Peanut Science (1978) 5, 13-16

The Incidence of Aflatoxin Found in Groundnuts (*Arachis hypogea* L.) Purchased From Markets in and Around Accra, Ghana[1]

S. Mintah and R.B. Hunter[2]

ABSTRACT

Eighty groundnut samples, representing both the Northern and Volta Region types, were purchased from markets in and around Accra. Extracts from each sample were assayed for the presence of aflatoxin. Of the samples examined, approximately half had aflatoxin levels exceeding that recommended as safe by FAO-WHO. The Volta Region conditions are much wetter at the time when the first season groundnuts are harvested. Damaged Volta Region drying conditions are much wetter at the time when the first season groundnuts are harvested. Damaged kernels had significantly higher levels of aflatoxin than whole and undamaged kernels. When damaged kernels were removed from the samples the remaining whole kernels were without exception below the tolerance level established by FAO-WHO.

Keywords: Peanuts, groundnuts, *Arachis hypogea* L., Aflatoxin, *Aspergillus flavus* Link ex Fr., mycotoxin, human hepatocarcinoma.

In 1960, concurrent outbreaks of disease in domestic animals in Europe and in East Africa focused attention on the importance of contamination of food and feed by fungus produced toxins. An outbreak of a disease (turkey X disease) among turkey poults in Britain was responsible for the loss of 100,000 birds in 1960. On postmortem examination the poults were found to have acute liver lesions. Within about a year of the mysterious turkey losses the problem was linked to a toxic material produced by *Aspergillus flavus* Link ex Fr. The toxic material was traced to a shipment of Brazilian groundnuts infected with A. *flavus*. Shortly after the problem with aflatoxins in the turkeys, toxic meals were shown to have hepatic effects on other domestic species (Sargeant *et al.*, 1961). Since 1960 a massive amount of literature has been accumulated on the toxicity of aflatoxins to various domestic and laboratory animals. For a detailed review of the literature, see Detroy, Lillehoj and Ciegler (1971). Most domestic and laboratory animals examined are susceptible to aflatoxin poisoning; however, toxicity is influenced by species, age, sex and nutrition. Normally, young animals are more susceptible than older ones and males more susceptible than females. In general, acute high level ingestion causes acute hepatic necrosis, whereas chronic low level ingestion causes hepatic fibrosis and/or primary hepatic cancer.

Direct evidence of the carcinogenicity of aflatoxins to humans has not been obtained. However, studies have been conducted that provide convincing evidence of a close association between the consumption of aflatoxin-containing foods and the occurrence of hepatocarcinoma in humans. A recent evaluation of the available data by van Rensberg *et al.* (1974) suggests that the incidence of hepatocarcinoma associated with aflatoxin exposure in humans is dose related.

Since the initial report in 1961, the natural occurrence of aflatoxins in peanuts and peanut meal has been reported by several investigators (Allcroft and Carnaghan, 1962; Allcroft and Lewis, 1963; Lopez and Crawford, 1967). Lopez and Crawford examined purchased packets of groundnuts sold in Uganda markets for human consumption. About 15% of the samples examined contained more than 1 ppm aflatoxin B_1 and 2.5% contained more than 10 ppm. The most badly contaminated nuts were obtained between May and July, a period when heavy rainfall directly preceded harvest of the new crop.

At the Tropical Products Institute in England, 1350 samples of whole groundnuts and 413 samples of groundnut meal and cake were examined for aflatoxin (Hiscocks, 1965). The samples came from 12 countries located in Africa, Asia and South America. Seventy-five percent of the whole groundnut samples and 91.3% of the groundnut meal samples contained aflatoxin with 3.3% and 42.0% respectively containing high to very high levels. Taber and Schroeder (1967) demonstrated the presence of aflatoxin in groundnuts grown in the United States.

The purpose of this study was to determine the levels of aflatoxins present in groundnuts grown in two areas of Ghana.

Materials and Methods

Ten groundnut samples were purchased from each of eight markets in the Accra area. The markets surveyed were Makola, Kaneshie, Salaga, London, Teshie, Nungua, Adabraka and Madina. All samples from a given market were purchased at the same time with the sampling time for all markets covering the period from July 1976 to October 1976. Of the ten samples collected from each market, five samples were the Northern type groundnuts and five were the Volta type. Northern groundnuts have a rough, light brown testa and are largely produced in the Northern and Upper Regions of Ghana. The Volta type has a smooth, deep brownish-red testa and is produced in the Volta Region. The five Northern and Volta samples purchased from each market were chosen at random from the nuts for sale at that market. In all cases sample size was 0.5 kg.

In addition to the 80 groundnut samples discussed above, an additional twenty samples, ten of the Northern and ten of the Volta type, were collected from the Makola and Keneshie markets. From each sample, damaged kernels (including those that were wrinkled or had parts of their testa removed) were separated from undamaged nuts.

