TOXICANTS, NATURAL

In addition to the many well-known major components (protein, fat, carbohydrate, and fiber) and trace nutrients (vitamins, minerals, and nonessential compounds), our food contains thousands of naturally present toxic compounds. Although these chemicals are in every meal we eat and are present in much greater quantities than residues of synthetic chemicals such as PCBs and pesticides, they have traditionally received relatively little attention compared to these well-known human-made chemicals. For thermore, the mechanisms of action of natural toxins—metabolite activation, interaction with critical cellular macromolecules—are different from those of synthetic toxins. In short, our bodies handle toxins similarly regardless of their origin. Many of the toxins presented here are known to cause or strongly suspected of causing cancer in laboratory animals and are therefore potentially carcinogenic in people. The human health risk posed by individual natural toxins varies considerably due to a variety of factors such as dose, inherent potency, variety of the diet, and presence of detoxifying factors. In any event, natural toxins pose a far greater health risk than do synthetic chemicals in our foods, despite the popular notion that "natural is good."

An issue of major concern is the relationship between diet and various human diseases, including cancer. As the single most important cancer variable to be identified from epidemiological studies, diet contributes to approximately 35% of the variation in cancer rates among individuals and populations in the United States. Other factors such as food additives, genetic predisposition, industrial pollution, and pesticide contamination play comparatively minor roles in human cancer rates. Excluding smoking, diet-related factors account for over 50% of all remaining cancer deaths in this country, or 150,000 food-related cancer deaths per year in the United States alone.

Natural toxins are defined here as naturally occurring substances in plants or other food products that exert undesirable or unhealthy effects when they are consumed. Although not intended to be an exhaustive, all-inclusive discussion of natural toxins, this section will center on those that are particularly well studied and characterized and are of current interest in food toxicology. The focus of this chapter will be not on bacterial toxins, but on three major categories of food toxicants: (1) toxins from plant or plant derived foods, (2) mycotoxins produced by fungi on grains, and (3) toxic substances created during cooking or other processing at the food (or induced toxins). An additional yet important category of induced toxins is endogenous toxic substances that may appear inadvertently in genetically manipulated plant materials that result from efforts to alter plant quality. This latter category will also not be considered here.

Our food also contains natural chemicals that can counteract and thereby prevent the adverse effects of many natural and synthetic toxins. Though much more work on these chemopreventive is needed, the data suggesting that some plant foods can actually reduce the incidence of certain types of cancer thus far are very encouraging. Hundreeds of animal and epidemiological studies have identi

fied several foods or specific compounds that offer protection against the carcinogenic effects of a wide variety of natural and synthetic chemicals. A few compounds have been shown to actually reverse the carcinogenic process in animals. As might be imagined, the field of chemoprevention is one of the most exciting areas in nutritional toxicology and cancer research. The reader is encouraged to consult a review on food chemopreventives.

NATURAL PLANT TOXINS IN FOODS

The following is a survey of some of the most well studied and characterized plant toxins.

Allyl Isothiocyanates

Allyl isothiocyanates are a group of major naturally occurring compounds that confer the pungent flavor to foods such as mustard and horseradish, where it is present at about 50 to 100 ppm. These compounds are in Brassica vegetables such as broccoli and cabbage, and in cassava and other tropical starchy foods, but at much lower concentrations. Normal dietary exposure to isothiocyanate-containing foods releases milligram amounts of isothiocyanates. Normal processing steps (chopping, rinsing, milling) render the food safe when washed with is discarded. In high doses, isothiocyanates are carcinogenic in rats but noncarcinogenic in bacteria. Isothiocyanates do not occur in foods per se but occur as glucosinolates conjugates that are hydrolyzed when the plant releases enzymes as it is disturbed, such as during chopping, processing, or ingestion (Fig. 1). The major concern with isothiocyanates is their gastric irritant properties in that they inhibit binding of isothiocyanates, the thyroid gland. Because insulin is required for the formation of the critical thyroid hormones (thyroxine T4 and triiodothyronine T3), isothiocyanate-induced by protein inactivation (i.e., an inadequate supply of isothiocyanate is a physical response to the thyroid attempts to compensate for reductions in both T4 and T3 production. Dendritic guinea is seen in geographical areas is India and Africa where consumption of poorly processed foods is coincident with insulin deficiency. Like many food toxins, allyl isothiocyanates are "double edged swords" in that they have been shown to be chemopreventive in certain animal testing protocols.

Caravamine

Despite their reputation as being the ultimate health food, alfalfa sprouts contain up to 15,000 ppm caravamine, an arginine analog that can substitute for this amino acid in cellular proteins, thereby altering their function. Caravamine is also produced in other legumes such as the jack bean. Caravamine inhibits nitric oxide synthetase (NOS), which induces heat shock proteins in human cells (in vitro). By virtue of its antimetabolite action, caravamine is under current consideration as an antimicrobial drug in combination with other antimetabolites such as 5-fluorouracil, but it has not yet been tested for carcinogenicity. Caravamine may cause autoimmune disorders such as lupus erythematosus in people (2). Primates fed alfalfa sprouts develop a severe toxic syndrome resembling human lupus.
Cyanogenic Glycosides

Seeds of apples, apricots, cherries, peaches, pears, plums, and quinces as well as almonds, sorghum, lima beans, cassava, corn, yam, chickpeas, cashew nuts, and kirsch contain compounds that are toxic due to their release of free hydrogen cyanide, which occurs when the plant tissue is disturbed during chopping, processing, or ingestion. These conditions initiate the hydrolysis of the glycoside by the action of β-glucosidases and other enzymes naturally present in the plant tissue and in the intestinal lumen. Although acid also initiates this process, it does not appear to occur in the digestive tract to any great extent, despite the acid environment in the stomach. Hydrolysis by β-glucosidases produces the sugar and a cyanohydrin, the latter spontaneously or enzymatically degrading to form free hydrogen cyanide (Fig. 2). There are several such cyanogenic glycosides, of which linamarin, amygdalin, and dhurrin are examples (Fig. 2). In the 1970s, amygdalin, as laetrile, was a fad remedy touted as a cure and/or preventive for cancer and other ailments. Underground “clashes” briefly flourished where patients were given large quantities of amygdalin or amygdalin-rich seeds and nuts.

