Project Number 77-N4

DFA OF CALIFORNIA

DFA AFLATOXIN RESEARCH PROJECT FOR ALMOND BOARD OF CALIFORNIA

by

George Stanley, Research Director

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Prior to 1973 the "CB Method" was the method of choice for aflatoxin analysis of almonds. It is a relatively complicated procedure and does not distinguish between positive and negative samples until the quantitation step is completed. This results in much wasted analytical time and labor due to the many negative samples that are carried through procedures required primarily for quantitation. Quality control and high-volume analytical laboratories needed a sensitive, simple, inexpensive aflatoxin detection method that could be used to rapidly screen out those samples containing no detectable aflatoxins and identify positive samples which require additional quantitation.

In 1973, an aflatoxin screening procedure called the Minicolumn Method became available and the DFA immediately tested it on almonds. This method proved to be faster and more sensitive than the "CB Method" with almonds. 20 to 30 Samples could be tested by one technician in an 8-hour day, whereas ony 6 - 10 samples could be tested by the CB Method. The DFA then decided to research the adoption of the Minicolum Method as a possible official method for almonds. Recognition by the AOAC (Association of Official Analytical Chemists) would mean that the method is an official U.S. Food & Drug Administration procedure recognized throughout the world.

In early 1977, the DFA began to acquire the data necessary to support the adoption of the Minicolumn Aflatoxin Detection Method. It was collaboratively tested on almonds by the DFA this year, and twenty laboratories throughout the United States and Canada participated in the research. Samples containing 0, 2, 5, 10 and 25 parts per billion (ppb) total aflatoxin were analyzed. Ninety-six per cent of the samples containing 5 - 25 ppb aflatoxin, and 83% of the negative samples were correctly identified. In October, at the AOAC meeting in Washington, D. C., the Minicolumn Method was adopted as official for the detection of total aflatoxin levels of 5 parts per billion and above in almonds.

Since 1972 the Western Regional Research Laboratory and the DFA have conclusively shown that manufacturing stock almonds may present a problem, while whole meats are relatively free from contaminants.

By continuing an aflatoxin monitoring program, the almond industry is demonstrating its concern for the aflatoxin problem and, at the same time, acquiring valuable data, demonstrating that top quality almonds on the market today rarely contain aflatoxin. We feel that due to the quality of California almonds and aflatoxin monitoring programs funded by the Almond Board of California, the industry has avoided costly lot-by-lot aflatoxin certifications, similar to those imposed upon the peanut industry.

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ANNUAL REPORT

TITLE: Project 77-N4 Aflatoxin Analytical Methods and Monitoring Programs

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1. Objectives:

Project 77-N4 has five objectives: (1) to obtain official status for the minicolumn aflatoxin detection method on almonds by conducting a collaborative study with the AOAC, (2) to participate in an aflatoxin analytical check sample program with the American Oil Chemists Society, (3) to monitor aflatoxin in almonds and almond products, (4) to analyze aflatoxin in almonds furnished by Dr. Doug Phillips, U.S.D.A., and (5) to analyze for concealed damage in samples submitted by Dr. Labavitch, U.C.D.

2. Interpretive Summary:

The minicolumn screening method for aflatoxins was collaboratively tested on naturally contaminated almonds. The nuts were extracted, cleaned up, and applied to a Velasco-type minicolumn. This allows for the detection of total aflatoxins (B_1, B_2, G_1, G_2) as a fluorescent band on the florisil layer of the column. The results of 20 collaborators from throughout the United States and Canada are presented. Samples containing 0, 2, 5, 10, and 25 parts per billion aflatoxin were analyzed. Ninety-six per cent of the samples containing 5-25 parts per billion total aflatoxins, and 83% of the negative samples were correctly identified. The method is recommended for adoption as official first action for detection of total aflatoxin levels of 5 parts per billion and above.

The DFA aflatoxin check sample program shows excellent correlation of our aflatoxin results to the many laboratories throughout industry and at the state and federal levels as well.

Aflatoxin contamination appears to be infrequent this year. No select sheller run almonds have been found to contain aflatoxin and only one sample of manufacturing stock almonds was positive. However, it contained 143 parts per billion which is much above the 20 parts per billion FDA guideline. Almond press cake meal continues to be overtolerance as the samples analyzed thus far contained 62 and 35 parts per billion.

Almond samples sent to this laboratory for aflatoxin analysis by Dr. Doug Phillips, U.S.D.A. have been completed as well as the almonds submitted by Dr. Labavitch, U.C.D., for concealed damage and aflatoxin.

3. Experimental Procedure:

Same as last year's annual report, except that 10 pound samples were drawn instead of 30 pound samples. This change was made due to the fact that the U. S. Food & Drug Administration has dropped their sample size for aflatoxin analysis from 30 to 10 pounds.

