Mutagenic Potential of Ammonia-Related Aflatoxin Reaction Products in a Model System

S.R. Haworth[a], T.E. Lawlor[a], E. Zeiger[b], L.S. Lee[c] and D.L. Park[*,d]
[a]Microbiological Associates, Inc., Rockville, MD 20850; [b]National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709, [c]U.S. Department of Agriculture, New Orleans, LA 70179, and [d]Food and Drug Administration, Washington, DC 20204

In a joint research effort, the Food and Drug Administration, the National Toxicology Program and the U.S. Department of Agriculture determined the mutagenic potential of aflatoxin reaction products following ammoniation of aflatoxin B_1 in a pressure chamber used to decontaminate aflatoxin-contaminated cottonseed meal. Uniformly ring-labeled (^{14}C)-aflatoxin B_1 was added to nonlabeled B_1, distributed on an inert carrier and treated with 4% ammonia at 40 psi, 100 C, for 30 min. Aflatoxin-derived decontamination reaction products were separated, and fractions having a high specific activity were tested for mutagenic activity using the *Salmonella*/mammalian-microsome mutagenicity assay (Ames test). When concentrations ranging from 3.3 to 100 μg per plate were tested, all fractions exhibited a similar mutagenic response. The observed mutagenic activity was 2,000–20,000 times less than that observed with nonammoniated aflatoxin B_1.

The use of cottonseed and cottonseed meal infected with *Aspergillus flavus* has been inhibited severely by their potential contamination by aflatoxins and the absence of an approved decontamination method for effective removal or inactivation of the toxins. In a joint research effort, the Food and Drug Administration, the National Toxicology Progam and the U.S. Department of Agriculture studied the mutagenicity of aflatoxin reaction products generated by the ammoniation conditions used (100 C for 30 min at 40 psi), which approximated those approved for commercial ammoniation of nonaflatoxin-contaminated meal (1,2). These studies demonstrated that treatment with ammonia significantly reduces aflatoxin levels and, based on data produced in the Ames test, the resulting aflatoxin decontamination products exhibited little or no mutagenic activity. The decontamination procedure reduced the aflatoxin B_1 concentration in the cottonseed meal from 4,000 to 4 μg/kg (1). The pressure chamber used in the study with cottonseed meal was also used to study the chemistry of the decontamination process (3). Lee et al. (3) mixed uniformly labeled aflatoxin B_1

TABLE 1

Mutagenicity of Aflatoxin B_1[a] and of Isolated Aflatoxin-Related Decontamination By-Products

		Average[b] TA100 revertants per plate											
		Extract concentration (μg/plate)											
Fraction control	Solvent	0.003	0.01	0.03	0.1	0.3	1.0	3.3	10	33	100	330	500
Acetone-soluble													
Fraction 1	104± 8								313± 8	500± 1	1258±145	1007±62	1202±93
Fraction 2[c]	71±10	81± 7	81±17	74± 7	69±9	81±10	106± 7	167±10	413±52	1383± 35			
Fraction 3	87±10	79±11	88± 8	72±22	69±9	90±12	111±14	185±10	389±23	1083± 35			
Acetonitrile-soluble													
Fraction 1	126±10								364±31	480±119	484± 33	526±38	450±15
Fraction 2	113± 9								172±13	227± 16	594± 52	736±28	563±36
Fraction 3	103± 7								160±28	190± 9	451± 21	478±30	362±33
Methanol-soluble													
Fraction 1	123±15								182± 7	265± 7	613± 30	613± 5	506±36
Fraction 2	128± 5								-[d]	-	-	-	-
Acidic methanol-soluble													
Fraction 1	115± 5								-[d]	-	-	-	-

[a]Aflatoxin B_1 tested at 0, 0.0018, 0.0056, 0.010, 0.032, 0.056 and 0.10 μg/plate induced 172±3, 270±28, 544±13, 743±34, 728±129 and 421±64 TA10 revertants per plate (spontaneous = 131 ± 7 revertants per plate).
[b]Mean (three plates) ± standard deviation.
[c]Contains MW 206 compound.
[d]A positive dose-related response was observed. However, a mold contaminant in the sample interfered with accurate enumeration of revertant colonies.

*To whom correspondence should be addressed at Department of Food Science and Nutrition, 309 Schantz Bldg., University of Arizona, Tucson, AZ 85721.