Cyanide is one of the most acutely toxic chemicals. It binds to and inactivates heme enzymes, specifically nitric-oxidative cytochrome oxidase, resulting in an acute, life-threatening anemia. The usual therapy is initiated with sodium nitrite, which induces methemoglobinemia, permitting the release of cyanide from heme proteins, followed by sodium thiosulfate, which acts as a substrate for rhodanese, an endogenous hepatic enzyme that catalyzes the conversion of free cyanide to the less toxic thiocyanate.

Cases of acute human poisoning from the cyanide released from certain varieties of lima beans, cassava, and bitter almonds are a regular occurrence (3). Due to the importance of cassava as a subsistence crop in Africa and South America, cyanogenic glycosides in that food probably represent the greatest health risk. High cyanide varieties of cassava, distinguished by their bitter taste, may contain over 600 ppm cyanide on a dry weight basis, whereas “sweet” varieties contain significantly less. Processing steps such as sun drying, soaking, boiling, and for

![Figure 1. Mechanism of formation of iso-thiocyanate gaseogens from glucosinolates.](image)

![Figure 2. Natural cyanogenic glycosides and mechanism of formation of hydrogen cyanide.](image)
Hydrazines and Other Toxins in Edible Mushrooms

Commercial mushrooms, such as the cultivated mushroom (Agaricus bisporus), the shiitake mushroom (Coriolus versicolor), and the false morel (Gyromitra occidentalis), all contain substantial amounts of compounds in the hydrazine family (Fig. 3). Many hydrazines are potent liver toxins and animal carcinogens. Commonly found in concentrations of 500 ppm in mushrooms, N-methyl-N-formylhydrazine is a lung carcinogen in mice and is also a carcinogen in hamsters. People eating a 108-gm serving of mushrooms (therefore ingesting 50 mg N-methyl-N-formylhydrazine) get nearly the same dose (for a pot kilogram body weight basis) that will cause cancer in mice on sustained daily exposure.

Shiitake and Agaricus mushrooms contain up to 2000 ppm agaritine, a metabolite product of which is dimethionine derivative C-potent carcinogen and a mutagen. The major carcinogenic hydrazine in the false morel, gyromitrin (acrolein-hydrate N-methyl-N-formylhydrazine), is also present in similar concentrations. Other carcinogenic hydrazines include pyrazinohydrazogenate (present in A. bisporus at 10 ppm) and 4-hydrasopenicyl benzeneimiazine (HMIB), the latter shown to induce DNA strand breaks, presumably through a carbon centered free radical intermediate, a possible mechanism of the carcinogenic action of hydrazines in general (6). Another carcinogenic hydrazine, methylhydrazine, is present in smaller concentrations (14 ppm).

Whole mushrooms have been shown in numerous studies to cause cancer in laboratory animals, but whether they are a significant cause of cancer in people is uncertain.

Hans fed a diet of whole A. bisporus mushrooms (30% of total diet) did not have a significant increase in tumors compared to control animals.

Toxic Substances in Spices and Flavoring Agents

Safral, estragol, myristicin, f-arsenic, pipernine, and sesquiterpene (Fig. 4) are closely related alkylbenzenes found in many spices, essential oils, and herbs. They are also present, in much lower levels, in pungent, parsley, and sesquiterpene oils. All are weak to moderate rodent hepatocarcinogens.

Oil of oregano (Sassafra albidum), which was once used to flavor root beer, contains approximately 85% saxifrage. The oil has been banned as a flavor additive since 1961 but Safral is also a minor, natural component of nutmeg, mace, star anise, cinnamon, and black pepper. Safral is also found in safflower oil. Sassafra seed is an ingredient in file powder used to make gumbo, a spicy Cajun stew. A procarricogenic, safral is activated by endogenous enzymes to a DNA reactive intermediate that forms covalent adducts with guanine in vitro (7). Estragole, a related aromatic flavor agent, is found in tarragon, basil, fennel, and is likewise a weak carcinogen.

Isosafrole, a component of ylang-ylang (Cananga odorata) oil, a flavorant and scent, is carcogenic in mice. Many of these alkylbenzenes interact with cytochrome P-450 (CYP)-mediated metabolism. For example, both safral and safral are powerful inducers of CYP 1A enzymes. Safral and isosafrole also inhibit CYP 2E1 enzymes and in so doing prevent against carbon tetrachloride liver toxicity in mice. f-arsenic is a major component of oil of calamus derived from the Azores calamus root (which is a folk remedy for indigestion) and was once used to flavor vermout and bitters. It is an intestitional carcinogen in rats. Myristin is a major flavor component of nutmeg, which is derived from the dried seed of the Myristica fragante. The world's principal commercial supply of nutmeg is grown in the Malay peninsula. Approximately 2% of nutmeg is myristin, which is present in the steam distilled volatile oil produced from the dried seeds. Mustik is a closely related spice, is derived from the outer coating of the seed. Myristin is also found in black pepper, parsley, and...
celery, dill, and carrots. Though not thought to be carcinogenic, large amounts of nutmeg, equivalent to two whole nutmeg seeds (6–15 g), are intoxicating and allegedly hallucinogenic, and large doses cause undesirable side effects such as tachycardia, flushed skin, and dry mouth. Because pure myristicin is not as hallucinogenic as nutmeg, other components in nutmeg are believed to contribute to its reported psychoactive properties.

