Korean J. Toxicol.
Vol. 6, No. 2, pp. 159-166, 1990

1990 Int'l Symp.
Chem. Tox. and Risk Assessment

HEALTH RISKS POSED BY MYCOTOXINS IN FOODS

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(Received September 9, 1990)
(Accepted September 20, 1990)

ABSTRACT: *The ability of many toxigenic fungi to invade and develop in a wide variety of raw ingredients of human diet renders human exposure to mycotoxins very difficult to avoid. Most of the energy-rich commodities, such as cereal grains, oil seeds, tree nuts, and dehydrated fruits, are susceptible to mycotoxin contamination. Mycotoxins therefore have been recognized as an important class of hazardous substances in the human food chain. Although human exposure to mycotoxins is largely through ingestion, inhalation and skin contact may also be significant under conditions other than consumption of foods. Human ingestion of mycotoxins is due to consumption of contaminated dietary ingredients and the edible tissues and products of domestic animals that have been exposed to mycotoxins in moldy feed. Large scale acute human mycotoxicoses, such as ergotism in France, alimentary toxic aleukia in Russia, yellow rice syndrome in Japan, endemic nephropathy in Balkan countries, and acute aflatoxin poisonings in India and Taiwan, have been well documented, indicating that mycotoxicosis is a global problem. In some incidents, hundreds of victims were killed and many more became seriously ill. The mycotoxins that have been implicated in the etiology of these human diseases include aflatoxins, citreoviridin, cyclopiazonic acid, ergot alkaloids, moniliformin, ochratoxin A, trichothecenes, tenuazonic acid, and zearalenone. Among these, aflatoxins have been also implicated in the etiology of human primary liver cancer in those high-incidence countries in Africa and southeast Asia. It is well recognized that cause-effect relationship between mycotoxins and human diseases is very difficult to establish, especially for the cancer connection. Careful risk assessment must be performed to determine whether a mycotoxin indeed warrants costly regulatory actions.*

Keywords: *Mycotoxins, human exposure, food chain, moldy feed, mycotoxicosis, ergotism, alimentary toxic aleukia, yellow rice syndrome, aflatoxins, citreoviridin, cyclopiazonic acid, ergot alkaloids, moniliformin, ochratoxin A, trichothecenes, tenuazonic acid, zearalenone, liver cancer, risk assessment.*

MYCOTOXINS AND THEIR OCCURRENCE

Mycotoxins are toxic, small molecular weight compounds produced by fungi. Under laboratory conditions, more than two hundred toxic fungal metabolites have been produced by pure cultures and characterized chemically and toxicologically (Cole and Cox, 1981). Of these, about twenty have been found to occur in foodstuffs at some degree of frequency, constituting the significant mycotoxins in foods and feeds (Pohland and Wood, 1987). They are produced by five genera of fungi that can grow and produce the mycotoxins in a wide variety of food commodities as listed in Table 1. Most of the energy-rich commodities, such as cereal grains, oil seeds, tree nuts, and dehydrated fruits, are susceptible to mycotoxin contamination. The mycotoxins listed represent the most important mycotoxins of food safety concern in northern America.

Mycotoxins therefore have been recognized as an important class of hazardous substances in the human food chain.

Table 1. The fungi and commodities related to major foodborne mycotoxins[a]

Fungus	Toxin	Dietary ingredient
Aspergillus toxins	Aflatoxin B1 Aflatoxin G1 Aflatoxin M1 Sterigmatocystin Ochratoxin A	Corn, corn products, peanuts, peanut products, cottonseeds, pumpkin, seeds, rice, beans, edible animal tissues, ham, bacon, kulen, sausage, milk, dairy products
Fusarium toxins	Deoxynivalenol Nivalenol Zearalenone T-2 toxin Diacetoxyscirpenol Moniliformin	Wheat, wheat products, corn, corn products, barley, barley products, rice, rye, oats, walnut
Penicillium toxins	Patulin Citrinin Penitrem A Cyclopiazonic acid	Fruits, fruit juices, corn, rice, cheese, walnuts
Alternaria toxins	Tenuazonic acid Alternariol methyl ether	Fruits, vegetables, apple & tomato products
Cleviceps toxins	Ergor alkaloids	Wheat, wheat products, rye

