Occurrence of Aflatoxin in Hypoallergenic Milk Substitutes

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ABSTRACT

Aflatoxin B1 was detected, and its identity confirmed, in hypoallergenic milk substitutes composed, among other things, of the following ingredients susceptible to possible aflatoxin contamination: soya protein isolate, soy and coconut oils, cornstarch, and corn syrup. Except for one determination all findings were under 1 ng of aflatoxin/ml of formula at drinking concentration. Manufacturers' reserve samples of soya protein isolate, the ingredient thought to be the most likely source of the aflatoxin, were found to be uncontaminated. Reserve samples of other ingredients were not examined. Because the hypoallergenic formula may be the major nutrient of an individual with a metabolic defect at infancy, a vulnerable stage of life, available control measures should be used to avoid aflatoxin-contaminated ingredients.

The association of mycotoxins with human and animal disease generally has been based on symptoms occurring shortly after ingestion. Such mycotoxicoses in animals have been well documented; few such situations have been reported for man. The extent of the aflatoxin hazard has been reviewed (6), but data in regard to man are limited.

In one study of a possible relation between dietary aflatoxin levels and primary liver cancer in Thai populations the investigators noted a relation between aflatoxin levels and the incidence of Reye's syndrome (7), an often fatal pediatric disease. We have been pursuing this observation with Reye's syndrome patients at the University of Mississippi Medical Center. Because of this interest and because the young of the species studied have been the most sensitive to the acute effects of aflatoxin (12), major dietary components of some of the patients were examined. This included a hypoallergenic milk substitute being used at the Medical Center.

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EXPERIMENTAL PROCEDURES

Sample preparation and distribution

A measured volume of liquid formula was freeze-dried in tared beakers; the beakers were then reweighed, the difference (ca 2.5 g) representing the freeze-dried weight of the formula. The first four samples were divided into equal lots; one lot of each formula was analyzed at the University of Mississippi Medical Center (UMMC), the other at the Bureau of Foods laboratories of the Food and Drug Administration (FDA). Subsequent samples were analyzed only at UMMC.

UMMC method

Freeze-dried samples were extracted using a method (1) of the Association of Official Analytical Chemists (AOAC), modified as follows to accommodate the small sample size. Lyophilizate, 20-33 mm diameter glass beads and 35 ml of acetone-water (7:3, v/v) were shaken together in a 125-ml Erlenmeyer flask at 300 rpm for 30 min. The content of each flask was vacuum filtered and the residue washed with acetone-water (10 ml). The filtrate was quantitatively transferred to a 100-ml beaker, evaporated to about 5 ml on a steam bath, and 2 ml of lead acetate solution (200 g Pb(OAc)2 • 3H2O • 3 ml HOAc, diluted to 1 liter) mixed in by swirling, followed by 2 ml of saturated sodium sulfate (ACS grade) solution. The mixture was transferred to a 13-ml centrifuge tube in a 13.5-cm radius rotor and operated 20 min at 2500 rpm. The supernatant fluid was decanted into a 30-ml separatory funnel, and defatted with 10-ml portions of hexane. The aflatoxins then were extracted with three 10-ml aliquots of chloroform. Pooled chloroform extracts were dehydrated over anhydrous granular sodium sulfate, evaporated to 1 ml and quantitatively transferred to a small test tube (10 x 75 mm). The extract was evaporated to near dryness with heat and finally to dryness under vacuum. The dried residue was dissolved in 50 or 100 ml of the HPLC elution solvent for analysis.

Liquid samples were extracted by the following modification: Liquid formula (20 ml) was mixed with lead acetate solution (2 ml), followed by saturated sodium sulfate solution (2 ml). The mixture was transferred to a 20-ml centrifuge tube in a 13.5-cm radius rotor and operated at 4000 rpm for 30 min. The supernatant fluid was transferred to a 200-ml separatory funnel, defatted with two 20-ml portions of hexane and then extracted with two 20-ml portions of chloroform. The pooled chloroform extracts were prepared for HPLC in the same manner as above for the freeze-dried samples.

Extracts were analyzed for aflatoxins B1, B2, G1 and G2 using a Waters Associates (Milford, MA 01757) high pressure liquid chromatograph. Sample components were separated on a purosil column (Waters Associates, Inc.) and detected by UV absorbance at 365 nm at sensitivities of 0.005-0.01 absorbance units full scale. The aflatoxins were identified by retention time and quantitated by peak height or peak area determined by digital electronic integration. The mobile
AFLATOXIN IN MILK SUBSTITUTES

RESULTS AND DISCUSSION

Aflatoxin B₁ was found in 3 of 4 samples taken from Medical Center stock, all of the same brand but bearing different production codes (Table 1). The original finding was confirmed by a second laboratory, including a chemical confirmation of the aflatoxin B₁ identity. The quantitative disparity observed is to be expected when measurements are made, as these were, with relatively small samples and close to the detection limits of the methods. Following this observation, 12 samples were picked up on the open market, eight of the same brand as used at the hospital, three of another brand and one of a third brand, all bearing different production codes. Aflatoxin was found in two of the open market samples - one of the original brand tested, and the other the single sample of the third brand.

The composition of the hypoallergenic milk substitutes (9) is given in Table 2. Of the components listed, possible sources of aflatoxin contamination are the soy protein isolate, coconut and soy oils, corn syrup, and cornstarch. Because of the usual source of the corn used and the distribution of any aflatoxin that might have been present into refinery products other than starch (10), the possibility of finding aflatoxin in cornstarch or corn syrup (made by hydrolysis of starch) is remote. Although not specified, the coconut and soy oils used must be refined to avoid undesirable flavors and odors. The usual refining process effectively removes any aflatoxins that might have been present from contaminated oilseed (2.4). Aflatoxin contamination of soybeans is not usually encountered (8), but if by chance aflatoxin-contaminated beans had been used, aflatoxin would have been concentrated in the soy protein isolate (9).

There are only three commercial sources of soy protein isolate. Each manufacturer was alerted to a possible problem. Two of the manufacturers sent us portions of their reserve samples, 30 from one and 23 from another. The third manufacturer, using in-house facilities and a method that we provided, analyzed 40 of his reserve samples. The method would have detected an aflatoxin B₁ level of 0.5 ng/g. Since the soy protein component is approximately 2% of the final formula weight, this detection level translates to 0.01 ng/ml of the formula, more than adequate to have detected in the protein the quantities found in the formula. No aflatoxin was detected in any of the samples tested by us or by the manufacturer. We did not attempt retrospective sampling of the other components susceptible to possible aflatoxin contamination.

There is no basis for judging whether the levels of aflatoxin B₁ encountered in these formulas present a risk of harm. However, since the formula can constitute the major nutrient of an individual with a metabolic defect, at the most vulnerable stage of life, there is reason to be ultraconservative in judgment. Controls for preventing incorporation of aflatoxin-contaminated ingredients are easily applied and an adequate supply of ingredients with no detectable aflatoxin (<0.5 ng/g) should be available.
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REFERENCES


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