The eighty market samples and the damaged and undamaged fractions of the additional 20 samples were all analyzed for

[1]Department of Crop Science, University of Ghana, P.O. Box 68, Legon, Ghana.

[2]Present address: Department of Crop Science, University of Guelph, Guelph, Ontario, Canada.

aflatoxin using the technique of Liem and Beljaars (1970). Each groundnut sample was ground to a paste and homogenized by mixing with a spatula. By quartering, 50 g of homogenized sample was weighed into a blender jar and 100 ml of methanol and 10 ml of distilled water were added. The mixture was blended at full speed for 3.5 minutes. The resulting slurry was thoroughly mixed with 30 ml of distilled water, transferred to a 250 ml centrifuge bottle and centrifuged for five minutes at 2500 rpm. Seventy ml of the clear extract was pipeted into a 250 ml separatory funnel and mixed with 20 ml distilled water. Ninety ml of chloroform was added and the mixture shaken for one minute. The bottom chloroform layer was collected and dried with anhydrous sodium sulphate. The drying agent was removed and the solution was evaporated to dryness. The residue was dissolved in 0.5 ml of chloroform and shaken vigorously. The final extract was equivalent to the extract from 25 g of sample and was ready for TLC analysis.

TLC plates were prepared by mixing 30 g silica gel H (Merck) with 64 ml distilled water in a Waring blender for 30 seconds. A gel layer 0.25 mm thick was spread on glass plates (20 x 20 cm). They were air dried and activated at 120 C for one hour before use. Prior to spotting the samples, the plates were developed in a chromatography tank with 100 ml diethyl ether to remove impurities present in the silica gel to the ether solvent front. The plates were spotted with 2, 3, 5 and 10 ul of sample extract along with 1, 2, 3, 5 and 10 ul of a standard solution of aflatoxin which contained 0.9 mg/ml B_1 and 0.6 mg/ml G_1. The solutions were spotted two cm from the bottom of the plate. The spotted plates were developed by the ascending technique in a dark room at a temperature of 25 C in an unequilibrated tank containing freshly mixed chloroform, trichloroethylene, n-amyl alcohol and formic acid (80 + 15 + 4 + 1 v/v). The plates were developed twice for 45 minutes each time.

After development, the plates were dried and examined in a darkroom at a distance of 30 cm from an ultraviolet lamp (356 nm). The fluorescence intensities of the sample extracts were compared with those of the standards. If the level of aflatoxin was higher than the standard solution, appropriate dilutions were made to the sample extract until it fell within the range of the standard. The concentrations of aflatoxins B_1 and G_1 were calculated using the formula:

$$\text{Aflatoxin content (ug/kg)} = \frac{S \times Y \times V}{W \times Z}$$

where

S = ul of aflatoxin standard equal to that of material being evaluated

Y = concentration of aflatoxin B_1 and G_1 standard in mg/ml

W = weight in gm of original sample contained in final extract

Z = ul of sample extract spotted to give fluorescence intensity equal to S, the aflatoxin standard

V = ul of solvent required to dilute final extract.

Two tests were used to confirm the presence of aflatoxin in the samples. In the first method, the locations of the fluorescent blue spots observed after TLC development were marked with a pencil. The plates were redeveloped in ether solution. Any movement of the spots suggested the spots were not due to aflatoxin (Anonymous, 1972). The second confirmatory test, developed by Przybylski (1975) involved spraying 25% sulphuric acid on developed plates. If the spots were aflatoxin they fluoresced yellow rather than blue or green.

Results and Discussion

The total aflatoxin levels (B_1 + G_1) found in the groundnut samples ranged from 3.0 ug/kg to 216 ug/kg (Table 1). It has been recommended that the level of aflatoxin in protein supplements eaten by humans at the rate of 100 g per day should not exceed 30 ug/kg of foodstuff (Anonymous, 1966). Approximately half of the 80 samples selected from the markets in the Accra area had aflatoxin equal to or exceeding this level. These findings suggest that a large number of the groundnuts available in the Accra area markets between July and October 1976 constituted a potential health hazard if consumed in large quantities on a regular basis.

The high incidence of aflatoxin on the groundnut samples examined should not be too surprising. In general, it appears that in those areas of the world like Ghana, where humidities are high and temperatures moderate to high and where harvesting, storage and marketing facilities are often inadequate, there is considerable frequency of aflatoxin occurrence (Detroy *et al.*, 1971). The molds involved are ubiquitous and when conditions are suitable, are readily capable of growth and aflatoxin production on groundnuts.

The Volta type groundnuts contained on the average significantly higher levels of aflatoxin than the Northern nuts (Table 1). Sixty-five percent of the Volta type samples had aflatoxin levels exceeding 30 ug/kg whereas only 30% of the Northern type exceeded this level.