4. Results:

Plan #1:

AOAC collaborative study on minicolumn: Five unknown samples containing 0, 2, 5, 10, and 25 parts per billion aflatoxin were sent to 20 collaborators in the U. S. and Canada. The following results were reported:

	0 ppb	2 ppb	5 ppb	10 ppb	25 ppb
% correct	83	65	93	100	95

The method has been accepted as official for almonds by the AOAC for the detection of total aflatoxin levels of 5 parts per billion and above.

Plan #2:

The following results are from the DFA-American Oil Chemistry Society (AOCS) aflatoxin check sample program:

		DFA (PPB)	AOCS mean (PPB)
Cottonseed meals -	Sample #1	23	22.5
	#2	standard check	
	#3	21	19.8
	#4	31	39.7
	#5	174	
	#6		
Corn	Sample #1	36	25.3
	#2	standard check	
	#3	20	24
	#4	18	16
	#5	8	
	#6		
Peanut Meal	Sample #1	37	26.7
	#2	standard check	
	#3	23	27.4
	#4	33	35.9
	#5	33	35.9
	#6	33	31.9

Plan #3:

a. Forty-nine Select S/R samples have been analyzed for aflatoxin and all were negative.

b. Thirteen manufacturing stock samples have been analyzed for aflatoxin and one was positive (143 parts per billion).

c. Sixteen hull samples have been analyzed for aflatoxin and five were found to be positive (20.8 parts per billion, 2.7 ppb, 3.6 ppb, 7.7 ppb, and 2 ppb).

d. Two press cake meal samples have been analyzed for aflatoxin and both were positive. One contained 62 parts per billion while the other contained 35 parts per billion.

Plan #4:

Fifty aflatoxin analyses were done for Dr. Doug Phillips.

Plan #5:

Eight free fatty acid, 38 roasting and 46 aflatoxin analyses were done for Dr. Labavitch, UCD.

5. Discussion:

Since 1973 the DFA of California has been working with the almond industry in an effort to find the least expensive procedure for aflatoxin analysis, in order to keep grower and packer costs down.

Prior to 1973 the "CB Method" was the method of choice for aflatoxin analysis of almonds. It is a relatively complicated procedure and does not distinguish between positive and negative samples until the quantitation step is completed. This results in much wasted analytical time and labor due to the many negative samples that are carried through procedures required primarily for quantitation. Quality control and high-volume analytical laboratories needed a sensitive, simple, inexpensive aflatoxin detection method that could be used to rapidly screen out those samples containing no detectable aflatoxins and identify positive samples which require additional quantitation.

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As early as 1974, I was concerned as to how I could be sure the DFA laboratory was releasing accurate aflatoxin data. In November of that year, I analyzed an unknown peanut sample sent out by U.S.D.A., Washington, D. C. and I found 31 parts per billion total aflatoxin. The mean result for 23 laboratories was 36.8 and the range was 25 - 52. I felt pretty good about that check sample, as the quantitation was done by thin-layer chromatography which has a built-in error of 20-50%.

We now use high-pressure liquid chromatography coupled with a fluorescent detector which has less than a 1% error for quantitation. This year DFA is participating in check sample programs administered by the American Oil Chemistry Society. The commodities we are evaluating are: peanut meal, corn, and cottonseed meal.

The results to date show excellent correlation of DFA results with laboratories in industry, government and at the federal level. It should be pointed out also that DFA is the only laboratory in the check sample program whose quantitation is being reported using a high-pressure liquid chromatograph coupled to a fluorescent detector. The other laboratories use thin-layer chromatography for quantitation. High-pressure liquid chromatography is one of the most powerful quantitation tools available and can detect aflatoxin down to 10 parts per trillion.

Crop year 1977-78 appears to be relatively insignificant as far as aflatoxin contamination is concerned, even though navel orange worm damage has been heavy. Up to now there has been good correlation between N.O.W. damage and aflatoxin. Perhaps this points to another necessary variable in the equation, moisture. This year we had hardly any significant rainfall at harvest time. Press cake meal continues to show aflatoxin at violative levels. Both this year and last, DFA has found aflatoxin in every press cake meal sample analyzed.

Future work could center on: (1) Modifications of the minicolumn procedure to make it faster, cheaper, and safer. (2) A collaborative study combining the minicolumn screening procedure and high-pressure liquid chromatography quantitation which some of our industry members now have. (3) Continued monitoring of Select S/R, manufacturing stock, hulls, and press cake meal. (4) Look at sugars and free fatty acids as causitive agents of concealed damage. (5) Larger scale studies of the effects of roasting and blanching. (6) Cost-evaluating ammoniation of almond press cake meal, and (7) Correlate worm damage and moisture to aflatoxin production.

Fig. 1. Susceptible period of split Nonpareil hulls to infection caused by inoculation with dry spores of Rhizopus stolonifer.