Piperine, an alkaloid present in high concentrations (10%) in black pepper (\textit{Piper nigrum} and other spp.), is largely responsible for the pungent "bite" of this condiment. Powdered \textit{P. cubeba} berries are added to cigarettes and smoked as a remedy for throat irritation, and oil derived from these berries is added to some throat lozenges. Reports of the cancer-causing ability of this compound are conflicting. Extracts of black pepper caused cancer in mice at several sites in skin painting tests, whereas orally ingested piperine did not. Furthermore, piperine is not mutagenic in a number of in vitro screening assays. Under appropriate conditions, however, piperine is chemically converted to potentially carcinogenic intermediates. In the presence of nitrite, piperine is nitrosated to form highly mutagenic nitrosamine intermediates in vitro that may have potential carcinogenic activity. Like the related allylbenzenes, piperine also affects CYP expression and activity. Piperine specifically inhibits CYP 2E1 while specifically inducing the expression and activities of CYP 1A and 2B 9.

The ingredient responsible for the pungency of red and yellow chili peppers (\textit{Capsicum annuum} and \textit{C. chinense}, and \textit{C. frutescens} is capsaicin, which represents up to 0.5% of the weight of the fruit. Aerosol capsaicin sprays are popular dog repellents for mail carriers. Topical creams containing capsaicin (0.025%) are commercially available as analgesics. Although its pain-relieving qualities are debatable, capsaicin has been shown to cause a local depletion of substance P, an endogenous neuropeptide known to transmit pain impulses. Thus, even though the physiological conditions causing pain may persist, capsaicin prevents pain impulses from reaching the brain.

Capsaicin may be a weak carcinogen. It is a bacterial mutagen in the Ames test and causes benign digestive tract adenomas in rats given lifelong dietary exposure at 0.03125%. Intraperitoneal injections of capsaicin causes the formation of sister chromatid exchanges and micronuclei in mice. Sister chromatid exchanges and micronuclei in mice.
Cytosine is a 6-mercaptopurine that binds to DNA and RNA, interfering with DNA replication and protein synthesis. It is commonly used in the treatment of acute lymphoblastic leukemia (ALL) and chronic lymphocytic leukemia (CLL). Cytosine arabinoside is a DNA alkylation agent that works by alkylating DNA, leading to the formation of DNA adducts that inhibit replication and transcription. Cytosine arabinoside is used to treat acute myeloid leukemia (AML) and chronic myeloid leukemia (CML). Cytosine arabinoside is also used in the treatment of hairy cell leukemia (HCL) and Kaposi's sarcoma associated with HIV infection. Cytosine arabinoside is administered intravenously, and its side effects include myelosuppression, nausea, vomiting, and mucositis. Cytosine arabinoside is available under the brand name Cytosar-U. Cytosine arabinoside is a guanine-containing nucleoside that is converted to Cytosine arabinoside monophosphate (Cytosine arabinoside monophosphate, Cytosine arabinoside monophosphate, Cytosine arabinoside monophosphate) by the enzyme nucleoside kinase. Cytosine arabinoside monophosphate is then converted to Cytosine arabinoside triphosphate (Cytosine arabinoside triphosphate, Cytosine arabinoside triphosphate, Cytosine arabinoside triphosphate) by the enzyme Cytosine arabinoside phosphokinase. Cytosine arabinoside triphosphate is a potent inhibitor of RNA synthesis and is cytotoxic to cells.
Human intoxication by PA containing plants is well recognized and reported in the medical literature and is endemic in Jamaica, India, and parts of Africa. Liver cirrhosis, amoebic disease, and liver cancer are linked to consumption of PA containing plants. Hispanic and Native American populations in the western and southwestern United States are at high risk for PA intoxications due to their traditional widespread use of herbs, occasional lack of confidence in traditional medicine, and, more commonly, lack of access to medical care.

Comfrey (Symphytum officinale) is a nearly universal herb commonly sold not only in health food stores and by herbalists but also in supermarkets. Since ancient Greek and Roman times, both leaves and roots have been used to make tea and compress pastes to treat a variety of external and internal diseases, such as wounds, skin disorders, and respiratory diseases. Numerous vegetarian recipes call for comfrey leaves in the preparation of souffles, salads, and broths. Comfrey leaves and roots contain up to 0.23% of PAs such as intermediate, scopoletine, synephrine, and others. Dietary containing powder from dried leaves and roots cause liver tumors in rats. Additionally, these pure PAs are animal carcinogens and bacterial mutagens. Several cases are cited in the medical literature of comfrey-related intoxications in people. The well-known toxicity and carcinogenicity of comfrey is such a significant cause for concern that the governments of Australia, Canada, Great Britain, and Germany either restrict comfrey’s availability or have banned its sale entirely. The U.S. Food and Drug Administration (FDA) has not yet acted to restrict the sale of PA containing foods.

Substances in Brassica Ferns

Brassica fern (Pantolium aquatilum, P. excisum, and others) is widely used as greens or in salads in places like New Zealand, Australia, Canada, the United States, and especially Japan. It is also a forage plant for sheep and cattle. Veterinarians first noticed severe toxicity—bladder cancer, bone marrow depression, severe leucopenia, pneumoconiosis, and a hemorrhagic syndrome—in livestock grazing on this plant. Brassica is a potent bladder, lung, and intestinal carcinogen in rodents. Feeding cows led to increased milk that was carcinogenic to rats, showing that human exposure may also occur through cow’s milk. Human consumption of brassica fern has been linked to an increased incidence of esophageal cancer in Japan. Paullinol, a potent non-steroidal glucoside (Fig. 7), often present at up to 1.3% dry weight, is thought to be the major carcinogen in brassica. Like other natural carcinogens, paullinol is alkylates DNA at codon 61 in the Ha ras oncogene (15).

