

# Survey of Aflatoxin M<sub>1</sub> Contamination of Dairy Products in Taiwan

LAN-CHI LIN, FANG-MING LIU, YOU-MIN FU AND DANIEL YANG-CHIH SHIH\*

Bureau of Food and Drug Analysis, Department of Health, Executive Yuan, 161-2 Kunyang St., Nangang District, Taipei City 115, Taiwan, R.O.C.

(Received: August 19, 2003; Accepted: November 4, 2003)

## ABSTRACT

Owing to their nutritional value, dairy products were popular in Taiwan. Aflatoxin M<sub>1</sub> (AFM<sub>1</sub>) was the metabolite of potential carcinogen aflatoxin B<sub>1</sub> and found in dairy products. An analytical method using immunoaffinity column for extraction and HPLC for quantification was developed for AFM<sub>1</sub>. The detection limits for fresh milk (pasteurized milk), milk powder and drinking yogurt (0.002, 0.02 and 0.005 ppb, respectively) were 10 times lower than that in the former survey. The recoveries of AFM<sub>1</sub> from fresh milk were  $83.3 \pm 2.9$  and  $89.5 \pm 2.9\%$  at 0.5 and 0.05 ppb spiked levels, respectively. Spiked 5 and 0.5 ppb AFM<sub>1</sub> in milk powder, the recoveries were  $86.0 \pm 1.9$  and  $88.7 \pm 1.9\%$ , respectively. The recoveries of AFM<sub>1</sub> from drinking yogurt were  $99.9 \pm 1.4$  and  $94.8 \pm 3.2\%$  at 0.5 and 0.05 ppb spiked levels, respectively. It was the first time AFM<sub>1</sub> was tested in drinking yogurt in Taiwan in this survey, and the performance for detecting AFM<sub>1</sub> in drinking yogurt was also evaluated through attending an international proficiency test. Our laboratory got a satisfactory result. In order to survey AFM<sub>1</sub> contents in dairy products, 44 samples of fresh milk, 45 samples of milk powder and 24 samples of drinking yogurt were collected from supermarkets, convenience stores and drug stores located in 23 counties of Taiwan from June to August, 2002. The results showed that AFM<sub>1</sub> was detected in 40 samples of fresh milk at 0.002–0.083 ppb level. AFM<sub>1</sub> was not found in all milk powders. AFM<sub>1</sub> was detected in 3 samples of drinking yogurt, at the level of 0.007, 0.009 and 0.044 ppb. According to the food sanitary standard regulation in Taiwan, the action levels of AFM<sub>1</sub> were 0.5 ppb, 5 ppb and not-detectable for fresh milk, milk powder and infant formula products, respectively. The 113 samples collected in this survey all met the regulation requirements.

Key word: aflatoxin M<sub>1</sub>, dairy product, immunoaffinity column, high performance liquid chromatography

## INTRODUCTION

Aflatoxins, a group of several toxic secondary fungal metabolites produced by some *Aspergillus* spp., are found in a wide variety of foods and feeds around the world. Aflatoxin M<sub>1</sub> (AFM<sub>1</sub>), a hydroxylated metabolite of the potential carcinogen Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) (Figure 1), occurs in milk from lactating animals consuming feed contaminated with AFB<sub>1</sub><sup>(1)</sup>. About 0.3–6.2% of AFB<sub>1</sub> in animal feed is transformed to AFM<sub>1</sub> in milk and a linear relationship has been found between intake of AFB<sub>1</sub> in contaminated feed and the AFM<sub>1</sub> content of milk in cows<sup>(2)</sup>. Following the withdrawal of the contaminated feed, AFM<sub>1</sub> levels in milk decrease to below the limit of detection within 72 hr<sup>(3)</sup>.

The potential hazardous human exposure to AFM<sub>1</sub> via consumption of milk and milk products has been demonstrated<sup>(4,5,6,7)</sup>. Both AFB<sub>1</sub> and AFM<sub>1</sub> can cause DNA

damage, gene mutation, chromosomal anomalies and cell transformation in mammalian cells *in vitro*, in insects, lower eukaryotes and bacteria<sup>(8)</sup>. However, AFM<sub>1</sub> is less carcinogenic and genotoxic than AFB<sub>1</sub><sup>(2)</sup>.

To protect consumers, particularly children, from contaminated dairy products, several countries have established legislation to regulate the levels of AFB<sub>1</sub> in feeds and AFM<sub>1</sub> in milk (Table 1). The Food and Drug Administration (FDA) of US has established an action level of 0.50 ppb in whole, low fat and skim milk<sup>(9)</sup>, whereas the EU has set a maximum admissible level of 0.05 ppb in raw milk, heat-treated milk, and milk for the manufacture of milk based products<sup>(10)</sup>. In Taiwan, the action levels in fresh milk, milk powder and infant formula dairy products are 0.5 ppb, 5 ppb and not-

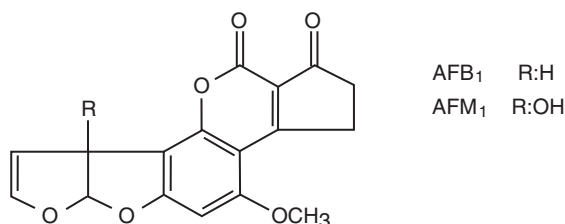


Figure 1. Chemical structures of AFB<sub>1</sub> and AFM<sub>1</sub>.

