Application of a Diphasic Dialysis Membrane Procedure for Surveying Occurrence of Aflatoxin M_1 in Commercial Milk

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Occurrence of aflatoxin M_1 was determined in 100 samples of commercial milk taken from a supermarket in Madrid (Spain) from January to June 1993. Aflatoxin M_1 levels were determined by a dialysis diphasic membrane procedure and high-performance thin-layer chromatography as the analytical method. Eighty-six samples contained <0.01 μg/L aflatoxin M_1, whereas 14 samples contained 0.02–0.04 μg/L.

Keywords: *Aflatoxin M_1; commercial milk; diphasic dialysis*

INTRODUCTION

Aflatoxin M_1 was the first metabolite of aflatoxin B_1 described for animals, and it appears in milk, urine, and several tissues after animal or human ingestion of aflatoxin B_1 (Allcroft and Carnaghan, 1963; Holzapfel et al., 1966; Campbell et al., 1970). Conversion of low dietary levels of aflatoxin B_1 into aflatoxin M_1, as studied by different authors, ranges between 0.2 and 3% (Patterson et al., 1980). Several authors have concluded that aflatoxin M_1 has hepatotoxic and carcinogenic properties (Purchase, 1967; Wogan and Pagialunga, 1974).

Since its discovery, most investigations on the occurrence of aflatoxin M_1 have been done with milk. However, the stability of this toxin during processing of dairy products allows detection of the toxin at different concentrations than those in the raw milk (Wiseman and Marth, 1983a,b; Stoloff et al., 1975; Blanco et al., 1993). Because of concern for human health, especially children's health, many countries have established regulatory limits for aflatoxin M_1 in milk and milk products. The most common limit for liquid milk is 0.05 μg/L, and in countries such as Austria and Switzerland, this amount is reduced to 0.01 μg/L for infant food (Van Egmond, 1991).

The aim of the present work was to test the performance of the diphasic dialysis (DD) technique, developed in our laboratory (Dominguez et al., 1992; Díaz et al., 1993), for investigating the occurrence of aflatoxin M_1 in commercial milk. This method has a recovery of 96%, a determination limit between 0.01 and 0.02 μg/L, and a detection limit of 0.002 μg/L, according to methodology described by the Commission of the European Communities (Heitzman, 1992).

MATERIALS AND METHODS

Milk Samples. Samples of commercial milk were taken from a supermarket in Madrid (Spain) at monthly intervals, during a period of 6 months (January to June 1993). These samples (n = 100) included two units of the same batch of all the trademarks available in the store (between seven and nine different trademarks in each of the steps). The study involved a total of 11 trademarks: 8 trademarks of whole UHT milk (64 samples), 2 of whole pasteurized milk (24 samples), and 1 of semiskimmed UHT milk (12 samples). All samples were stored at 4 °C and analyzed immediately upon opening.

Instruments. Shaking was accomplished with a controlled environment incubator shaker (Model G25; New Brunswick Scientific, Edison, NJ). Detection and quantification were done with a high-performance thin-layer chromatography (HPTLC) system and a densitometer (TLC/HPTLC Scanner II, Camag, Muttenz, Switzerland).

Materials and Reagents. Dialysis tubing (Visking size 20/32) was obtained from Serva (Feinbiochemical, Heidelberg, Germany). Reagents used, chloroform, methanol, and anhydrous sodium sulfate, were all analytical grade. Aflatoxin M_1 in the crystalline form was obtained from Sigma (St. Louis, MO), prepared as a standard solution dissolved in chloroform, and calibrated by spectrophotometry (Scott, 1990). Because aflatoxins are carcinogenic to humans, safety precautions were observed (Scott, 1990).

Extraction Procedures. Analysis of aflatoxin M_1 in milk was performed by the DD procedure, which has been previously published (Díaz et al., 1993). Aflatoxin M_1 from 50-mL samples of milk was extracted with 70 mL of chloroform placed in a hydrated dialysis tube (60-cm length). Samples and dialysis tube were placed in flasks and extraction was accomplished in 5 h by shaking (150 rpm) at 37 °C. Then, the dialysis tube was removed, and the chloroform extract was filter-dried through anhydrous sodium sulfate and concentrated in a rotary evaporator to a final volume of 200 μL.

HPTLC Conditions. Determination of aflatoxin M_1 was made with high-resolution 10 × 10-cm silicagel plates without fluorescence indicator (Merck, Darmstadt, Germany). Spots of duplicate samples (20 μL) and standards are deposited as usual, and plates were developed in an unsaturated vertical chamber at room temperature, with chloroform:methanol (95:5, v/v) as developing solvent.

Aflatoxin M_1 was quantitated by a densitometer Camag TLC Scanner II equipped with Cats 3 software (Camag) at an excitation wavelength of 350 nm and an emission cutoff of 400 nm.