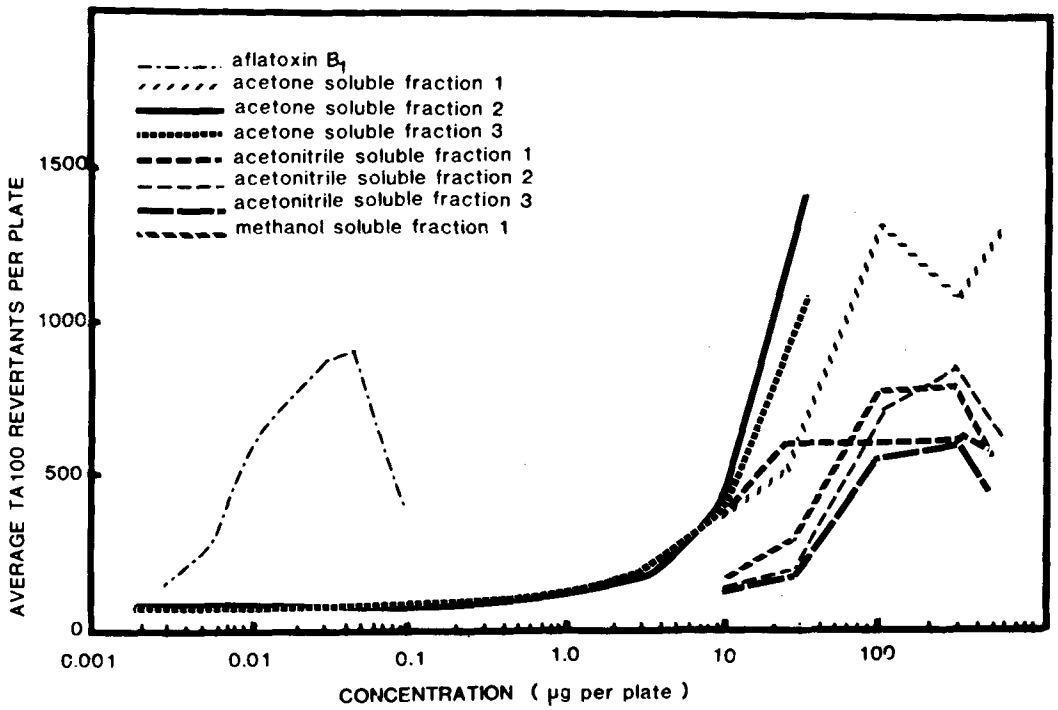

FIG. 1. Dose responses of tester strain TA100 to aflatoxin B_1 and isolated aflatoxin B_1-related decontamination products. (Average of three plates per dose.) Graph generated from data in Table 1.

with nonlabeled B_1, distributed the mixture on an inert carrier and subjected the mixture to ammoniation at 100 C for 30 min at 40 psi. They demonstrated that the ammoniation process resulted in the structural alteration of over 99% of the aflatoxin B_1. Approximately 20% of the aflatoxin B_1 degradation products was identified as an MW 206 compound, approximately 60% consisted of fragments of aflatoxin B_1, each having an MW <200, and the remaining 20% was lost as volatile compounds.

The present study was undertaken to evaluate the mutagenic potential of the ammoniated aflatoxin reaction products separated in the Lee et al. (3) study.

MATERIALS AND METHODS

Sample preparation. The fractions tested in this study were those isolated and purified by Lee et al. (3).

Mutagenicity determination. The Ames test (preincubation method) was conducted essentially as described by Haworth et al. (4). The mutagenicity of each extract was determined using *Salmonella typhimurium* tester strain TA100 (obtained from B.N. Ames, University of California, Berkeley, California) in the presence of Aroclor 1254-induced Sprague-Dawley rat liver homogenate. Each extract was dissolved in dimethylsulfoxide (DMSO) and subsequently was serially diluted with DMSO. Fifty-µl aliquots from the appropriate dilutions were then plated in triplicate, as were concurrent appropriate positive and solvent controls.

At least five dose levels of each extract were plated; the maximum dose level plated for each extract was 500 µg/plate.

RESULTS AND DISCUSSION

Results of the Ames test on the various ammoniation fractions are presented in Table 1. All fractions tested exhibited some degree of mutagenicity, but all were significantly less mutagenic than aflatoxin B_1. All of the extracts exhibited some cytotoxicity to the tester strain over the dose range tested.

Purified aflatoxin B_1 was judged mutagenic at a concentration of 0.005 µg/plate, whereas the acetone-, acetonitrile- and methanol-soluble fractions required approximately 10–33 µg to elicit a similar response. Figure 1 shows the mutagenic response for aflatoxin B_1 and for the reaction products formed by ammoniation of aflatoxin B_1.

Lee et al. (5) and Cucullu et al. (6) reported the formation of two major products when pure aflatoxin B_1 reacted with ammonium hydroxide under elevated temperature and pressure: (i) a nonfluorescent phenol of MW 286 (aflatoxin D_1) that lacks the lactone carbonyl moiety, and (ii) a similar compound of MW 206 that lacks the cyclopentenone ring. In the current study, only the MW 206 entity was observed; no aflatoxin D_1 was formed. The fraction containing the MW 206 degradation product exhibited a positive mutagenic response at 10 µg/plate.

The amount of conversion of aflatoxin B_1 to aflatoxin D_1 and to the 206 MW compound was approximately 30% and 23%, respectively, when reactions were carried out in model systems with no meal present (3, 5, 6). In peanut and cottonseed meals, however, the average conversion of aflatoxin B_1 to D_1 was 0.35% and no MW 206 compound was detected (7). Lee et al. (8) also reported a conversion rate of <1% for B_1 to D_1

in a peanut meal matrix. Park et al. (1) observed similar results where neither aflatoxin D_1 nor the 206 MW decontamination reaction product was found in the ammoniation of cottonseed meal. In a follow-up study, Lawlor et al. (2) reported that only one fraction, representing 0.16% of the original aflatoxin contamination, exhibited a mutagenic response.

These studies indicate that more mutagenic activity is observed when aflatoxin B_1 is ammoniated in the model system without meal matrix than when actual aflatoxin B_1-contaminated meal matrix is ammoniated. Ammoniation of aflatoxin-contaminated cottonseed and peanut meals significantly lowers aflatoxin contamination levels (9). We have shown that mutagenic compounds are formed when pure aflatoxin is ammoniated in a model system; however, these compounds were not found in the actual ammoniated meal matrix study of Lawlor et al. (2). Therefore, the meal matrix constituents apparently influence the formation of ammoniated aflatoxin by-products. Thus, the model system used by Lee et al. (3) may be inappropriate for characterizing aflatoxin by-products actually produced in an ammoniated meal matrix.

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