\* Author for correspondence. Tel: 886-2-26531265; Fax: 886-2-26531268; E-mail: shihdyc@nlf.gov.tw

Table 1. Regulatory limits for AFM<sub>1</sub> in various countries

| Country     | Milk (ppb)       | Infant formula (ppb) |
|-------------|------------------|----------------------|
| Argentina   | 0.5 <sup>a</sup> | 0.1                  |
| Austria     | 0.05             | 0.01                 |
| Brazil      | 0.5              | 0.01                 |
| France      | 0.2              |                      |
| Germany     | 0.05             | 0.01                 |
| Italy       | 0.05             | 0.05                 |
| Netherlands | 0.05             | 0.05                 |
| Switzerland | 0.05             | 0.01 <sup>b</sup>    |
| US          | 0.50             |                      |
| EU          | 0.05             |                      |
| Taiwan      | 0.5              | Not-detectable       |

<sup>a</sup>Sum of B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub> and M<sub>1</sub>.

<sup>b</sup>Sum of B<sub>1</sub> and M<sub>1</sub>.

detectable, respectively<sup>(11,12)</sup>.

Monitoring surveys are frequently conducted in several countries all over the world to determine the levels of AFM<sub>1</sub> in milk and milk products. In Taiwan, 161 raw milk and milk powder had been examined according to the AOAC method in 1986, and no AFM<sub>1</sub> contamination in these samples was found<sup>(13)</sup>. Using a silica-gel column to extract and clean up all 110 dairy samples, 44 of 50 (88%) fresh milk, 15 of 25 (60%) milk powder, 6 of 10 (60%) condensed milk and 0 of 25 cheese samples were detected the contamination of AFM<sub>1</sub><sup>(14)</sup>. Fu determined the AFM<sub>1</sub> in milk and milk powder using an immunoaffinity column and fluorescence measurement. AFM<sub>1</sub> was detected in 3 of 25 (12%) fresh milk and 0 of 25 milk powder samples<sup>(15)</sup>. However, no survey has referred to the occurrence of AFM<sub>1</sub> in drinking yogurt. The objectives of this work were to establish a more sensitive method to determine the AFM<sub>1</sub> in dairy products (especially in drinking yogurt) and to understand the contamination of AFM<sub>1</sub> in fresh milk, milk powder and drinking yogurt in Taiwan.

## MATERIALS AND METHODS

### I. Materials

#### (I) Sample collection

The feed in Taiwan is much easier to be contaminated with AFB<sub>1</sub> due to the warm and humid weather in summer; therefore, this survey was conducted during June ~ August. The samples were collected from supermarkets, convenience stores and drug stores of 23 counties in Taiwan. Forty-four samples were fresh milk, 45 samples were milk powder (of which 21 were infant formula) and 24 samples were drinking yogurt. The minimum sample size was 500 mL or g. The samples were conserved in a refrigerator during the transfer to the laboratory. In the laboratory, fresh milk and drinking yogurt samples were stored in a refrigerator ( $5 \pm 2^\circ\text{C}$ ), and milk powder samples were stored at a temperature below  $25^\circ\text{C}$ . All samples were analyzed before the expiration date.

#### (II) Reagents

AFM<sub>1</sub> standard was purchased from Supelco (Bellefonte, PA., U.S.A.). The immunoaffinity columns AflaM<sub>1</sub><sup>TM</sup> were purchased from Vicam (Watertown, MA., U.S.A.). Sodium hydroxide was reagent grade. Acetonitrile and methanol used for the liquid chromatographic mobile phases were of LC grade. Distilled, deionized water was used throughout the procedure.

### II. Methods

#### (I) Samples preparation

The procedures were modified from Dragacci *et al.*<sup>(16)</sup>

and Tuinstra *et al.*<sup>(17)</sup> Fresh milk was warmed before analysis to ca  $37^\circ\text{C}$  in a water bath, and then stirred gently with a magnetic stirrer to disperse the fat layer. Ten grams of milk powder (including infant formula) were dissolved with 50 mL of warm water, and mixed with stirring until a homogeneous mixture was obtained. The solution of milk powder was transferred to a 100-mL volumetric flask and diluted to the mark. Liquid milk (fresh milk and the solution of milk powder) was centrifuged for 10 min at  $2000\times g$  to separate the fat, and the upper fat layer was discarded. The samples were filtered through one or more paper filters, and at least 50 mL of the filtrate was collected. Twenty-five grams of drinking yogurt was warmed as fresh milk and the pH was adjusted to 6.5 with 0.1N NaOH before passing samples through the clean-up column.

#### (II) Immunoaffinity clean up

Fifty mL of liquid milk and pH-adjusted drinking yogurt were passed at about 1-2 mL/min through an AflaM<sub>1</sub><sup>TM</sup> immunoaffinity column fitted with a 60-mL syringe reservoir. Twenty mL of water was used to wash the loaded immunoaffinity column at a steady flow rate. The column was blown to dryness with a stream of nitrogen, and AFM<sub>1</sub> was eluted with 4 mL of acetonitrile. The acetonitrile eluate was evaporated to dryness under a gentle stream of nitrogen, and the residue was redissolved by vortexing with 2 mL of mobile phase, which was then filtered through a  $0.45\ \mu\text{m}$  microfilter for HPLC analysis.

