Aflatoxins
CAS No. 1402-68-2

Known to be a human carcinogen
First Listed in the *First Annual Report on Carcinogens* (1980)

Carcinogenicity

Aflatoxins are *known to be human carcinogens* based on sufficient evidence of carcinogenicity in humans. Early evidence for the carcinogenicity of aflatoxins in humans came from descriptive studies that correlated geographic variation in aflatoxin content of foods with geographic variation in the incidence of liver cancer (hepatocellular carcinoma, or primary liver-cell cancer). Studies in Uganda, Swaziland, Thailand, Kenya, Mozambique, and China demonstrated strong, significant positive correlations between estimated aflatoxin intake or aflatoxin levels in food samples and the incidence of liver cancer. In the United States, a 10% excess in hepatocellular cancer was observed in the Southeast, where the estimated average daily intake of aflatoxin was high, compared with the North and West, areas with low aflatoxin intake. In a case-control study in the Philippines, individual levels of aflatoxin in the diet were estimated retrospectively, and the risk of liver cancer increased significantly with increasing aflatoxin consumption. Interpretation of these studies is complicated by potential confounding due to hepatitis B virus infection, which is endemic in many of the study areas and is known to cause hepatocellular carcinoma. In studies that took into account the prevalence of chronic hepatitis B infection, aflatoxin exposure remained strongly associated with liver cancer. Chinese studies in which chronic hepatitis B prevalence did not appear to fully explain differences in liver cancer rates were reviewed, and it was concluded that both estimated dietary levels of aflatoxins and measured urinary levels of aflatoxins and their metabolites were related to the remaining variance in hepatocellular cancer incidence. In a study in Swaziland, estimated aflatoxin intake based on levels in food samples was strongly correlated with liver cancer incidence; in this study, geographic variation in aflatoxin exposure better explained the variation in liver cancer incidence than did variation in the prevalence of hepatitis B infection (IARC 1987, 1993). The International Agency for Research on Cancer (IARC) concluded in 1987 that there was sufficient evidence in humans for the carcinogenicity of naturally occurring aflatoxins (IARC 1987). This conclusion was reaffirmed in two subsequent reevaluations (IARC 1993, 2002). These reevaluations considered the results of several cohort studies in China and Taiwan, which reported associations between biomarkers for aflatoxin exposure (aflatoxin metabolites in the urine and aflatoxin-albumin adducts in the blood) and hepatocellular carcinoma; the association remained when the analyses controlled for hepatitis B infection.

The findings in humans are supported by studies in experimental animals. Mice, rats, and other experimental animals exposed to aflatoxins by various routes developed tumors at multiple tissue sites. Oral administration of aflatoxin mixtures or aflatoxin B_1 alone (in the diet, by gavage, or in drinking water) caused hepatocellular or cholangiocellular liver tumors in all species tested except mice; these included rats, hamsters, marmosets, tree shrews, and monkeys. In addition, renal-cell and colon tumors occurred in rats, lung adenoma in mice, and liver and osteogenic sarcoma and gall-bladder and pancreatic adenocarcinoma in monkeys. Aflatoxin B_1 administered by intraperitoneal (i.p.) injection caused liver tumors in infant mice, adult rats, and toads. Aflatoxin B_1 administered by i.p. injection to pregnant and lactating rats caused tumors of the liver, digestive tract, urogenital system, and nervous system in the mothers and offspring. Aflatoxin mixtures administered by subcutaneous (s.c.) injection caused injection-site sarcomas in rats and mice. Aflatoxins B_2, G_1, and M_1 also caused liver tumors in experimental animals, but generally at lower incidences than did aflatoxin mixtures or aflatoxin B_1 alone. In rats, aflatoxin G_1 also caused kidney tumors when administered orally and a low incidence of injection-site sarcomas when administered by i.p. injection. In one experiment with fish, oral administration of aflatoxin G_2 did not cause liver tumors. Both enhancement and inhibition of aflatoxin's carcinogenicity were observed following co-administration of aflatoxins with various diets, viruses, parasites, known carcinogens, and other chemicals (IARC 1976, 1993). IARC (1993) concluded that there was sufficient evidence in experimental animals for the carcinogenicity of naturally occurring mixtures of aflatoxins and aflatoxins B_1, G_1, and M_1; limited evidence for the carcinogenicity of aflatoxin B_2; and inadequate evidence for the carcinogenicity of aflatoxin G_2. In its 2002 evaluation, IARC reported on several more recent studies suggesting that animals (woodchucks, tree shrews, and transgenic mice heterozygous for the *p53* tumor-suppressor gene) infected with hepatitis B virus were more sensitive to the carcinogenic effects of aflatoxin than were uninfected animals. IARC (2002) concluded that these studies confirmed the carcinogenicity of aflatoxins in experimental animals.