Solanum Alkaloids

Members of the family Solanaceae contain a variety of toxic steroidal glycoalkaloids. Potatoes (Solanum tuberosum) are an important food staple in many parts of the world, and under certain conditions they produce a variety of glycoalkaloids. Concentrations of solanum alkaloids vary considerably between species and strains of potatoes, but many important lectins include wound damage, fungal or bacterial infection, exposure to light, and whether the potatoes have sprouted. The major glycoalkaloids are solanine and a-chinin (Fig. 8), which may be present at concentrations over 100 ppm. Like physostigmine, solanine and a-chinin are potent inhibitors of the enzyme acetylcholine esterase. Higher amounts of a solanine and a-chinin are present in the potato greens (top). Healthy potatoes contain negligible amounts of these toxins.
Episodes of human poisoning by green potatoes have been documented. Fatalities from solanum alkaloids are rare, but gastrointestinal symptoms—gastric pain, weakness, nausea, vomiting, and labored breathing—that are consistent with acetylcholinesterase inhibition are more common. These symptoms have been duplicated in clinical trials with human volunteers. Studies have indicated that the acetylcholinesterase inhibitory activity of solanine is probably insufficient to cause these toxic effects, which are probably due to the combined toxicity of solanine with other cholinesterase inhibitors in the potato, such as chaconine.

Most cases of human poisoning and deaths have occurred in Europe, but they are occasionally seen in the Western Hemisphere. Livestock fed damaged potatoes or peel, greens, or sprout are occasionally poisoned. A small number of studies in which animals are fed toxic doses of blighted potatoes or pure glycoalkaloid have indicated that these compounds may have weak teratogenic activity. For example, solanine and chaconine, and their aglycone derivatives solamargine, induced cranial malformations (exencephaly, encephalohoele, and anophthalmia) in Syrian hamsters (16). In that study, solandrine was much stronger teratogen than solanine and chaconine, which were classified as weakly teratogen. The teratogenic and embryotoxic effects of solanine and chaconine appear to be synergistic (17).

**Caffeic Acid and Chlorogenic Acid**

High concentrations (often over 1.500 ppm) of caffeic acid and its quinic acid conjugate, chlorogenic acid (Fig. 9), occur in an extremely wide range of vegetables (e.g., lettuce, potatoes, radishes, and celery), fruits (e.g., grapes, berries, eggplant, and tomato), and seasonings (e.g., cyme, basil, anise, caraway, rosemary, tarragon, marjoram, sage, and dill). Coffee is especially rich in caffeic and chlorogenic acid, as well as other compounds, such as caffeine. A cup of coffee contains about 190 mg of chlorogenic acid. Other minor conjugates of caffeic acid also exist.

Chlorogenic acid is hydrolyzed in the gastrointestinal tract to caffeic and quinic acids. In people, caffeic acid is metabolized to the α-methylated derivatives ferulic, diphlosteric, and vanillic acids and meta-hydroxyphenyl derivatives, which are excreted in the urine. Caffeic acid inhibits 5-lipoxygenase, which is a key enzyme in the biosynthesis of various eicosanoids such as leukotrienes and thromboxanes. These eicosanoids are mediators of a wide variety of physiological and disease states and are involved in immunoregulation, asthma, inflammation, and platelet aggregation. At high doses (2% in the diet), caffeic acid causes forestomach squamous cell papillomas and carcinomas in both sexes of rats and mice, renal tubular cell hyperplasia in male rats and female mice, and eyelid type II cell tumors in male mice (18). Chlorogenic acid is a bacteria mutagen but has not been tested for carcinogenicity.

**Coumarin and Furmarcoumarin**

Coumarin (Fig. 9) is a natural anticoagulant found in a variety of plant foods such as cabbage, radish, and spinach and its flavoring agents such as lavender and sweet woodruff (Dipsacus odoratus). The latter is an essential herb in making German May wine. Coumarin is found in herb teas based on tarrax beans (Dipsacus odoratus) and sweet clover
(M. albus and M. officinalis) called mellite. (In fact, the native caumarin originates from comaro, the Carribean name for fenka beans.) Purified caumarin was once used as a food additive but was banned by the FDA after it was discovered that high doses cause liver damage in test animals. Caumarin has also been reported to cause bile duct carcinomas in rats. The anticoagulant action of caumarin is based on its interference with the action of vitamin K in the synthesis of clotting factors II, VII, IX, and X. The anticoagulant action of caumarin was discovered when cows grazing on sweet clover developed hemorrhaging and internal bleeding. Shortly after it was found that rodents (which were used to bioassay the then-unknown toxic principle in clover) were extremely susceptible to it, however, the newly isolated 3 (4-acetylbenzoyl) 4-hydroxycumarin, known as warfarin (a named derived from the acronym for Wisconsin Alumni Research Fund), was developed as a rodent bait. Known as coumarin, warfarin is also used in human medicines as a blood thinning agent and to prevent the formation of blood clots.

Poratrices are a group of phototoxic furancumarins widespread in a number of plant families such as Asparagaceae (formerly Umbelliferae—celery and parsnip), Rutaceae (eg, bergamot, limes, cloves), and Moraceae (eg, figs). Celery contains 100 ppb pyrrolizine, whereas parsley contains approximately 40 ppm. When activated by sunlight, poratrices are mutagenic, presumably due to their ability to form interstrand and protein cross links with DNA. Many members of this chemical family are carcinogenic as well, including 5-methoxytryptamine and 8-methoxytriptamine (also called methoxsalen and xanthoxin, respectively, Fig. 9). Methoxsalen, in addition to forming DNA crosslinks, causes specific mutations in the tumor suppressor gene p53. Dietary exposure to poratrices is probably not a significant health risk; however, the margin of safety is thought to be narrow. Human volunteers who ingested 300 g of celery roots (with a total phototoxic furancumarin content of 28 ppm) experienced no skin reactions after UVA exposure, and the blood levels of poratrices methoxsalen, and 5-methoxytryptamine were below the analytical detection limit.