#### (III) HPLC analysis

The LC system consisted of a Shimadzu (Kyoto, Japan) LC-10AT<sub>VP</sub> pump, a RF-10A<sub>XL</sub> fluorescence detector, and a Waters 717<sub>plus</sub> autosampler (Waters, MA., U.S.A.), all under the control of the Shimadzu SCL-10A<sub>VP</sub> system. Data acquisition was performed on an SISC program. The column ( $150 \times 4.6\ \text{mm}$ , Cosmosil 5C18-AR,  $5\ \mu\text{m}$ , Nacalai, Japan) was maintained at  $30^\circ\text{C}$ . Injection volume was 200  $\mu\text{L}$  and the detector wavelength settings were 365 nm (excitation) and 435 nm (emission). The mobile phase, water/acetonitrile/methanol (68/24/8, v/v/v), was pumped at a constant flow rate of 1.0 mL/min.

#### (IV) Calibration

AFM<sub>1</sub> standard was diluted with mobile phase to prepare a series of working solutions containing 0.002-10 ng AFM<sub>1</sub>/200  $\mu\text{L}$ . A calibration curve was constructed by plotting the peak area for each standard against the mass of AFM<sub>1</sub> injected. Slope and intercept data of the calibration curve were used to compute the quantity of the analyte in sample extracts.

#### (V) Validation

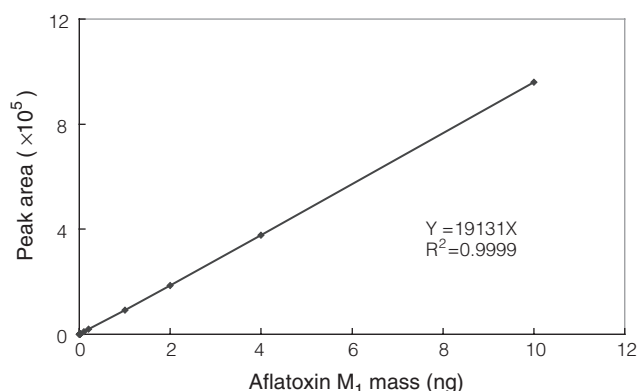
Analytical methods for AFM<sub>1</sub> determination from

three kinds of dairy products were validated in-house. Two each of AFM<sub>1</sub>-free fresh milk and drinking yogurt samples were spiked with 0.05 and 0.5 ppb AFM<sub>1</sub> standards. Two AFM<sub>1</sub>-free milk powders were spiked with 0.5 and 5 ppb AFM<sub>1</sub> standards, then dissolved with warm water. Then the following procedures were according to those described above. The method detection limit (MDL) of the chromatographic procedure was measured according to the Standard Operation Procedure for the MDL determination of Bureau of Food and Drug Analysis<sup>(29)</sup> as follows:

AFM<sub>1</sub> standards of serial concentrations were injected into and analyzed by the LC system. A curve was constructed by plotting the peak area for each standard against the mass of AFM<sub>1</sub> injected. The concentration where the slope of the standard curve changed markedly was defined as IDL (instrument detection limit). To obtain the MDL, an AFM<sub>1</sub> standard at a concentration equal to 1~5 times IDL was spiked into AFM<sub>1</sub>-free samples, followed by the analytical procedures described above. Standard deviation (Sa) was calculated from 7 replicates. An AFM<sub>1</sub> standard at the concentration equal to 3 times Sa was spiked into AFM<sub>1</sub>-free samples, followed by the analytical procedures described above. Standard deviation (S) was calculated from 7 replicates. The bigger one of Sa<sup>2</sup> and S<sup>2</sup> was chosen as the numerator. If the ratio was smaller than 3.05, the pooled standard deviation (S<sub>pool</sub>) and MDL were calculated using the following regulations :