Additional Information Relevant to Carcinogenicity

In humans and susceptible animal species, aflatoxin B_1 is metabolized by cytochrome P-450 enzymes to aflatoxin-8,9-epoxide, a reactive form that binds to DNA and to albumin in the blood serum, forming adducts. The levels of the major aflatoxin B_1 adducts (the N^7-guanine and serum albumin adducts) that have been detected in humans and susceptible animal species are comparable. The 8,9-epoxide metabolite can be detoxified through conjugation with glutathione, mediated by the enzyme glutathione *S*-transferase (GST). The activity of GST is much higher (by a factor of 3 to 5) in animal species that are resistant to aflatoxin carcinogenicity, such as mice, than in susceptible animal species, such as rats. Humans have lower GST activity than either mice or rats, suggesting that humans are less capable of detoxifying aflatoxin-8,9-epoxide. Studies of rats and trout treated with chemopreventive agents have shown a reduction of aflatoxin B_1-guanine adducts and incidence of liver tumors.

Aflatoxin causes genetic damage in bacteria, in cultured cells from humans and experimental animals, and in humans and experimental animals exposed to aflatoxin *in vivo*. Types of genetic damage observed include formation of DNA and albumin adducts, gene mutations, micronucleus formation (a sign of chromosome damage or loss), sister chromatid exchange, and mitotic recombination. Metabolically activated aflatoxin B_1 specifically induced G to T transversion mutations in bacteria. G to T transversions in codon 249 of the *p53* tumor-suppressor gene have been found in human liver tumors from geographic areas with high risk of aflatoxin exposure and in experimental animals (IARC 1993, 2002).

Properties

Aflatoxins are toxins produced by fungi of the genus *Aspergillus* that grow on grains and other agricultural crops. The four major types are designated aflatoxin B_1 (molecular weight = 312.3), B_2 (mol wt = 314.3), G_1 (mol wt = 328.3), and G_2 (mol wt = 330.3), based on their fluorescent color when exposed to ultraviolet light (B = blue fluorescence, G = yellow-green fluorescence). Aflatoxin M_1, which may be found in the absence of other aflatoxins, is a major metabolic hydroxylation product of aflatoxin B_1. Aflatoxins are slightly soluble in water, soluble in moderately polar organic solvents, and insoluble in nonpolar solvents. They are unstable when exposed to oxidizing agents, ultraviolet light, or solutions with a pH below 3 or above 10. Aflatoxins decompose at their melting points, which are between 237°C (G_1) and 299°C (M_1), but are not destroyed under normal cooking conditions. They can be completely destroyed by autoclaving in the presence of ammonia or by treatment with bleach (IARC 1976, 1993).

Use

Aflatoxins are used solely for research purposes. They are naturally occurring contaminants formed by certain fungi on agricultural crops and were first discovered in the 1960s (IARC 1976).

Production

Aflatoxins are produced by several fungus species in the genus *Aspergillus. A. flavus* and *A. parasiticus* are responsible for most aflatoxin contamination of food crops worldwide. Although these species have similar geographical ranges, *A. parasiticus* is less widely distributed and is rare in Southeast Asia. *A. flavus* is the most widely reported fungus in foodstuffs. *A. australis*, which occurs in the Southern Hemisphere, is the only other species that may be an important source of aflatoxins. Both *A. flavus* and *A. parasiticus* occur in the warm temperate regions of the United States, but are less abundant there than in tropical regions. *A. flavus* is uncommon in cool temperate regions. Both *A. flavus* and *A. parasiticus* produce aflatoxins B_1 and B_2, and *A. parasiticus* also produces aflatoxins G_1 and G_2. The relative proportions and amounts of the various aflatoxins on food crops depend on the *Aspergillus* species present, pest infestation, growing and storage conditions, and other factors. Contamination generally is higher on crops grown in hot, humid tropical climates, but does occur in temperate climates and varies from year to year. Pre-harvest aflatoxin levels increase during droughts, and post-harvest levels increase when crops are not properly dried before storage or are not protected from insect and rodent infestations. Rapid post-harvest drying and storage in an area with a moisture content less than 10% can eliminate most contamination (IARC 1976, 1993, 2002).