[a]Information derived from Pohland and Wood (1987)

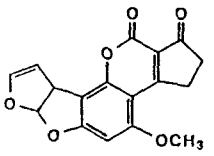
HUMAN EXPOSURE

Mycotoxins are ingested by humans through the ingestion of the susceptible food commodities. The occurrence of mycotoxins in such a wide variety of food commodities renders it very difficult to avoid exposure to some kinds of mycotoxins,

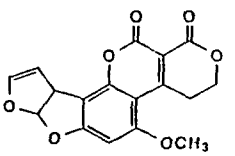
especially at low doses. Exposure usually takes place involving mixtures of a number mycotoxins rather than single mycotoxins. Although human exposure to mycotoxins is largely through ingestion, inhalation and skin contact may also be significant under conditions other than consumption of foods (Burg *et al.*, 1981; Sorenson *et al.*, 1984). Human ingestion of mycotoxins is through dietary ingredients that are contaminated with mycotoxins and through products of domestic animals that contain metabolites of mycotoxins ingested by the animals from moldy feed.

HEALTH EFFECTS

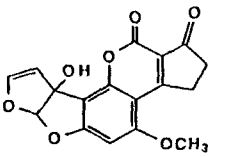
The mycotoxins represent a class of naturally occurring compounds of diversified chemical structures, as illustrated in Fig. 1. Due to the diversity of their chemical structures, mycotoxins collectively are capable of producing a wide spectrum of toxic effects in laboratory animals as listed in Table 2. Some of the toxic effects of mycotoxins in



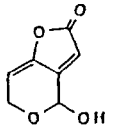
Aflatoxin B_1



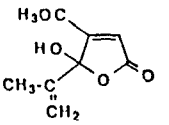
Aflatoxin G_1



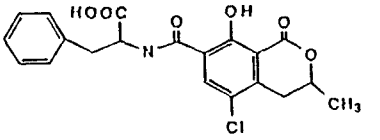
Aflatoxin M_1



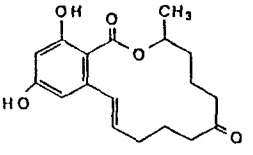
Patulin



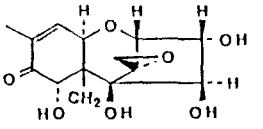
Penicillic acid



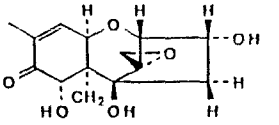
Ochratoxin A



(S)-Zearalenone



Nivalenol



Deoxynivalenol

Fig. 1. The structures of some foodborne mycotoxins

Table 2. The spectrum of toxic effects of mycotoxins in experimental animals

Hepatotoxicity, Nephrotoxicity, Neurotoxicity, Immunotoxicity, Tremogenicity, Vomition, Excessive salivation, Hemorrhagenicity, Estrogenicity, Abortion, Carcinogenicity, Teratogenicity

animal models are consistent with the characteristic symptoms seen in some documented human mycotoxicoses. Other effects may be an indication of human diseases yet to be discovered and documented.

Many human diseases have been documented or suspected as mycotoxicoses. Those that are well documented include such well-known mycotoxicoses as ergotism in Europe, alimentary toxic aleukia in Russia, yellow rice syndrome in Japan, endemic nephropathy in the Balkans, and acute aflatoxicosis in southeast Asia, indicating that mycotoxicosis is a global problem. In some incidents, hundreds of victims were killed and many more became seriously ill. The mycotoxins that have been implicated in the etiology of human diseases include aflatoxins, citreoviridin, cyclopiazonic acid, ergot alkaloids, moniliformin, ochratoxin A, trichothecenes, tenuazonic acid, and zearalenone. Mycotoxins have also been associated with cancer of various organs in humans including liver, kidney, esophagus, and uterus (Pohland and Wood, 1987).