MYCOTOXINS

Mycotoxins are a diverse group of mold-produced chemicals that elicit a wide range of toxic responses in animals.
and humans. Postharvest contamination of various food crops by mycotoxigenic fungi is a common problem, with approximately 25% of the world's food supply potentially contaminated by mycotoxins annually. The severity of mycotoxin contamination of agricultural commodities varies from year to year, depending on factors such as excessive moisture in the field and in storage, temperature extremes, humidity, drought, variations in harvesting practices, and insect infestation. Although the actual resultant economic loss to agriculture is difficult to determine with accuracy, it is considerable.

Many mycotoxins have been implicated in outbreaks of human diseases. Some are potent animal and presumed human carcinogens. In domestic animals, such as dairy cattle, swine, and poultry, mycotoxin contamination is known to reduce growth efficiency, lower feed conversion, lower reproductive rates, impart resistance to infectious diseases, reduce vaccination efficacy, and induce pathogenic damage to the liver and other organs. For these reasons, mycotoxins pose a major threat to public and animal health. A few classes of mycotoxins that are problems in foods will be considered here.

Aflatoxin B₁
Aflatoxin B₁ (AFB₁) represents a group of potent mycotoxins produced by strains of the filamentous fungus Aspergillus flavus and A. parasiticus. Of the Aspergillus mycotoxins, AFB₁ has generated the greatest concern and has stimulated the most research because of its extreme toxicity and its widespread occurrence in staple foods and feeds (such as corn, peanuts, and cottonseed). For these reasons, the U.S. FDA regulates AFB₁ in foods. The current action level, the concentration above which the commodity is condemned and discarded, is 20 ppb of total aflatoxins. This regulatory value was established in the 1960s, in large part from the analytical detection limits at that time. The action level for the major AFB₁ metabolite present in milk and milk products, aflatoxin M₁ (AFM₁), is 0.5 ppb in fluid milk. Other regulatory guidelines for AFB₁ include 20 ppb in corn for dairy cows, 300 ppb in corn for finishing beef cattle and swine, and 100 ppb for breeding stock. Permissible AFB₁ concentrations in cottonseed for beef cattle, swine, and poultry is 300 ppb. These regulatory concentrations generally preclude detectable AFB₁ in the various products from these animals. Worldwide, established tolerances for AFB₁ in animal feeds range from 10 to 500 ppb.

Prevention of Aspergillus intoxication in foods and feeds is the most desirable method of reducing contamination. Despite the best agricultural practices, however, AFB₁ contamination is mostly unavoidable. Thus, several methods of reducing postharvest product contamination have been developed. Some of these methods involve early identification and segregation of grossly contaminated kernels of corn or peanuts, or electronic devices to identify and reject grains that exhibit fluorescence due to AFB₁. Aflatoxin results in nearly complete elimination of aflatoxins and associated toxicity in commodities, and it is suitable for treating large batches of product (10). This method has not yet been approved by the FDA for interstate shipments, but it is in use in some states. Another experimental strategy is
the use of inorganic absorbent feed additives that prevent absorption of mycotoxins in animals. Hydrolyzed sodium cellulose xanthinmum (TASCAN), an FDA-approved antitack agent, significantly reduces AFB1 bioavailability as well as many of its specific toxic effects in pigs (20).

The many toxic effects of AFB1 are initiated by its conversion, principally by hepatic and extrhepatic microsomal CYPs, to a variety of metabolites (Fig. 10). The reported carcinogenic intermediates are the AFB1-8,9-epoxide. Previously because of its extreme reactivity (it has a half life of approximately 0.5 s), the AFB1-8,9-epoxide has been isolated only indirectly from biological systems as adducts of glutathione-ECSSH DNA bases, or other macromolecules. Most other metabolite products are less toxic than parent AFB1. The most prevalent of which is AFB2, so named for its appearance in the milk of dairy cows that consume AFB1-contaminated feeds. Other detoxified metabolites produced from the CYP oxidation of AFB1 include aflatoxin Q1 (AFQ1) and aflatoxin P1 (Fig. 10). Soluble NADPH dependent (cytochrome enzymes reduce AFB1 to produce aflatoxin Afl,a which is nearly as toxic, mutagenic, and carcinogenic as the parent compound. Thus AFB1 is not considered a detoxified metabolite. Because the reduction is reversible, AFI is postulated to represent a storage form of AFB1.

The most critical detoxification AFB1 route is via glutathione S-transferase (GST) using GSH as cofactor. Species whose GST has a high affinity for the AFB1-8,9-epoxide are generally protected from the toxic and carcinogenic effects of AFB1, regardless of how well the epoxide is formed by CYPs. The AFB1-8,9-epoxide is a substrate for GST, producing some form of nontoxic AFB1-GSH adduct that is often the simple sulfhydryl derivative of AFB1-GSH. Aflatoxin B1 may also be detoxified via conjugation with sulfates and glucuronic acid. The AFB1-8,9-epoxide may also be catalytically (by epoxide hydrolase) or spontaneously hydrolyzed to the AFB1-8,9-dihydrodiol. A soluble AFB1, aldo keto reductase (AFAR) has also been isolated from liver and extrhepatic tissues from human, rat, and other species that have strong affinity for reducing the AFB1, diol, thereby contributing to epoxide detoxification (21).

