Aflatoxin M_1. Occurrence in Stored and Freshly Harvested Corn

Aflatoxin M_1 (50 ppb) has been found in a ground blended sample of stored corn collected in Illinois that contained 1600 ppb of aflatoxin B_1. Aflatoxin M_1 was also detected in seven samples of freshly harvested corn from the Southeast containing 210–3200 ppb of B_1. Individual fluorescing corn kernels and pieces were collected from four lots of corn for M_1 analysis: white stored corn, freshly harvested yellow corn, yellow stored corn, and acid-treated yellow stored corn. Aflatoxin M_1 could be detected in kernels and pieces that contained more than 1000 ppb of B_1, and its identity was confirmed by the acetate and hemiacetal derivatives.

Aflatoxin M_1 has not been reported as occurring naturally in corn. In fact, the only commodities other than milk in which M_1 has been detected were peanuts and pistachio nuts, both in pickouts (Waltking, 1975). *Aspergillus flavus* and *A. parasiticus* produce such low yields of M_1 relative to aflatoxin B_1 (ratio of M_1 to B_1 is 0.01–0.0025) (Hesseltine et al., 1970) that no one would expect to detect M_1 in corn containing low levels of B_1. However, lots of corn more highly contaminated have been surveyed (Shotwell et al., 1975b; Lillehoj et al., 1975). We now report the occurrence of aflatoxin M_1 in 12 lots of corn from several sources.

EXPERIMENTAL SECTION

Sample Preparation. Each 10-lb sample of corn was ground in a 12-in. Raymond hammer mill with screens containing $^1/_8$-in. round-hole perforations. Each ground sample was blended 15–30 min in a Hobart planetary mixer, A200 (12-qt capacity) or in a Twin Shell Blender (PK-LB-6948).

Aflatoxin Determination. The 10-lb ground corn samples were assayed for aflatoxins B_1, B_2, G_1, and G_2 by the Official First Action Method of the Association of Official Analytical Chemists (AOAC, 1975a). Individual kernels were analyzed by the method described by Shotwell et al. (1974). Amounts of aflatoxin in partially purified extracts of corn samples or extracts of kernels were determined by thin-layer chromatography (TLC) on plates coated with Adsorbosil-1 (0.5 mm). Plates were developed with water–acetone–chloroform (1.5:12:88, v/v) (Stubblefield et al., 1969), for B_1 and B_2, or with isopropyl alcohol–acetone–chloroform (5:10:85, v/v) (AOAC, 1975b), for M_1. Aflatoxins on TLC plates were determined by fluorodensitometry.

Confirmatory Tests. The presence of aflatoxin M_1 in extracts was confirmed by preparation of both hemiacetal and acetate derivatives (AOAC, 1975c).

RESULTS AND DISCUSSION

We detected aflatoxin M_1 for the first time in corn in kernels freshly harvested in 1973 that had been collected for a field study (Lillehoj et al., 1975). Of the 73 kernels containing aflatoxin B_1, 34 of the most highly contaminated had M_1 at levels ranging from 43 to 1700 ppb (micrograms per kilogram). Of eight aflatoxin-contaminated kernels collected from a "hot spot" that developed in artificially dried yellow corn stored in 1973 in Illinois (Shotwell et al., 1975a), two had M_1 (1400 ppb, 40 ppb). Only one out of 21 kernels selected from isobutyric acid treated yellow corn (Bothast et al., 1976) had aflatoxin M_1 (240 ppb), but these kernels had lower levels of B_1 than those from other sources. Out of 18 kernels of stored white corn selected from the 1971 crop, 11 contained M_1 (27–270 ppb).

All individual kernels with more than 10 000 ppb of aflatoxin B_1 had detectable M_1 (Table I). Aflatoxin M_1 was detected in one-half to two-thirds of the kernels with 1000–10 000 ppb of B_1. By the method we used, aflatoxin M_1 was not detectable in many kernels having less than 1000 ppb of B_1, probably because of TLC interferences. Extracts of individual kernels were subjected to TLC without a purification step so more extraneous material was present to obscure M_1 on plates. Levels of M_1 varied from 27 to 1700 ppb with 71% of the kernels containing less than 300 ppb. The average level of M_1 in the kernels was 2.6 ± 2.0% of that of aflatoxin B_1. The two most highly contaminated kernels contained aflatoxins at levels of 230 000 ppb of B_1, 13 000 ppb of B_2, 1400 ppb of M_1, and 310 000 ppb of B_1, 17 000 ppb of B_2, and 1700 ppb of M_1.