Since humans normally avoid heavily moldy foods, human health problems resulting from exposure to acute levels of mycotoxins are relatively rare. The greatest concern today is probably the potential cancer risks posed by carcinogenic mycotoxins such as aflatoxins. Many believe that the strong interest in mycotoxins shown by the scientific community and regulatory agencies in the last 25 years was due to the discovery in the early 1960's of the potent carcinogenicity of aflatoxins, especially aflatoxin B_1 (Wogan and Newberne 1967) and the subsequent detection of the toxins in a wide variety of food commodities (Stoloff, 1976). Aflatoxin B_1 is now recognized by the International Agency for Research on Cancer (IARC) as a human carcinogen (IARC, 1987), involved in the etiology of human primary liver cancer in those high-incidence countries in Africa and southeast Asia. The potent carcinogenicity of sterigmatocystin and ochratoxin A in experimental animals have also been well noted in the literature (IARC, 1987, Kanizawa, 1984). Mycotoxins as a whole have been suspected as a significant class of naturally occurring carcinogens in foods (Ames *et al.*, 1987). It is noteworthy however that, after more than two decades of active research, there are only three mycotoxins (aflatoxin, sterigmatocystin, and ochratoxin A) that are, based on the criteria of IARC, clearly shown to be carcinogenic to experimental animals and that human carcinogenicity can only be established for aflatoxin B_1. Caner risks posed by mycotoxins, therefore, should be assessed with discretion and not be overstated.

RISK ASSESSMENT

It is well recognized that cause-effect relationship between mycotoxins and human diseases is very difficult to establish, especially for carcinogenesis which involves a latency period for disease development. Careful risk assessment must be performed to determine whether a mycotoxin indeed warrants costly regulatory actions.

Aflatoxin B_1 was not designated as a human carcinogen by IARC until 1987, despite the enormous volume of information available on this mycotoxin. The difficulty in the designation of aflatoxin B_1 as a human carcinogen is a good illustration that identification of the causative agent of a human disease is not easy. The evidence that a certain form of cancer is caused by a mycotoxin, or a human disease is a mycotoxicosis, requires all of the following five criteria being satisfied:

1. Occurrence of the mycotoxin in food supplies
2. Human exposure to the mycotoxin
3. Correlation between exposure and incidence
4. Reproducibility of characteristic symptoms in experimental animals
5. Similar mode of action in humans and animal models

Of these criteria the most important evidence indicating that a mycotoxin is involved in the etiology of a form of human cancer is the correlation between the mycotoxin in question and the incidence of the disease. The "sufficient evidence" of a chemical agent to be considered as a human carcinogen as specified by IARC is "a positive relationship between exposure to the agent and the cancer in question in which chance, bias, and confounding could be ruled out with reasonable confidence." For human populations, this correlation can only be obtained through epidemiological studies. Unfortunately, the results of epidemiological studies oftentimes become issues of controversy, due to problems in the methods used.

To date, aflatoxins are the only class of mycotoxins that has been a subject of extensive epidemiological studies and risk assessment. Animal studies have indicated that aflatoxins are capable of inducing tumors in liver, kidney, intestine, colon, and lung, with liver being the principal and most sensitive target organ (Newberne and Rogers, 1981). Therefore, all the epidemiological studies on aflatoxins have been directed toward liver-cell carcinoma (LCC).

Most of the evidence that implicates aflatoxin in the etiology of LCC comes from epidemiological studies conducted in six African and Asian countries where LCC is a major form of cancer. Quantitative population studies in Thailand and four African countries (Van Rensburg, 1986), involving a total of 17 regions, showed a strong correlation, both inter-regionally and within a single country, between the unadjusted combined crude LCC rates for both sexes and the estimated dietary aflatoxin intake based on current levels of aflatoxin contamination of prepared food (Van Rensburg *et al.*, 1985). A recent cohort study conducted in a southwest province of China (Yeh *et al.*, 1989) also gave a strong support for the aflatoxin theory of human LCC. In four regions surveyed in the China study, the age-adjusted prevalence of HBsAg was about constant, and yet the mortality rate due to liver cancer differed considerably in good correlation with the levels of exposure to aflatoxin.