The electrophilic and highly reactive AFB1, 8,9-epoxide is reportedly responsible for the carcinogenic and mutagenic action of AFB1. Intermolecular bonds to cellular nucleophiles such as DNA. Activated AFB1 binds exclusively to guanine residues, and the AFB1, N7 guanine (AFB1, N7-Gua) adduct is the most predominant (Fig. 10). Additional adducts have been isolated, of which the "ring opened" derivative of AFB1, N7-Gua, the formamido-glycine or, AFB1, FAyP, is the most common. In hepatic DNA from livers of rats injected with AFB1, approximately 80% of the adducts present are AFB1, N7-Gua, whereas the AFB1, FAyP comprises approximately 7% (22). The formation of these adducts is the presumed first step in the development of heritable mutations from which tumors may arise. Repair of these genetic lesions occurs in living cells enzymatically or spontaneously, and the repair adduct is excreted in the urine. The ring opened adduct appears to be more resistant to DNA repair enzyme. In rats treated with a single dose of AFB1, the AFB1, N7-Gua was rapidly removed with an apparent half-life of 7.1 h, whereas other adducts, such as FAyP, remained rathure more slowly (23).

Aflatoxin B1 is a potent acute toxin that primarily targets the liver. The primary lesions include hemorrhagic necrosis, fatty infiltration, and bile duct proliferation. In pigs, guinea pigs, and dogs, these effects are found most commonly in the centrilobular region, whereas in chickens and rats, the pericentral region is the site of action. Various species show considerable variation in susceptibility to the acute effects of AFB1, and no species appears to be totally resistant. Poultry, rainbow trout, rats, and monkeys are particularly sensitive to the acute effects of AFB1. Mice and hamsters are much less sensitive (24).

Aflatoxin B1 is carcinogenic in a wide variety of animals. As is the case following acute exposures, the major target organ is the liver. Although tumors in other organs result from long-term dietary exposure to AFB1, Aflatoxin B1 at 1.4 ppm fed over a 14 month period resulted in 14% in incidence of hepatocellular carcinoma in rainbow trout. The most sensitive animal species known to the carcinogenic effects of this mycotoxin. By contrast, only a 5% tumor in incidence was observed during a similar time frame in Fischer rats exposed to 5 ppm.

Epidemiological data indicate that at least in sub-Saharan Africa and Southeast Asia, where AFB1 contamination in foods is considerable, dietary AFB1 is an important risk factor for human hepatocellular carcinoma (HCC). In these geographical areas there is a linear relationship between levels of AFB1 contamination of food and the incidence of HCC. A factor that complicates epidemiology is that the incidence of hepatitis B virus infection, which is another reported factor for HCC in humans, is also high in these regions. Specific biomarkers of human exposure to AFB1, such as adducts of DNA and serum albumin, have proven to be valuable tools in the study of the diet and AFB1 in human cancer. Measurement of these biomarkers in samples of blood or urine has allowed a direct determination of actual AFB1 exposure in populations, which is an improvement over performing random dietary analysis for AFB1 and imprecise dietary recall surveys.

Although the majority of interest in possible health effects of AFB1 has focused on its dietary exposure to AFB1, workers in food and grain production, harvest, transport, and processing industries are also exposed to considerable amounts of airborne, respirable AFB1, contaminated grain dusts. For example, airborne dust sampled in a corn processing plant contained 107 mg/m^3 AFB1, and the daily occupational exposure to this toxin was estimated to be between 40 and 850 mcg (25). In another survey, concentra tions of AFB1, in smaller, more easily handled airborne grain particles were found to contain more AFB1, than did larger grain particles. AFB1, in particles under 7 um were as high as 1,814 mcg, whereas particles in the size range of 7 to 11 um had an average content of 695 pg (26).

Some studies indicate that inhalation exposure to AFB1 may result in adverse health effects to these exposed. Aflatoxin-contaminated peanut dusts have been associated with liver and lung cancer in Dutch peanut-processing workers who were continuously exposed to be-
Figure 10. Major metabolites and fate of aflatoxin B₁ in animals.
Trichothecenes

Another chemically and toxically significant class of mycotoxins are the trichothecenes, which are a group of more than 150 structurally related compounds produced by several genera of fungi, the most common of which are Fusarium sporotrichoides and F. graminearum. Trichothecenes are common contaminants of grains such as corn, wheat, and barley. In the United States, corn from the upper Midwest is especially affected. Low temperature, high moisture, and humidity appear to increase toxin production. Trichothecenes possess a sesquiterpenoid tetra cyclic 12,13 epoxy-trichococne skeleton. Examples of important trichothecenes include T-2 toxin, nivalenol, and deoxynivalenol (Fig. 11). T-2 toxin was the first trichothecene discovered in grains, but of the trichothecenes, the incidence of deoxynivalenol (DON; vomitoxin) is more widespread worldwide.

The most acutely toxic trichothecene is verrucarin J, which has a LD50 of 0.5 mg/kg (iv) in the mouse. T-2 and nivalenol are only slightly toxic; LD50 values are 5.2 and 4.1 mg/kg, respectively. The in vitro toxicity of T-2 and nivalenol are approximately 10 times that of deoxynivalenol. In a variety of in vitro systems, T-2 toxin consistently has been the most toxic, followed by nivalenol and then deoxynivalenol (28).

Acute trichothecene toxicity is characterized by vomiting, diarrhea, and inflammation. Dermal irritation, food refusal, abortion, and hematological sequelae such as anemia and leukopenia are also common. In cattle, doses of T-2 toxin at 0.04 ppm for 20 days results in death with bloody feces, enteritis, and abnormal rumen gases. In poul try, trichothecenes at levels as low as 5 ppm cause enteritis and reduced body weight gains. Decreased egg production and shell quality result when poultry are fed a diet of 20 ppm T-2. Trichothecenes also are toxic when administered dermally and cause symptoms similar to those seen when these mycotoxins are administered orally.