There was no difference in the levels of aflatoxin found in the groundnuts purchased from the various markets. However, in every market the Volta type nuts had higher levels of aflatoxin than the Northern types.

The difference between the aflatoxin content in the Volta and Northern nuts may result from the climatic conditions prevailing at each location during harvesting, drying and storage. Although *A. flavus* may invade the developing fruit of the groundnut prior to digging time (Diener *et al.*, 1965), invasion by the fungus and subsequent aflatoxin production probably occurs primarily after harvest when shells and kernels begin to dry (Austwick and Ayerst, 1963). In the Northern and Upper Regions of Ghana, groundnuts are sown in June and harvested from September to November. During the harvest

Table 1. Aflatoxin levels (ug/kg of foodstuff) in Northern and Volta types of groundnuts purchased from eight markets in the Accra area.

	Northern type groundnuts		Volta type groundnuts	
Market	Mean of five samples	Range of five samples	Mean of five samples	Range of five samples
Makola	13	3 - 27	42	14 - 100
Kaneshie	12	4 - 22	50	13 - 99
Salaga	28	3 - 88	78	15 - 141
Adabraka	33	5 - 71	59	10 - 116
Teshie	26	5 - 58	69	10 - 187
Nungua	27	10 - 47	67	25 - 145
Madina	41	5 -133	86	10 - 216
Mean	27 a*	3 -133	62 b	10 - 216

* Means with different letters are significantly different at the 0.05 level of probability.

period rainfall and relative humidity are low. Under these conditions the groundnuts are more easily and quickly dried to moisture levels unfavourable to fungus growth and toxin production (Feuell, 1966).

In the Southern Sector (Volta Region) of Ghana there are two seasons of cultivation. In the first season the groundnuts are sown from March to April and harvested from June to August. During this period rainfall and relative humidities are high. Such conditions make drying of the nuts difficult and provide conditions conducive to *A. flavus* growth (Diener and Davis, 1967). Groundnuts grown in the second season are planted from September to October and harvested from December to February. Conditions at this time of year are less conducive to *A. flavus* growth and development. If the Volta type groundnuts sampled in this study were from the first season crop it may, at least in part, account for the higher levels of aflatoxin in the Volta nuts.

Table 2. Aflatoxin levels (ug/kg of foodstuff) in the damaged and undamaged whole nut portions of groundnuts selected from individual market samples purchased from two markets in the Accra Area.

	Makola market		Kaneshie market	
Sample	Damaged kernels	Undamaged kernels	Damaged kernels	Undamaged kernels
1	77	18	101	10
2	47	10	24	ND*
3	178	12	79	12
4	105	2	29	18
5	20	5	178	ND*
6	29	ND*	94	14
7	87	12	69	7
8	44	2	20	ND*
9	79	18	14	ND*
10	129	ND*	103	24
Mean	80 a**	8 b	71 a	9 b

* ND no aflatoxin detected.

** Means with different letters are significantly different at the 0.05 level of probability.

Other factors such as varietal differences, harvesting techniques, drying techniques and storage methods could also be important factors conditioning the amount of aflatoxin found in groundnuts. Varietal differences have been reported by Suryanarayana and Tulpule (1967). Shank (1976) points out that the incidence and severity of contamination can be greatly reduced by improving harvesting and handling methods. Practices should be adopted that minimize physical injury to the crop at harvesting. In addition, it is necessary to achieve rapid drying once harvesting has taken place. Shank suggests practices such as tying freshly harvested plants to stakes rather than drying them on the ground can be helpful in reducing aflatoxin. Crops that are being dried should be covered during periods of rain. Once dried, the nuts should be protected from moisture by storage under dry conditions.

Obviously the best method of coping with the potential health problems associated with aflatoxin contamination of foods and feeds is to prevent growth and development of *A. flavus* from the outset. However, once the nuts have been contaminated, methods should be sought to detoxify the nuts. Heat treatments under normal atmospheric conditions have been unsuccessful (Carnaghan, 1964). Analysis has shown that in contaminated samples the aflatoxin is not uniformly present on all kernels. In fact, in most instances a relatively small percentage of the kernels are contaminated (Austwick and Ayerst, 1963; Cucullu et al., 1966).

When samples purchased from the market were cleaned by hand removal of damaged kernels, there were no instances where the cleaned samples had levels of toxin above 30 μg/kg (Table 2). The damaged kernels in all cases contained higher levels of aflatoxin than the non-damaged portion of the sample.

During the survey it was noted that many of the market women remove some of the damaged and moldy kernels prior to the sale of the groundnuts. The physical removal of damaged kernels should be encouraged both in the market and at home.