$$S_{\text{pool}} = [(6S^2 + 6Sa^2) / 12]^{1/2}$$

$$\text{MDL} = 2.681 \times S_{\text{pool}}$$



**Figure 2.** Calibration curve of AFM<sub>1</sub> analyzed by HPLC.

**Table 2.** Recoveries of AFM<sub>1</sub> in dairy products

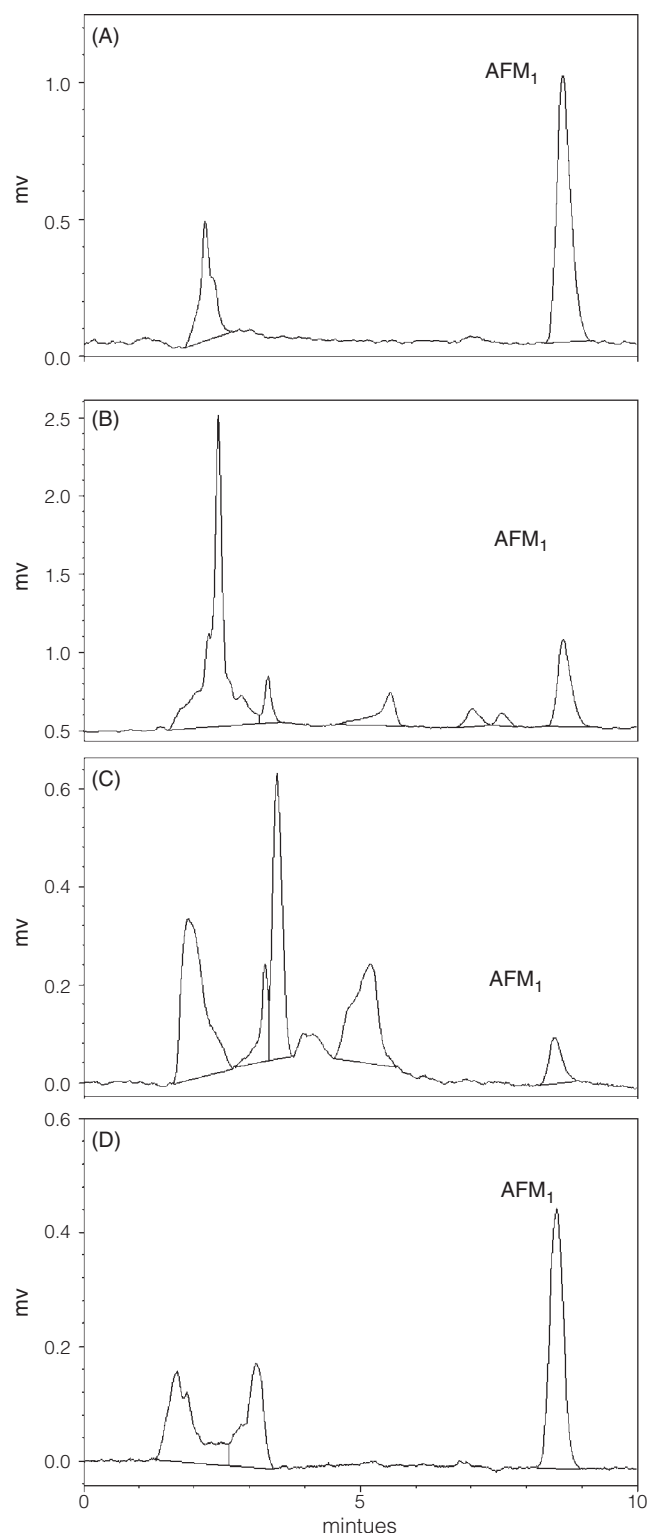
| Dairy product   | Spiked levels (ppb) | Recovery <sup>a</sup> (%) | CV <sup>b</sup> (%) |
|-----------------|---------------------|---------------------------|---------------------|
| Fresh milk      | 0.05                | 89.5 ± 2.9                | 3.2                 |
|                 | 0.5                 | 83.3 ± 2.9                | 3.5                 |
| Drinking yogurt | 0.05                | 94.8 ± 3.2                | 3.4                 |
|                 | 0.5                 | 99.9 ± 1.4                | 1.4                 |
| Milk powder     | 0.5                 | 88.7 ± 1.9                | 2.1                 |
|                 | 5                   | 86.0 ± 1.9                | 2.2                 |

<sup>a</sup>Average of triplicate analysis ± standard deviation.

<sup>b</sup>CV: coefficient of variation.

## (VI) Quantification

AFM<sub>1</sub> mass concentration of the test sample was calculated using the following equation:



**Figure 3.** HPLC chromatograms of (A) AFM<sub>1</sub> standard, (B) AFM<sub>1</sub>-spiked fresh milk, (C) AFM<sub>1</sub>-spiked milk powder, (D) AFM<sub>1</sub>-spiked drinking yogurt. HPLC conditions: Cosmosil 5C18-AR; mobile phase, water/acetonitrile/methanol (68/24/8, v/v/v) flow rate, 1.0 mL/min.

$$W_m = W_a \times (V_f/V_i) \times (1/V_s)$$

Where  $W_m$  = the numerical value of AFM<sub>1</sub> mass concentration in the test sample in ppb (ng/g or ng/mL);  $W_a$  = the numerical value of the amount of AFM<sub>1</sub> corresponding to the area of AFM<sub>1</sub> peak of the sample extract (ng);  $V_f$  = the numerical value of the final volume of redissolved eluate (μL);  $V_i$  = the numerical value of the final volume of injected eluate (μL);  $V_s$  = the numerical value of volume or mass of prepared test portion passing through the column (mL or g).

Express the results to 3 significant figures.

#### (VII) Proficiency test

Our Laboratory participated in the “Analysis of Aflatoxin M<sub>1</sub> in yogurt” (Food Analysis Performance Test Assessment Scheme, Series 4, Aflatoxins Analysis, Round 4) proficiency test held by the Central Science Laboratory (Sand Hutton, York, U.K.) in February, 2002. The sample was analyzed as described above.

## RESULTS

### I. Analytical Method Performance

#### (I) Calibration

Within the calibration range of 0.002–10 μg/200 μL of AFM<sub>1</sub>, the HPLC responses (peak area) positively regressed with injected AFM<sub>1</sub> mass and gave a correlation coefficient of  $R^2 = 0.9999$ , as shown in Figure 2.

