, none of the imported baby milk powders nor any of the evaporated milk samples tested contained detectable levels of AF M_1.

The control of various types of imported milk should be expanded because, at present, the number of imported samples being analyzed is small. Furthermore, the control on locally produced milk should be continued and the monitoring of AF M_1 should be extended to locally produced and imported dairy products.

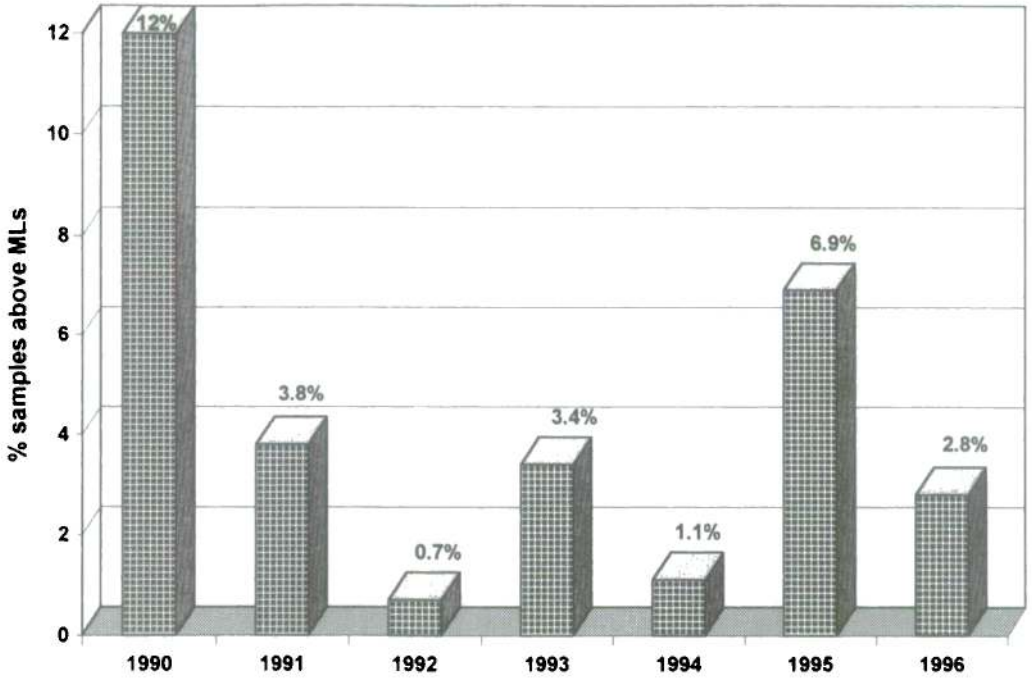

Figure 5. Aflatoxin control 1990–1996, violation rates for nondairy foodstuffs. The numbers of samples (*N*) analyzed per year are 700, 1645, 1590, 1196, 906, 1562, and 1108 for the years 1990 through 1996, respectively. Analytical data for 1990 and 1991 are not included in this paper, but their violation rates are included in this figure to show the general trend.

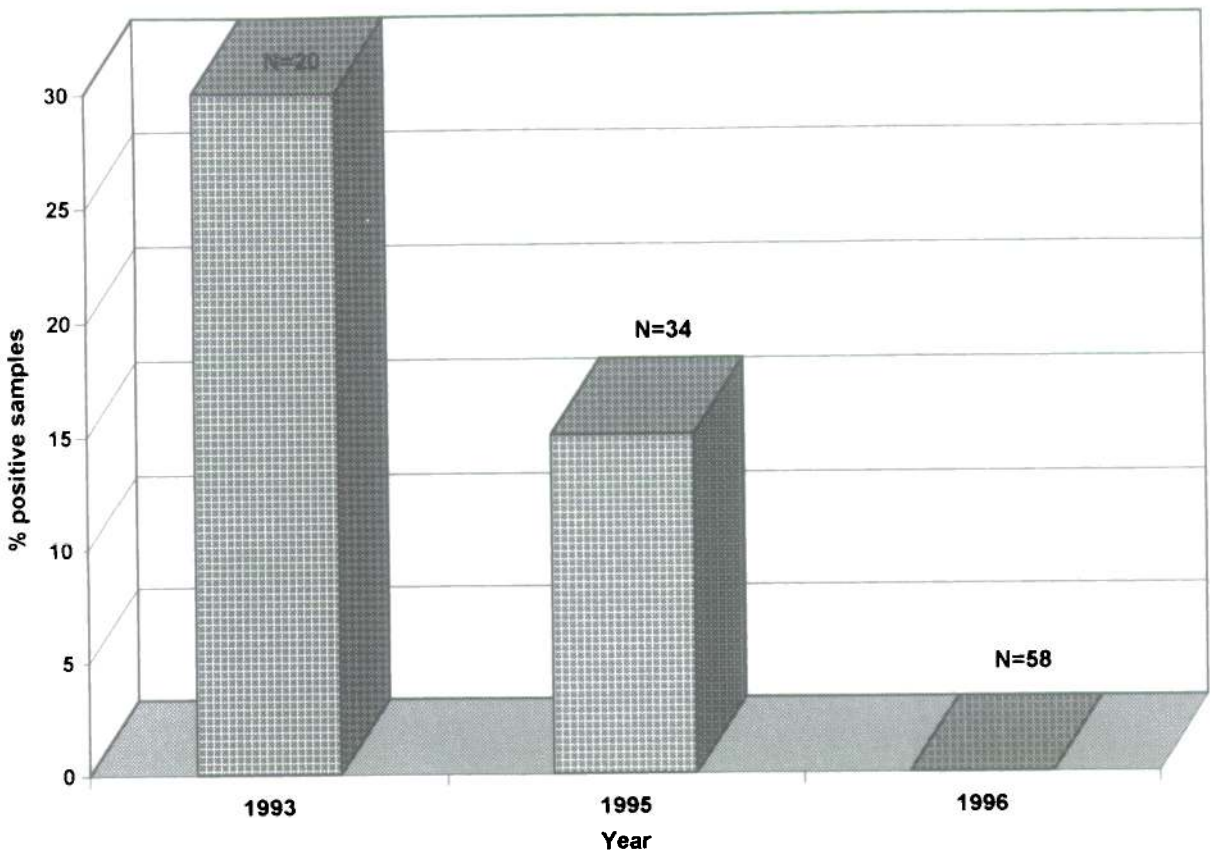

Figure 6. Incidence of aflatoxin M_1 in milk in 1993, 1995, and 1996. *N* is the number of samples analyzed per year. Positive samples were those with detectable levels of AF M_1, that is, >0.005 μg/kg by the IAC/HPLC method (13).

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