Several widespread cases of human trichothecene poisoning have occurred. Known as alimentary toxic aleukia, the disease follows a multisystem pathogenesis. Shortly after ingestion of contaminated cereal grains, initial symptoms include a burning sensation in the mouth, tongue, throat, esophagus, and stomach and gastrointestinal distress. Severe hemolytic effects, such as leukopenia and granulopenia, may then follow, which may progress to white cell counts as low as 100/μl of blood. Continuous exposure to trichothecenes results in rash on the skin and elsewhere that may progress to severe necrotic lesions.

Trichothecenes have widespread adverse effects on synthesis of proteins and other intracellular and on membrane and immune functions. Trichothecenes inhibit all steps in protein synthesis: initiation, elongation, and termination. Protein synthesis inhibition likely is a result of binding of the toxins to ribosomes. The binding of T-2 to ribosomes is specific and saturable (0.3 mM), and the ribosome is a single 60S molecule bound per ribosome (20).

The antifungal nature of T-2 has led same to believe that it could be incorporated into the lipid of protein and/or ribosome of cellular plasma membranes, thereby interfering with membrane function. Mycelia treated with T-2 toxin had reduced uptake of calcium, glucose, inositol, and tyrosine within 10 min of exposure, effects apparently independent of protein synthesis inhibition.

The toxins significantly alters several immune parameters, and the major effects appear to be associated with the cellular immune response. Specifically, effects such as an inhibition of the mitogen response, reductions of protein, DNA and RNA synthesis in Concanavalin A-treated normal spleen cells, thymus atrophy, and reductions in plaque-forming spleen cells have been demonstrated. In animals, decreases in resistance to many challenges that are cellular immune dependent have been observed, such as to bacterial, fungal, and mycobacterial infections and skin grafts.

Zearalenone

Zearalenone (ZEN) is a phenolic monosaccharide lactone (Fig. 1) produced by strains of Fusarium, primarily by F. graminearum and F. sporotrichoides, it is a natural contaminant of corn, wheat, barley, oats, rye, sorghum, and hay. As with most mycotoxins, significant ZEN production is promoted by high humidity and low temperatures, rain conditions in the upper Midwest during autumn harvest. Zearalenone is a nearly universal corn contaminant and is often found in the same samples with trichothecenes. Despite its dissimilarity to steroidal compounds, ZEN produces potent phytoestrogenic responses in susceptible animals. Thus the toxicity of this compound is unique among known mycotoxins.

The animal most affected by ZEN is swine, but other animals, such as cattle, poultry, and laboratory animals, are affected to a lesser degree. Symptoms of ZEN poisoning include uterine enlargement and swollen vulvas and teat veins. In pigs, symptoms of hyperestrogenism generally appear when contamination of ZEN in corn exceeds 1 ppm, but it can occur as low an 0.1 ppm. In ruminants, ZEN exposure results in decreased ovulation rate and cycle length and an increase in duration of estrus. Young male pigs exposed to ZEN undergo symptoms of feminization, such as enlarged nipples and penile atrophy.

Zearalenone causes significant adverse reproductive effects in animals. For example, dairy heifers exposed to ZEN have reduced conception rates; in rams, ZEN (10 mg/kg in the diet) causes decreased growth rate, feed intake, fertility, fetal resorptions, stillbirths, abortion and fetal bone malformations. ZEN reduces egg production in hens. In newborn female mice, ZEN (1 μg daily for 5 days) results in significant dose dependent reproductive tract

alterations at 8 month posttreatment; the majority of treated animals lacked corpus lutea and uterine glands and exhibited squamous metaplasia of the uterine luminal epithelium. Ovariectomized ZEN-treated animals showed none of these ovary dependent alterations.

The mechanism of the estrogenic effect of ZEN appears to be mediated via binding of ZEN or metabolite(s) to the cytoplasmic estrogen receptor. In rat uterine tissue, trans- and co-ZEN and two ZEN derivatives compete with 17β-estradiol for binding with the cytoplasmic receptor, but with a lesser affinity than that of estradiol. Accordingly, ZEN appears to have a greater affinity for estrogen receptors from animals that are more susceptible to the estrogenic effects of the mycotoxin. The affinity of ZEN to uterine and ovine estrogen receptors follows the pattern pig, rat, and then chicken.

Fumonisins

Fumonisins are a group of recently discovered mycotoxins that have been associated with toxicity and mortality in horses and pigs following ingestion of Fusarium-contaminated corn-based feeds. Fumonisin B₁ (FB₁) is the most toxic representative of the fumonisins (Fig. 11). One such animal disease is the neurotoxic syndrome Equine Leukoencephalomalacia (ELEM), which is characterized by bilateral paralysis, nervousness, lameness, ataxia, and an inability to eat or drink. The principal pathologic lesions include severe cerebral edema, focal malacia, and liquefaction of cerebral white matter. The onset of such severe symptoms can be as short as a few hours. ELEM syndrome often is episodic and nearly always is associated with ingestion of moldy corn.