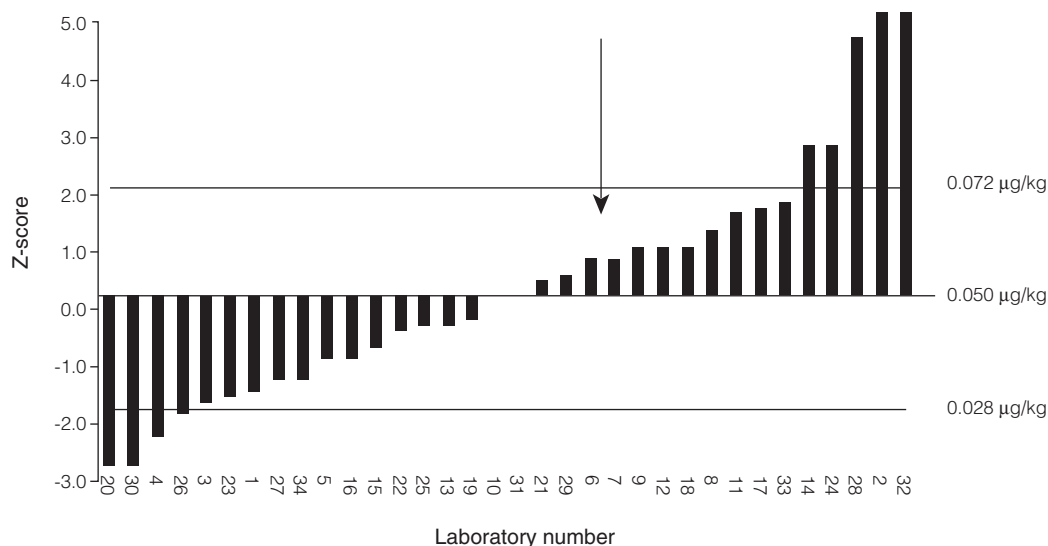
#### (II) Validation

The MDLs of fresh milk, milk powder and drinking yogurt were 0.002, 0.02 and 0.005 ppb, respectively. Specificity of the AFM<sub>1</sub> peaks were clearly shown in HPLC chromatograms of Figure 3, which showed the absence of interfering signals at the AFM<sub>1</sub> retention area for fresh milk, milk powder and drinking yogurt. The recoveries of AFM<sub>1</sub> in fresh milk, milk powder and drinking yogurt were 83.3–89.5, 86.0–88.7, and 94.8–99.9%, respectively, as shown in Table 2.

#### (III) Proficiency test

Thirty-four of 43 participants delivered their detection results and 25 participants received the “satisfied result” ( $|z| < 2$ ). The z-scores of all 34 participants were shown in Figure 4. Our laboratory number was 7, and the z-score was 0.7.

#### II. Survey Results (Table 3)



**Figure 4.** Z-score distribution of participants in the “Analysis of AFM<sub>1</sub> in yogurt” proficiency test. (The number of our laboratory was 7 as the arrow indicated)

**Table 3.** AFM<sub>1</sub> contents of dairy products

| Dairy product   | No. of samples | No. of positive samples (%) | AFM <sub>1</sub> contents (ppb) |           |          |
|-----------------|----------------|-----------------------------|---------------------------------|-----------|----------|
|                 |                |                             | 0.002~0.01                      | 0.01~0.05 | 0.05~0.1 |
| Fresh milk      | 44             | 40 (90.9)                   | 10                              | 29        | 1        |
| Milk powder     | 24             | 0 (0)                       | 0                               | 0         | 0        |
| Infant formula  | 21             | 0 (0)                       | 0                               | 0         | 0        |
| Drinking yogurt | 24             | 3 (12.5)                    | 2 <sup>a</sup>                  | 1         | 0        |
| Total           | 113            | 43 (38.1)                   | 12                              | 30        | 1        |

<sup>a</sup>Between 0.007 and 0.01 ppb.

**Table 4.** Incidence and levels of AFM<sub>1</sub> contamination in fresh milk

| Country                  | Year      | No. of samples | No. of positive samples (%) | AFM <sub>1</sub> (ppb)    |
|--------------------------|-----------|----------------|-----------------------------|---------------------------|
| Brazil <sup>(19)a</sup>  | 1992      | 52             | 4 (7.7)                     | 0.073 ~ 0.370             |
| Italy <sup>(20)</sup>    | 1995      | 159            | 136 (85.5)                  | < 0.001 ~ 0.11            |
| Italy <sup>(21)</sup>    | 1996      | 161            | 125 (77.6)                  | < 0.001 ~ 0.024           |
| Tailand <sup>(22)</sup>  | 1995~1996 | 250            | 249 (99.6)                  | 47 more than 0.5 ppb      |
| Taiwan <sup>(15)</sup>   | 1996      | 25             | 3 (12.0)                    | 0.08 ~ 0.33               |
| Korea <sup>(7)</sup>     | 1997      | 70             | 39 (55.7)                   | 0.015 ~ 0.052             |
| Kuwait <sup>(23)</sup>   | 1998      | 30             | 13 (43.3)                   | 0.01 ~ 0.21               |
| Spain <sup>(25)</sup>    | 1998      | 47             | 14 (29.8)                   | 0.02 ~ 0.1                |
| Portugal <sup>(26)</sup> | 1999      | 101            | 85 (84.2)                   | 2 more than 0.05 ppb (2%) |
| Greece <sup>(24)</sup>   | 1999~2001 | 136            | 113 (83.0)                  | 0.005 ~ 0.05              |
| Brazil <sup>(27)</sup>   | 1999~2000 | 139            | 29 (20.9)                   | 0.05 ~ 0.24               |
| Taiwan <sup>b</sup>      | 2002      | 44             | 40 (90.9)                   | 0.002 ~ 0.083             |