The validity of the dose-response relationship demonstrated by these epidemiological studies has been questioned because of the problems in the determination of dose values using population averages, the use of current exposure data to represent previous ones, and the incompleteness of liver cancer incidence records in developing nations (Wagstaff, 1985; Stoloff, 1986; 1899). The dose values were determined by multiplication of the average concentration of aflatoxin B_1 in the food

samples analyzed with an average daily food intake divided by an average adult body weight. The validity of these values is questionable in that the distribution of aflatoxin in food samples are highly uneven, the composition of human daily diet is rather different, and daily food intake and body weight vary individually. A valid dose determination should be on an individual basis but the techniques to determine such individual dose values have not been available until very recently.

Indeed, the correlation between human LCC and aflatoxin has not been consistently found in all epidemiological problems, probably due to the abovementioned uncertainties and methodological problems. For example, in a retrospective study conducted in the U.S.A., rural white males from the Southeastern, Northern, and Western regions were selected for the comparison of lifetime risk of death from liver cancer with past dietary exposure to aflatoxin (Stoloff, 1983). For an approximately 100-fold inter-regional difference in aflatoxin intake, there was only a 6-10% increase in excess risk of death due to liver cancer in the expected direction of comparison, indicating a very low probability that aflatoxin is the cause of liver cancer in the U.S. rural white males.

EXPOSURE ASSESSMENT USING BIOMARKERS

Active work has been in progress in recent years in the use of DNA and protein adducts of aflatoxin B_1 as biomarkers to assess individual exposure to aflatoxin to confirm the correlation between aflatoxin and human LCC. It has been established that aflatoxin B_1 upon metabolic activation is transformed to an epoxide active form which may form adducts with DNA or with proteins (Swenson *et al.*, 1975). Some of the adducts are sufficiently persistent to be measured in tissues or in excreta weeks to months after exposure. The persistent aflatoxin B_1-DNA adducts (ADA), which has been identified as a formamidopyrimidine derivative of the N^7-guanyl DNA adduct of aflatoxin B_1 (FAPY-ADA), has proven to be a useful biomarker for the assessment of human dosimetry of aflatoxin B_1. Two recent limited studies by Garner *et al.*, (1988) and Hsieh *et al.* (1988), both using the enzyme-linked immunosorbent assay (ELISA) method involving monoclonal antibodies against FAPY-ADA, have revealed that most of the hepatoma patients in Czechoslovakia (7 out of 8) and Taiwan (9 out of 9) that were monitored had the ADA in their livers.

Similarly, it has been demonstrated that aflatoxin B_1 binds quantitatively in relation to dose to peripheral blood albumin in rats (Sabbioni *et al.*, 1987). Upon repeated exposure, accumulation of binding occurs and the level of albumin binding parallels the binding to liver DNA (Wild *et al.*, 1986). The major albumin adduct in rats has been characterized as an aflatoxin B_1-lysine residue. The half-life of albumin in humans is about 20 days and thus aflatoxin B_1-albumin appears useful as a biomarker for assessment of exposure to aflatoxin B_1. In a recent study (Wild *et al.*, 1990), blood samples obtained from individuals from Thailand and Kenya, where AF exposure is likely high, and from France, where AF exposure is likely low, were assayed for AF-albumin adducts by ELISA and HPLC methods. The aflatoxin B_1-albumin adducts were found only in the former two countries. The exposure assessment at an individual level using

DNA and protein adducts so far are consistent with the theory that aflatoxin is involved in the etiology of human LCC.

CONCLUSION AND RECOMMENDATION

From the complexity seen in the identification of aflatoxin B_1 as a causative agent of human liver cancer, one can appreciate the degree of uncertainty associated with the cause-effect relationship between mycotoxins and human diseases. Therefore, one may recommend that (a) for regulatory agencies, evidence for the involvement of mycotoxins in human diseases needs very careful substantiation to justify massive investment in their regulatory actions; and (b) for an individual household, moldy foods and poorly controlled fermented foods should be avoided as much as possible.

ACKNOWLEDGEMENTS

The author gratefully acknowledges the dedication and tireless effort of Dr. Louis Chang that has made the mission of this ACTS delegation possible. The author's research in this area has been supported by the Western Regional Research Project in Food Safety, W-122.

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