Literature Cited

1. Allcroft, R., and R.B.A. Carnaghan. 1962. Groundnut toxicity *Aspergillus flavus* toxin (aflatoxin) in animal products; preliminary communication. Vet Record 74: 863-864.
2. Allcroft, R., and G. Lewis. 1963. Groundnut toxicity in cattle: experimental poisoning of calves and a report on clinical effects in older cattle. Vet Record 74: 487-493.
3. Anonymous. 1966. Mycotoxins in foods. Joint FAO/WHA expert committee on nutrition. Seventh Report, Rome, Italy. pp. 55-56.
4. Anonymous. 1972. Methods of aflatoxin analysis. Trop. Prod. Inst. Bull. No. G70.
5. Asao, T., G. Buchi, M.M. Abdel-Kader, S.B. Chang, E.L. Wick, and G.N. Wogan. 1963. Aflatoxins B and G. J. Amer. Chem. Soc. 85: 1706-1707.
6. Asao, T., G. Buchi, M.M. Abdel-Kader, S.B. Chang, E.L. Wick, and G.N. Wogan. 1965. The structures of aflatoxins B_1 and G_1. J. Amer. Chem. Soc. 87: 882-886.
7. Austwick, P.K.C., and G. Ayerst. 1963. Groundnut microflora and toxicity. Chem. and Ind. 41: 55-61.
8. Carnaghan, R.B.A. 1964. Some biological effects of aflatoxin. Proc. Roy. Soc. Med. 57: 414-416.
9. Cucullu, A.F., L.S. Lee, R.Y. Mayne, and L.A. Goldblatt. 1966. Determination of aflatoxins in individual peanuts and peanut sections. J. Amer. Oil Chem. Soc. 43: 89-92.

10. Detroy, R.W., E.B. Lillehoj, and A. Ciegler. 1971. Aflatoxin and related compounds. In Microbial toxins. Ciegler, A., Kadis, S. and Ajl, S.J. (eds.) Fungal toxins. VI. Academic Press, New York and London.

11. Diener, U.L., and N.D. Davis. 1966. Aflatoxin production by isolates of *Aspergillus flavus*. Phytopathol. 56: 1390-1393.

12. Diener, U.L., and N.D. Davis. 1967. Limiting temperature and relative humidity for growth and production of aflatoxin and free fatty acids by *Aspergillus flavus* in sterile peanuts. J. Amer. Oil Chem. Soc. 44: 259-263.

13. Diener, U.L., C.R. Jackson, W.E. Cooper, R.J. Sliper, and N.D. Davis. 1965. Invasion of peanut pods in the soil by *Aspergillus flavus*. Plant Dis. Rep. 49: 931-935.

14. Feuell, A.J. 1966. Toxic factors of mould origin. Can. Med. Ass. J. 94: 574-581.

15. Hiscocks, E.S. 1965. The importance of molds in the deterioration of tropical foods and feedstuffs. In Mycotoxins in Foodstuffs, G.H. Wogan (ed.). pp. 15-26. M.I.T. Press, Cambridge, Massachusetts.

16. Joffe, A.Z. 1969. Aflatoxin produced by 1626 isotoles of *Aspergillus flavus* from groundnut kernels and soils in Israel. Nature 221: 492.

17. Liem, D.H., and R.R. Beljaars. 1970. Note on a rapid determination of aflatoxins in peanut and peanut products. J. Ass. Offic. Anal. Chem. 53: 1064-1066.

18. Lopez, A., and M.A. Crawford. 1967. Aflatoxin content of groundnuts sold for human consumption in Uganda. The Lancet 2: 1351-1354.

19. Przybylski, W. 1975. Formation of aflatoxin derivatives on thin layer chromatographic plates. J. Ass. Offic. Anal. Chem. 58: 163-164.

20. Sargeant, K., A. Sheridan, J. O'Kelly, and R.B.A. Carnaghan. 1961. Nature. Lond. 192: 1096-1097.

21. Shank, R.C. 1976. Mycotoxins in the environment. In Trace substances and health, a handbook, Part 1. Newberne, P.M. (ed). Marcel Dekker Inc., New York. pp. 67-158.

22. Suryanarayana, K.R., and P.G. Tulpule. 1967. Varietal differences of groundnut in production of aflatoxin. Nature 214: 738-739.

23. Taber, R.A., and H.W. Schroeder. 1967. Aflatoxin-producting potential of isolates of the *Aspergillus flavus - oryzae* group from peanuts *(Arachis hypogaea)*. Appl. Microbiol. 15: 140-144.

24. van Rensberg, S.J., J.J. van der Watt, I.F.H. Purchase, L.P. Coutinho, and R. Markham. 1974. Primary liver cancer rate and aflatoxin intake in a high cancer area. S. Afr. Med. J. 48: 2508a-2508d.

25. Wogan, G.N., G.S. Edwards, and P.M. Newberne. 1971. Structure - activity relationships in toxicity and carcinogenicity of aflatoxins ad analogs. Cancer Res. 31: 1936-1942.