<sup>a</sup>Reference.<sup>b</sup>Data of this survey.**Table 5.** Incidence and levels of AFM<sub>1</sub> contamination in milk powder

| Country                 | Year      | No. of samples | No. of positive samples (%) | AFM <sub>1</sub> (ppb) |
|-------------------------|-----------|----------------|-----------------------------|------------------------|
| Italy <sup>(20)a</sup>  | 1995      | 97             | 81 (83.5)                   | < 0.001 ~ 0.10         |
| Italy <sup>(21)</sup>   | 1996      | 92             | 50 (54.3)                   | < 0.001 ~ 0.08         |
| Tailand <sup>(22)</sup> | 1995~1996 | 13             | 2 (15.4)                    | Not more than 0.5 ppb  |
| Taiwan <sup>(15)</sup>  | 1996      | 25             | 0                           | 0                      |
| Korea <sup>(7)</sup>    | 1997      | 50             | 35 (70.0)                   | 0.032 ~ 0.342          |
| Brazil <sup>(28)</sup>  | 1997      | 300            | 33 (11.0)                   | 0.1 ~ 1.0              |
| Kuwait <sup>(23)</sup>  | 1998      | 19             | 0                           | 0                      |
| Taiwan <sup>b</sup>     | 2002      | 45             | 0                           | 0                      |

<sup>a</sup>Reference.<sup>b</sup>Data of this survey.**Table 6.** Incidence and levels of AFM<sub>1</sub> contamination in yogurt

| Country                | Year | No. of samples | No. of positive samples (%) | AFM <sub>1</sub> (ppb) |
|------------------------|------|----------------|-----------------------------|------------------------|
| Italy <sup>(20)a</sup> | 1995 | 114            | 91 (79.8)                   | < 0.001 ~ 0.50         |
| Italy <sup>(21)</sup>  | 1996 | 120            | 73 (60.8)                   | < 0.001 ~ 0.032        |
| Korea <sup>(7)</sup>   | 1997 | 60             | 31 (51.7)                   | 0.017 ~ 0.124          |
| Kuwait <sup>(23)</sup> | 1998 | 5              | 1 (20.0)                    | 0.01 ~ 0.21            |
| Taiwan <sup>b</sup>    | 2002 | 24             | 3 (12.5)                    | 0.007 ~ 0.044          |

<sup>a</sup>Reference.<sup>b</sup>Data of this survey.

### (I) Fresh milk

Among the 44 fresh milk samples, 3 samples were manufactured by small scale farms and sold only in local supermarkets. The remaining 41 samples were manufactured by larger scale food companies and sold to other counties. AFM<sub>1</sub> above the MDL of 0.002 ppb was present in 40 of 44 (90.9%) fresh milk samples, among which 10 were between 0.002 and 0.01 ppb, 29 were between 0.01 and 0.05 ppb, 1 was between 0.05 and 0.1 ppb (0.083 ppb).

### (II) Milk powders and infant formula

The milk powders and infant formula were all goods imported. No AFM<sub>1</sub> was detected in the 24 milk powders and 21 infant formula according to the MDL of 0.02 ppb.

### (III) Drinking yogurt

Two of 24 samples of drinking yogurt were manufac-

tured by small scale food processing companies and sold only in local supermarkets. The remaining 22 samples were manufactured by larger scale food companies and sold to other counties. The incidence of AFM<sub>1</sub> in drinking yogurt (12.5%) was lower than that in fresh milk. Three of 24 drinking yogurt samples were contaminated by AFM<sub>1</sub> at the concentrations of 0.007, 0.009 and 0.044 ppb.

## DISCUSSION

The retention time of AFM<sub>1</sub> in the HPLC chromatograms was shorter in this survey (8~9 min) than that in the former survey (13~14 min)<sup>(15)</sup>. The MDL was 10 times lower.

Fu<sup>(15)</sup> surveyed the AFM<sub>1</sub> content in fresh milk, and found that 3 of 25 (12%) samples were positive. The column Fu used (AFLAPREP M, Vicam) was different from what we used in this survey, and the MDL was 0.05 ppb, which was much higher than those in this survey.



Though the principles of these two surveys were similar, the sensitivity of the HPLC system in this survey was elevated and the MDL was lowered to 0.002 ppb. According to the MDL of 0.05 ppb, only 1 of 44 (2.3%) fresh milk was found to be positive in this survey. Hence, the AFM<sub>1</sub> contamination status in fresh milk was not serious. According to Visconti *et al.*<sup>(18)</sup>, most of such results should be attributed to the poor sensitivity of the analytical methods used. Today, the increased efficiency of the extraction procedures and the increased accuracy of the analytical methods and better equipments resulted in much lower limits of detection, thereby a significant increase in the percentage of positive samples.