RESULTS

Aflatoxin M_1 was detected in commercial milk samples by the DD methodology. Of 100 samples analyzed, 86 contained <0.01 μg/L, whereas 14 had >0.01 μg/L. Aflatoxin M_1 content (μg/L) was 0.02 (seven samples), 0.03 (five samples), and 0.04 (two samples). Of the 14 positive milk samples, two were pasteurized whole milk and 12 were UHT whole milk. No aflatoxin M_1 was detected in semiskimmed UHT milk (24 analyzed samples).

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Table 1. Distribution of Aflatoxin M_1 (Micrograms per Liter) among Sampling Steps and Trademarks

trademark[a]	sampling 1	2	3	4	5	6
A WP	–[b]	–	–	–	–	–
B WP	–	–	–	–	–	0.02
						0.03
C WU	–	–	–	–	–	–
D WU	–	–	–	–	–	–
E WU	–	–	–	–	*[c]	*
F SSU	–	–	–	–	–	–
G WU	0.03	0.03	–	0.02	0.02	–
	0.02	0.04		0.02	0.04	
H WU	–	0.02	*	*	*	*
		0.03				
I WU	–	–	*	–	*	*
J WU	*	*	–	–	*	–
K WU	*	*	*	*	0.02	–
					0.03	

[a] WP, whole pasteurized; WU, whole UHT; SSU, semiskimmed UHT. [b] –, Aflatoxin M_1 was not detected in both samples. [c] *, Samples not available.

We detected contaminated milk samples in five of the six sampling steps performed. Distribution of positive samples among them was as follow: 2 of 18 in sampling one; 4 of 18 in sampling two; 0 of 16 in sampling three; 2 of 18 in sampling four; 4 of 14 in sampling five; and 2 of 16 in sampling six (Table 1).

Aflatoxin M_1 was detected in samples originated from 4 of the 11 trademarks examined. It is remarkable that 8 of the 14 contaminated samples come from only one of the UHT whole milk trademarks, which also included the 2 samples with the maximum content of the toxin detected (0.04 μg/L).

Results of analysis of two units of the same lot were always both positive and negative, and contamination levels showed little differences (Table 1).

DISCUSSION

Milk and milk products in several countries have been widely surveyed for the natural occurrence of aflatoxin M_1. In Spain, there are few surveys on the subject, and those based on TLC techniques showed the following results. In 1984, Jodral et al. detected aflatoxin M_1 in 72 of 1150 samples of different types of milk, with a minimum level of 0.05 μg/L. However, these authors did not find the toxin in 330 samples of sterilized milk analyzed. Burdaspal et al. (1983) found aflatoxin M_1 in seven of 95 samples of different types of milk, ranging from 0.02 to 0.04 μg/L. These results are in accordance with those found in our study. A survey by Blanco et al. in 1988 found that 14 of 47 UHT milk samples collected in a small dairy plant contained aflatoxin M_1 levels ranging from 0.02 to 0.10 μg/L. The difference between these results and those obtained by us may be due to the origin of the samples. In fact, 10 of the trademarks used in our survey came from large dairy plants where milk from different places is mixed and aflatoxin M_1 may be diluted.

Macho et al. (1992), using an HPLC technique, found aflatoxin M_1 at 0.010–0.025 μg/L in 13 of 94 samples and at 0.025–0.050 μg/L in one of 94 samples of milk analyzed. These authors employed the analytical procedure described by Mortimer et al. (1987), which has determination limit of 0.0005 μg/L. These results may be in accord with ours if one takes into account differences between analytical techniques.

Finally, Jalón et al. (1994) found from aflatoxin M_1 at 0.01 to 0.02 μg/L in 10 of 61 samples and at 0.02–0.04 μg/L in two of 61 samples of raw milk, as well as at 0.01–0.02 μg/L in four of 33 samples and at 0.02–0.04 μg/L in one of 33 samples of sterilized milk.

In summary, although a formal sampling method has not been used in this study and the percentage of aflatoxin M_1-contaminated milk samples in Spain could be not well estimated, our results using DD are very close to those previously reported and they confirm that it is necessary to pay attention to this subject. Spain has no legislation yet about the content of aflatoxin M_1 in dairy products and levels obtained in this and previous studies are below the levels stated for liquid milk in other countries. However, it is important to consider that one of the surveyed trademarks shows constant contamination and therefore poses a major risk for habitual consumers of this product.

Analysis of two samples of the same batch confirms that the toxin is practically homogeneously distributed in the milk (Van Egmond, 1991). Consequently sampling does not pose problems for determination of aflatoxin M_1 (Van Egmond, 1989). Finally, DD has been shown to be a simple and useful analytical technique that can be used to survey milk for aflatoxin M_1 contamination.

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