CC/NUMBER 2
JANUARY 11, 1988

This Week's Citation Classic®

Shotwell O L, Hesseltine C W, Stubblefield R D & Sorenson W G. Production of aflatoxin on rice. *Appl. Microbiol.* 14:425-8, 1966.
[Northern Regional Research Laboratory, Agricultural Research Service, US Department of Agriculture, Peoria, IL]

Aflatoxin B_1, a carcinogenic mycotoxin, was produced for animal feeding tests by growing *Aspergillus flavus* on the solid substrate rice. Isolation and recrystallization resulted in analytically pure aflatoxin B_1 for analytical standards and chemical studies. [The *SCI*® indicates that this paper has been cited in over 245 publications.]

Odette L. Shotwell
Northern Regional Research Center
Agricultural Research Service
US Department of Agriculture
1815 North University Street
Peoria, IL 61604

October 16, 1987

In the early 1960s aflatoxins were found to occur in the US; their carcinogenicity had been established in laboratory animals by British workers. In a cooperative effort by chemists (R.D. Stubblefield and me) and microbiologists (C.W. Hesseltine and W.G. Sorenson), we developed the solid-substrate fermentation process with an isolate of the *Aspergillus flavus* group (NRRL 2999) as well as the analytical, isolation, and purification procedures for aflatoxin B_1 described in the cited paper.

The Agricultural Research Service (ARS) of the US Department of Agriculture (USDA) decided to conduct feeding studies to assess the hazards of aflatoxin to farm animals. I was asked by Alex Keyl and Harlow Hall, ARS, to prepare the needed 75 g of aflatoxin B_1. I panicked at the prospect of preparing such a sample of crystalline B_1, then thought to be one of the most potent carcinogens known. We were located in a back building where facilities were not ideal. I suggested, out of desperation, that the study would more nearly simulate field aflatoxicosis if fermented rice containing aflatoxin B_1 were fed instead of crystalline B_1. All of the secondary metabolites and products formed by the fungi would be present in the feed. To my relief, Keyl and Hall agreed. Our research group only had to determine the concentrations of aflatoxin formed so as to prepare feeds containing specified amounts of toxin. Over the years it has become evident that purified mycotoxins do not cause the adverse effects expected at toxin levels observed in field mycotoxicosis.

By the time aflatoxin became commercially available, we had produced almost 200 g of aflatoxin B_1 at various stages of purity, and we often provided samples to other researchers. Grocery stores in our area were sometimes overwhelmed by our demand for rice. For five or six years, we were also the sole source of aflatoxin M_1 (also carcinogenic), a coproduct of the B_1 fermentation.[1] Aflatoxin M_1 occurs in milk and urine of animals ingesting feed containing aflatoxin B_1, causing problems in the dairy industry.

The B_1 fermentation technique was extended to other mycotoxins. Scientists were unable to determine the chronic and acute effects of T-2 toxin, a trichothecene, on livestock and poultry until H. Burmeister[2] produced gram quantities of T-2. He inoculated moistened white corn grits with *Fusarium tricinctum* and isolated T-2 toxin after three weeks of incubation. Ochratoxin A, a nephrotoxic mycotoxin, was produced on wheat inoculated with *A. ochraceus*; extracts were purified by preparative high-performance liquid chromatography to obtain toxin for feeding studies.[3] Whole kernel corn was the substrate used for a study on the production of the estrogen zearalenone on a number of hybrid and inbred corn lines.[4]

Recently, solid-substrate fermentations for production of mycotoxins and enzymes, as well as the more traditional fermentations leading to food products, were reviewed.[5] Advantages of solid-substrate fermentations in the production of mycotoxins are numerous. Yields of many mycotoxins are higher on solid substrates than in liquid culture, which is not surprising because they occur naturally on cereal grains and oilseeds. Temperature and moisture levels necessary for optimum mycotoxin formation in the field can be determined by solid-substrate fermentations. Isolations are easier to prepare than from liquid culture because liters of water do not have to be removed from milligrams of product. Only one ingredient is necessary to supply nutrients for the fermentation. However, there are at least two disadvantages to solid-substrate fermentations: they are not suitable for studying the biosynthetic pathways for formation of mycotoxins, and it is difficult to scale up a solid-substrate fermentation that is carried out in shaken Fernbach flasks. Also, the scaled-up process requires a great deal of energy.

In 1980 the research team I led received the Distinguished Service Award from the USDA for its work on mycotoxins. In 1982 I was honored by receiving the Harvey W. Wiley Award from the Association of Official Analytical Chemists for mycotoxin research.

1. **Stubblefield R D, Shannon G M & Shotwell O L.** Aflatoxins M_1 and M_2: preparation and purification. *J. Amer. Oil Chem. Soc.* 47:389-90, 1970. (Cited 50 times.)
2. **Burmeister H.** T-2 toxin production by *Fusarium tricinctum* on solid substrate. *Appl. Microbiol.* 21:739-42, 1971. (Cited 50 times.)
3. **Peterson R E & Ciegler A.** Ochratoxin A: isolation and subsequent purification by high pressure liquid chromatography. *Appl. Environ. Microbiol.* 36:613-4, 1978.
4. **Shannon G M, Shotwell O L, Lyons A J, White D G & Garcia-Aguirre G.** Laboratory screening for zearalenone formation in corn hybrids and inbreds. *J. Assn. Offic. Anal. Chem.* 63:1275-7, 1980.
5. **Hesseltine C W.** Solid state fermentation—an overview. *Int. Biodeterior.* 23:79-89, 1987.

ZA8

CC/AB+ES