In comparison, the incidence of AFM<sub>1</sub> contamination in fresh milk in recent studies ranged from 7.7 to 99.6%, with means of AFM<sub>1</sub> contents from 0.01 to 0.5 ppb, as shown in Table 4<sup>(7,15,19,20,21,22,23,24,25,26,27)</sup>. In this survey, the incidence of AFM<sub>1</sub> contamination in fresh milk was very high, 90.9% of the samples were positive. However, Table 3 showed that AFM<sub>1</sub> was not detected in 9.1% of the fresh milk sample, detected at low levels (0.002 to 0.05 µg/L) in 88% of the samples and none of these positive samples were above the action level of 0.5 ppb. This supports the view that a high incidence of low-level contamination and infrequent high contamination levels in commercial milk could be caused by only a few contaminated samples entering the bulk milk supply<sup>(20)</sup>.

The incidence of AFM<sub>1</sub> in milk powder in recent studies ranged from 0 to 84%, with AFM<sub>1</sub> mean contents from 0 to 1.0 ppb, as shown in Table 5<sup>(7,15,20,21,22,23)</sup>. The 45 milk powder samples (including 21 infant formula) collected in this survey were all imported from America, Japan, New Zealand and the Netherlands. The same as the result of a former survey held in 1996<sup>(15)</sup>, no AFM<sub>1</sub> was detected in the milk powder samples. Though there were trace levels of AFM<sub>1</sub> contamination found in the milk powder samples in the recent survey, none of these positive samples was detected at levels above the action level of 5.0 ppb, and it seems that the milk powders exported to Taiwan were free of AFM<sub>1</sub>. Since Taiwan became a member of WTO, there will be more and more milk powders imported from different countries to Taiwan. Therefore, related surveys should be continued in the future.

Owing to its nutritional value, yogurt, especially drinking yogurt, has become more and more popular in Taiwan. However, the content of AFM<sub>1</sub> is not reduced in the fermentation process if AFM<sub>1</sub>-contaminated milk is used. The concentration of AFM<sub>1</sub> could even be increased due to the condensation process<sup>(20)</sup>. The incidence of AFM<sub>1</sub> in yogurt in recent studies ranged from 20 to 80%, with AFM<sub>1</sub> mean contents from 0 to 0.50 ppb, as shown in Table 6<sup>(7,20,21,22,23)</sup>. In this survey, it was the first time AFM<sub>1</sub> was tested in drinking yogurt in Taiwan. Though there was AFM<sub>1</sub> contamination in drinking yogurt, the content was low (0.007 to 0.044 ppb). Till now, the action level of AFM<sub>1</sub> in yogurt has not been set in Taiwan.

## CONCLUSION

By combining immunoaffinity column for cleanup and LC with fluorescence for detection, the AFM<sub>1</sub> contents in dairy products could be measured with good recoveries and satisfactory MDL. Through participation in the "Analysis of Aflatoxin M<sub>1</sub> in yogurt" in 2002, our laboratory was among the 34 laboratories which received "satisfied result" in the proficiency test.

In this survey, it was revealed that trace levels of AFM<sub>1</sub> were detected in dairy products such as fresh milk and drinking yogurt in Taiwan. However, none of these positive samples was above the action level of 0.5 ppb.

## ACKNOWLEDGMENTS

This survey was supported by project DOH91-FD-2054 from Department of Health.

## REFERENCES

1. DeLong, H., Vles, R. O. and VanPelt, J. G. 1964. Milk of mammals fed an aflatoxin containing diet. *Nature* 202: 466-467
2. World Health Organization. 2002. Evaluation of certain mycotoxins in food. WHO Technical Report Series 906: 8-27
3. Van Egmond, H. P. 1989. Current situation on regulations of mycotoxins. Overview of tolerances and status of standard methods of sampling and analysis. *Food Addit. Contam.* 6: 139-188
4. Scott, P. M. 1989. Mycotoxigenic fungal contaminants of cheese and other dairy products. In "Mycotoxins in Dairy Products". pp. 193-259. van Egmond, H. P., ed. Elsevier. New York, U. S. A.
5. Gourama, H. and Bluerman, L. B. 1995. *Aspergillus flavus* and *Aspergillus parasiticus*: aflatoxingenic fungi of concern in foods and feeds: a review. *J. Food Prot.* 58: 1395-1404
6. Galvano, F., Galofaro, V. and Galvano, G. 1996. Occurrence and stability of aflatoxin M<sub>1</sub> in milk and milk products. A worldwide review. *J. Food Prot.* 59: 1079-1090
7. Kim, E. K., Shon, D. H., Ryu, D., Park, J. W., Hwang, H. J. and Kim, Y. B. 2000. Occurrence of aflatoxin M<sub>1</sub> in Korean dairy products determined by ELISA and HPLC. *Food Addit. Contam.* 17: 59-64.
8. Standard Committee of Foods (SCF). 1994. Opinion on aflatoxins, ochratoxin A and patulin. pp. 1-6. 94th Meeting of the SCF, 22-23 September 1994. European Commission. Brussels, Belgium.
9. US Food and Drug Administration. 1996. Sec. 527.400 whole milk, low fat milk, skim milk-aflatoxin M<sub>1</sub>(CPG 7106.210). In "FDA Compliance Policy Guides" pp. 219. FDA. Washington DC., U. S. A.

