Aflatoxins

CAS No. 1402-68-2

Known to be human carcinogens

First listed in the *First Annual Report on Carcinogens* (1980)

Carcinogenicity

Aflatoxins are *known to be human carcinogens* based on sufficient evidence of carcinogenicity from studies in humans. Aflatoxins were listed in the *First Annual Report on Carcinogens* as *reasonably anticipated to be human carcinogens* based on sufficient evidence of carcinogenicity from studies in experimental animals and limited evidence of carcinogenicity from studies in humans; however, the listing was revised to *known to be human carcinogens* in the *Sixth Annual Report on Carcinogens* in 1991.

Cancer Studies in Humans

Early evidence for the carcinogenicity of aflatoxins in humans came from epidemiological studies (a case-control study and 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 of primary liver-cell 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, levels of aflatoxin in the diets of individuals were estimated retrospectively, and the risk of liver cancer increased significantly with increasing estimated 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 primary liver-cell cancer (IARC 1987, 1993).

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 the prevalence of chronic hepatitis B did not appear to fully explain differences in rates of primary liver-cell cancer were reviewed, and it was concluded that the remaining variance in liver-cancer incidence was related both to estimated dietary levels of aflatoxins and to measured levels of aflatoxins and their metabolites in the urine. 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 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 primary liver-cell cancer; the association remained when the analyses controlled for hepatitis B infection.

Studies on Mechanisms of Carcinogenesis

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, 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).

In humans and susceptible animal species, aflatoxin B_1 is metabolized by cytochrome P450 enzymes to aflatoxin-8,9-epoxide, a reactive form that binds to DNA and to albumin in the blood serum, forming adducts. Comparable levels of the major aflatoxin B_1 adducts (the N^7-guanine and serum albumin adducts) have been detected in humans and susceptible animal species. 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. In studies of rats and trout, treatment with chemopreventive agents reduced the formation of aflatoxin B_1–guanine adducts and the incidence of liver tumors.

Cancer Studies in Experimental Animals

Aflatoxins caused tumors in several species of experimental animals, at several different tissue sites, and by several different routes of exposure. Oral administration of aflatoxin mixtures or aflatoxin B_1 alone (in the diet, by stomach tube, or in the drinking water) caused liver tumors (hepatocellular or cholangiocellular tumors) in all species tested except mice; these included rats, hamsters, marmosets, tree shrews, and monkeys. In addition, kidney (renal-cell) and colon tumors occurred in rats, benign lung tumors (adenoma) in mice, and tumors of the liver, bone (osteogenic sarcoma), gallbladder, and pancreas (adenocarcinoma) in monkeys. When administered by intraperitoneal injection, aflatoxin B_1 caused liver tumors in infant mice, adult rats, and toads. Aflatoxin B_1 administered by intraperitoneal 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 injection caused tumors at the injection site (sarcoma) 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 tumors (sarcoma) when administered by intraperitoneal injection. 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 experimental animals infected with hepatitis B virus (woodchucks, tree shrews, and transgenic mice heterozygous for the *p53* tumor-suppressor gene) were more sensitive to the carcinogenic effects of aflatoxin than were unin-

fected animals. IARC (2002) concluded that these studies confirmed the carcinogenicity of aflatoxins in experimental animals.

Properties

Aflatoxins are toxins produced by fungi in the genus *Aspergillus* that grow on grains and other agricultural crops. They exist as colorless to pale-yellow crystals at room temperature (IARC 1976, 1993). They are slightly soluble in water and hydrocarbons, soluble in methanol, acetone, and chloroform, and insoluble in nonpolar solvents. Aflatoxins are relatively unstable in light and air, particularly in polar solvents or 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. Physical and chemical properties of aflatoxins are listed in the following table.

Property	Information
Melting point	237°C to 299°C[a]
Log K_{ow}	0.5[b]
Water solubility	3.150 g/L at 25°C[b]
Vapor pressure	1.25×10^{-10} mm Hg at 25°C[b]

Sources: [a]IARC 1993, [b]ChemIDplus 2009.

The four major types of aflatoxins are designated aflatoxin B_1 (molecular weight = 312.3), B_2 (molecular weight = 314.3), G_1 (molecular weight = 328.3), and G_2 (molecular weight = 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.

Use

Aflatoxins are used solely for research purposes. They are naturally occurring contaminants formed by certain fungi on agricultural crops, 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 of less than 10% can eliminate most contamination (IARC 1976, 1993, 2002).

Aflatoxins are not manufactured in commercial quantities but may be produced in small quantities for research purposes. Total annual production was reported to be less than 100 g (IARC 1993, 2002). No U.S. suppliers for aflatoxins were identified in 2009 (ChemSources 2009).

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 also are sources of potential exposure. Although aflatoxin levels generally are higher during periods of drought, surveys by the U.S. Food and Drug Administration detected aflatoxins in fewer than half of samples collected from feedstuffs even in drought years (Price *et al.* 1993).

Median total aflatoxin concentrations 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 concentration of 4 µg/kg (range = 0 to 30 µg/kg) and a median total aflatoxin concentration of 8 µg/kg (range = 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). 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). Aflatoxins were detected 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 (Ghosh *et al.* 1997). Dust samples collected from 28 U.S. farms during harvest and unloading, animal feeding, and bin cleaning contained aflatoxins at concentrations ranging from 0.00004 to 4.8 µg/m^3 (Selim *et al.* 1998). The lowest concentrations were detected during harvest and unloading, and the highest 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 level of detection (< 0.000002 µg/m^3) to 0.00013 µg/m^3 (Brera *et al.* 2002).

Regulations

Environmental Protection Agency (EPA)

Resource Conservation and Recovery Act

Listed as hazardous constituents of waste.

Food and Drug Administration (FDA, an HHS agency)

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 to 300 ppb.

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