Table I. Relationship between Aflatoxin B_1 Level and Detectable Aflatoxin M_1 in Individual Corn Kernels

Aflatoxin B_1, ppb	No. of kernels assayed	No. containing M_1	Aflatoxin M_1 levels, ppb
<1 000	66	4	27-150
1 000-4999	19	11	44-190
5 000-10 000	6	4	110-230
>10 000	29	29	40-1700
Total	120	48	

Table II. Aflatoxins (ppb) in Ground Corn Samples

Sample no.	Aflatoxin B_1	Aflatoxin B_2	Aflatoxin M_1
1[a]	1600	290	7
2	210	15	1
3	250	19	2
4	610	46	2
5	3200	290	35
6	500	48	3
7	500	69	3
8	260	25	1

[a] Sample 1 had been stored 6 months; the rest of the samples were freshly harvested corn.

The question arose whether aflatoxin M_1 could be detected in bulk samples of corn lots. Only the more highly contaminated corn samples had detectable M_1 (Table II). We analyzed eight samples having the highest levels of B_1 (210–3200 ppb) that were available. One sample came from the "hot spot" that developed in a bin of yellow corn (Shotwell et al., 1975a). The others were samples of freshly harvested yellow corn from the Southeast, crop year 1973 (Lillehoj et al., 1975). Very low levels of aflatoxin M_1 were detected. Although aflatoxin M_1 is more polar than B_1, M_1 can be eluted from the silica gel columns used in the approved method (AOAC, 1975a) with ethanol–chloroform (1.5:98.5, v/v) (Stubblefield et al., 1970). The elution solvent used in the analytical method is methanol–chloroform (3:97, v/v) (AOAC, 1975a).

Even though the toxicity of M_1 is similar to that of aflatoxin B_1 (Sinnhuber et al., 1974), the presence of M_1 in corn has little practical significance. It is present in levels lower than B_1 and also lower than the error in the determination of B_1 by the AOAC Official Method (Shotwell and Stubblefield, 1972).

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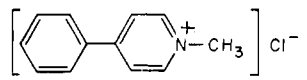
Determination of Cyperquat (1-Methyl-4-phenylpyridinium Chloride) Residues in Soil by Gas–Liquid Chromatography

Catalytic hydrogenation of cyperquat (PtO_2:cyperquat, <2) resulted in the formation of 1-methyl-4-phenylpiperidine and 1-methyl-4-cyclohexylpiperidine. However, hydrogenation with an increasing amount of the catalyst (PtO_2:cyperquat, ≥2) produced only the latter compound. The development of the method for cyperquat residues in soils was based on the formation of 1-methyl-4-cyclohexylpiperidine which gave a single symmetrical gas chromatographic peak. The method involves catalytic hydrogenation of the acid extract of soil, extraction of the material into hexane, and analysis by gas–liquid chromatography. Recoveries of the herbicide added to soil at 0.5- and 1-ppm levels were 77.1 and 85.2%, respectively. The method has been used for the determination of field applied cyperquat.

Cyperquat (I) is a new postemergence herbicide and is reported to give good control of purple and yellow nutsedge in various crops (Gulf Oil Chemical Co., 1975). The compound is available as a chloride salt and is soluble in water. It ionizes completely in aqueous solution into a reactive cation which may quickly disappear from solution



1-methyl-4-phenylpyridinium chloride
I

on contact with soil particles.

With the increasing interest in cyperquat for controlling nutsedge weeds in corn and soybeans (Hamill, 1975), it became of considerable interest to determine the level of the herbicide residues in soil. The possibility exists that the herbicide may remain in soil for some time after spraying the crop. A need was therefore felt to develop a sensitive analytical method for the determination of cyperquat residues in soils. Such a method is reported in this paper. The principle of the method is similar to that recently described for determining paraquat (1,1'-dimethyl-4,4'-bipyridinium dichloride) and diquat (1,1'-ethylene-2,2'-bipyridinium dibromide) residues in soils (Khan, 1974). The method involves catalytic hydrogenation of the acid extract of soil, extraction of the material into organic solvents, and analysis by gas–liquid chromatography.

MATERIALS AND METHODS

Chemicals. All solvents were pesticide grade and used as received. Platinum oxide (Adam's catalyst) was purchased from Matheson Coleman and Bell Inc., Norwood, Ohio. An analytically pure sample of cyperquat was supplied by Gulf Oil Chemicals Co., Merriam, Kan.

Hydrogenation of Cyperquat. A simple apparatus similar to that described by Vogel (1966) was used for hydrogenation. Ten milligrams of cyperquat dissolved in about 30 ml of methanol was taken in a hydrogenation flask containing 20 mg of platinum oxide (PtO_2). Hydrogenation was carried out at room temperature for 2 h.