Aflatoxins are not manufactured in commercial quantities but are produced in small quantities for research purposes. Total annual production is less than 100 g (IARC 1993, 2002). In 2003, the number of U.S. suppliers for specific types of aflatoxins ranged from three to ten (ChemSources 2003).

Exposure

The general population is exposed to aflatoxins primarily by eating contaminated food. Aflatoxin-producing fungi commonly grow on corn and other grains, peanuts, tree nuts, and cottonseed meal; however, *A. parasiticus* is rarely found in corn. Meat, eggs, milk, and other edible products from animals that consume aflatoxin-contaminated feed are additional sources of potential exposure. Although aflatoxin levels generally are higher during periods of drought, FDA surveys found detectable levels of aflatoxins in fewer than half of samples collected from feedstuffs even in drought years (Price *et al.* 1993).

Median levels of total aflatoxins in corn samples collected in the United States between 1978 and 1983 ranged from less than 0.1 to 80 µg/kg (IARC 1993). Data on contamination of foods compiled in 1995 from 90 countries reported a median aflatoxin B_1 level of 4 µg/kg (ranging from 0 to 30 µg/kg) and a median total aflatoxin level of 8 µg/kg (ranging from 0 to 50 µg/kg) (IARC 2002). The estimated daily dietary intake of aflatoxins in the southeastern United States (based on samples collected from 1960 to 1979) was 2.7 ng/kg of body weight, which was substantially less than the daily intake estimated for periods before 1960 (197 ng/kg for 1910 to 1934 and 108 ng/kg for 1935 to 1959). The time-weighted average daily intake for 1910 to 1979 was 110 ng/kg for the Southeast, but only 0.34 ng/kg for the North and West (Bruce 1990).

Nursing infants may be exposed to aflatoxins in breast milk (Zarba *et al.* 1992). For example, aflatoxins were detected in 90 of 264 breast-milk samples collected from nursing mothers in Africa but were not detected in 120 samples collected from nursing mothers in Kiel, Germany. Aflatoxin M_1 was most frequently detected in breast milk, at concentrations varying seasonally from 0.02 to about 1.8 µg/L, but aflatoxin B_1 was found at the highest concentration, 8.2 µg/L (Somogyi and Beck 1993). Biomarkers that may be used to assess aflatoxin exposure include the aflatoxin-DNA adduct in urine and the aflatoxin-albumin adduct in blood serum (Weaver *et al.* 1998).

Occupational exposure to aflatoxins occurs by inhalation of dust generated during the handling and processing of contaminated crops and feeds. Therefore, farmers and other agricultural workers have the greatest risk of occupational exposure. Of 45 animal-feed production plant workers in Denmark, 7 had detectable levels of aflatoxin B_1 in their blood after working for four weeks in the factory or unloading raw materials from ships (Autrup *et al.* 1993). Ghosh *et al.* (1997) reported detecting aflatoxins (at concentrations of 0.00002 to 0.0008 µg/m^3) in respirable dust samples collected in workplace and storage areas at rice and corn processing plants in India. Selim *et al.* (1998) collected dust samples from 28 farms in the United States during harvest and unloading, animal feeding, and bin cleaning and found aflatoxin concentrations ranging from 0.00004 to 4.8 µg/m^3. The lowest concentrations were detected during harvest and unloading, and the highest concentrations during bin cleaning. Both area and personal samplers were used to determine airborne concentrations of aflatoxins B_1, B_2, G_1, and G_2 in dust samples collected from three food-processing plants (for cocoa, coffee, and spices) in Tuscany, Italy. Concentrations ranged from below the detection level (less than 0.000002 µg/m^3) to 0.00013 µg/m^3 (Brera *et al.* 2002).

Regulations

EPA

Resource Conservation and Recovery Act:

Listed as a Hazardous Constituent of Waste

FDA

Ingredients susceptible to contamination with aflatoxins must comply with FDA rules in the manufacturing and processing of food

Carbohydrase may be safely used in the production of dextrose from starch, provided that aflatoxin is not present

Action levels for aflatoxins in foods and animal feed range from 0.5-300 ppb

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