10. European Communities. 2001. Commission regulation (EC)No. 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs. Official J. European Communities L77, 44: 1-16.
11. Department of Health, Executive Yuan. 1993. Tolerance of Aflatoxins in Food. DOH FOOD No. 8189322. January 4, Taipei. (in Chinese)
12. Department of Health, Executive Yuan. 1993. Sanitary Standard in Infant's Formula. DOH FOOD No. 8189322. January 4, Taipei. (in Chinese)
13. Wang, C. C. 1986. Study on the aflatoxin M<sub>1</sub> contaminated raw milk and milk powder. 2nd report of Food Sanitary Management. DOH. Taipei, Taiwan. (in Chinese)
14. Ong, K. P. and Fu, Y. M. 1989. Determination of aflatoxin M<sub>1</sub> in dairy products. Technical Report of National Laboratory of Foods and Drugs. NLFD, DOH. Taipei, Taiwan. (in Chinese)
15. Fu, Y. M. 1996. Determination of aflatoxin M<sub>1</sub> in milk and milk powder using immunoaffinity column and fluorescence. *J. Food Drug Anal.* 4: 175-183.
16. Dragacci, S. and Grosso, F. 2001. Immunoaffinity columns cleanups with liquid chromatography for determination of aflatoxin M<sub>1</sub> in liquid milk: collaborative study. *J. AOAC Int.* 84: 437-443.
17. Tuinstra, L. G., Roos, A. H. and van Trijp, J. M. 1993. Liquid chromatography determination of aflatoxin M<sub>1</sub> in milk powder using immunoaffinity columns for cleanup: interlaboratory study. *J. AOAC Int.* 76: 1248-154.
18. Visconti, A., Bottalico, A. and Solfrizzo, M. 1985. Aflatoxin M<sub>1</sub> in milk in southern Italy. *Mycotoxin Res.* 1: 71-75
19. De Syllos, C. M., Rodriguez-Amaya, D. B. and Carvalho, P. R. N. 1996. Occurrence of aflatoxin M<sub>1</sub> in milk and dairy products commercialized in Campinas, Brazil. *Food Addit. Contam.* 13: 169-172.
20. Galvano, F., Galofaro, V., De Angelis, A., Galvano, M., Bognanno, M. and Galvano, G. 1998. Survey of the occurrence of aflatoxin M<sub>1</sub> in dairy products marketed in Italy. *J. Food Prot.* 61: 738-741.
21. Galvano, F., Galofaro, V., Ritieni, A., Bognanno, M., De Angelis and Galvano, G. 2001. Survey of the occurrence of aflatoxin M<sub>1</sub> in dairy products marketed in Italy: second year observation. *Food Addit. Contam.* 18: 644-646.
22. Saitanu, K. 1997. Incidence of aflatoxin M<sub>1</sub> in Thai milk products. *J. Food Prot.* 60: 1010-1012.
23. Srivastava, V. P., Bu-Abbas, A., Alla-Basuny Al-Johar, W., Al-Mufti, S. and Siddiqui, K. J. 2001. Aflatoxin M<sub>1</sub> contamination in commercial samples of milk and dairy products in Kuwait. *Food Addit. Contam.* 18: 993-997.
24. Rossi, V., Govaris, A., Varagouli, A. and Botsoglou, N. A. 2002. Occurrence of aflatoxin M<sub>1</sub> in raw and market milk commercialized in Greece. *Food Addit. Contam.* 19: 863-868.
25. Blanco, J. L., Dominguez, L., Gomez-lucia, E., Garayzabal, J. F. F., Garcia, J. A. and Suarez, G. 1998. Presence of aflatoxin M<sub>1</sub> in commercial ultra-high temperature treated milk. *Appl. Envi. Micro.* 54: 1622-1623.
26. Martins, M. L. and Martins, H. M. 2000. Aflatoxin M<sub>1</sub> in raw and ultra high temperature-treated milk commercialized in Portugal. *Food Addit. Contam.* 17: 871-874.
27. Garrido, N. S., Iha, M. H., Santos Ortolani, M. R. and Duarte Favaro R. M. 2003. Occurrence of aflatoxin M<sub>1</sub> and M<sub>2</sub> in milk commercialized in Ribeirao Preto-SP, Brazil. *Food Addit. Contam.* 20: 70-73.
28. Oliveria, C. A. F., Germano, P. M. L., Bird, C. and Pinto, C. A. 1997. Immunochemical assessment of aflatoxin M<sub>1</sub> in milk powder consumed by infants in Sao Paulo, Brazil. *Food Addit. Contam.* 14: 7-10.
29. Wisconsin Department of Natural Resources. 1986. Appendix B to part 136-Definition and procedure for determination of the method detection limit- revision 1.11. Code of Federal Regulations. Wisconsin, U. S. A.