Several studies have passively linked fumonisins to ELEM. A majority of horses fed a corn-based feed containing 37 to 122 ppm FB₁ developed fatal ELEM (20). Hepatic involvement is often coincident with central nervous involvement in horses and swine. Intravenous or dietary administration of FB₁ causes both pulmonary edema and hydrothorax in swine (21). In swine, lower doses of FB₁ result in a slowly progressive hepatic necrosis, whereas higher doses result in acute pulmonary edema coincident with hepatic toxicity. That fumonisins are potent inhibitors of sphingosine biosynthesis in cultured hepatocytes has been postulated to account for both
the hepatotoxic and central nervous system effects of this toxin. Besides the neuromuscular and hepatotoxic effects, FB₁ is a potent rat liver carcinogen. Initial studies showed that a diet containing culture filtrate inoculated with F. maeotilutax was carcinogenic in rats. Dietary FB₁, or only initiators, glutathione peroxidase (+/- CT x Hepatic fibs), but also promotes those induced by compounds such as diethylnitrosamine (32). Long-term dietary FB₁, (50 ppm) induces a high incidence (80%) of HCC along with metasta- ses to the heart, lungs, or kidneys (33). Symptoms of hepatic involvement such as macrovascular cirrhosis and cholangitis are also observed. Later studies have led to the conclusion that FB₁ is probably only a modest ini- tiator of liver tumors, because: CT x fibs and hepatocel- lular nodules were observed only after prolonged feeding of very high (10%) FB₁ in rats. It was postulated that the carcinogenic effect of FB₁ likely involves promotion and the selection of initiated hepatocytes, events that occur during the post-initiation phase of hepatocarcinogenesis. Addi- tionally, FB₁ carcinogenicity does not appear to involve inter- action with DNA. Neither FB₁, nor F₂, leads to undetectable DNA repair in primary rat hepatocyte cultures treated ei- ther in vitro or in vivo (34). It is therefore possible that the carcinogenic activity of fumonisins is mediated via epige- netic mechanisms, such as is the case of procarcinogenic pro- liferators.

Research on the health effects of fumonisins is rela- tively modest, and information on the mechanism of hu- man toxicity or on the mechanisms underlying species sensitivity is only beginning to be compiled. Obviously, more information is needed to provide a fuller understand- ing of the extent of the adverse effects of fumonisins on human and animal health. Because corn is a staple in many parts of the world, and because Fumonisium contami- nation of corn is nearly universal, it is likely that huma- nes may be involved in human toxicosis and other health effects. Fumonisium-contaminated corn has been epidemi- ologically associated with human esophageal cancer in some regions of South Africa.

Ergot Alkaloids

Documented since the Middle Ages, the fungus Claviceps purpurea has caused periodic outbreaks of mycotoxicoses in many parts of the world. This mildew, which grows in grasses and cereal crops, is relatively prevalent in over- wintered rye and wheat. Growth of the fungus takes the form of hard, black purple sclerotia (or ergots) that ger- minate in the spring. Claviceps produces several ergot al- kaloids, which are derivatives of lysergic acid dihy- droxyamine (eq. 12). The major effect of these compounds is vasospasm, resulting in a reduction of blood flow and subse- quent gangrene of the feet, legs, and hands. Symptoms of ergot poisoning include a burning sensation in the arms and legs, gangrene, abortion, vomiting, diarrhea, weak- ness, and sometimes hallucinations.

Ochratoxins

Ochratoxins are produced by Aspergillus and ochro- toxans and selected Penicillium species. Ochratoxin A (eq. 12), which is the most toxicologically significant of the ochratoxins, is produced in a variety of cereals (wheat, barley, oats, corn), dry beans, peanuts, and thyme. Ochratoxin A (OAT A), a potent nephrotoxin in nearly all animals studied and a probable etiologic agent for Balkan endemic nephropathy (BEN), a fatal chronic renal disease. The syndrome, which is associated with nephritis and associated urinary tu- mors, is especially prevalent in Bulgaria, Romania, and Yugoslavia. Pigs exposed to ochratoxin A experimentally developed a nephropathy strikingly similar to BEN.

Surveys have repeatedly shown that a significant per- centage of food supplies from several Balkan countries are contaminated with ochratoxin A. In addition to the afore- mentioned cereal and other products, ochratoxin is often found in animal products such as sewage, biomass, and hams.

HETERO CYCLIC AMINES IN COOKED FOODS

It has been known for years that heating food produces several classes of toxic compounds. For example, polycyclic aromatic hydrocarbons, the same compounds present in cigarette smoke, are also produced by barbecuing meat, chicken, and fish. More recently, however, a new class of heat-produced compounds, the heterocyclic amines (HCA), have been discovered and characterized. Like polycyclic aromatic hydrocarbons, HCA are produced by pyrolysis reactions that occur under ordinary cooking conditions. HCA have been found in meat, fish, and poultry. Compo- nents in raw meat that react to form HCA include creatine, sugars, and amino acids. Both creatinine and amino acids appear to be rate limiting, but the role of sugars in formation of HCA is uncertain (35). Cooking practices in- volving higher temperatures, such as flame broiling or pan-frying, produce higher amounts of these toxicants, which is stable in food, and a major storage situation. In addition to temperature, cooking time and type of food are factors important in HCA formation.

More than 20 HCA have been isolated from cooked foods, nearly all of which are powerful mutagens, and in most cases animal carcinogens. In fact, HCA are among the most potent bacterial mutagens known. Some of the most common and intensively studied include IQ (2-amino- 3-methylimidazo[4,5-f]quinoline), methyl IQ (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-f]quinoline), PhIP (2-amino-1 methyl-5 phenylimidazo[4,5,6]pyridine), Trp-P1 (1-amino-1,4-dimethyl-5H-pyrindazin-3-8-dione), and Chl P1 (2-amino-6-methylpyridinol-2.3.2',3'-dini- dzazole) (eq. 13). Heterocyclic amines are well absorbed from the intestine and, like many other natural carci- nogens, require hepatic metabolic activation for toxicologic activity (see 49). Cytotoxicity and genotoxic activity of HCA in mammalian cell lines, human Hep G2 cells, and primary liver cells have been observed in the absence of metabolic activation (40-4090).

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Figure 12. Chemical structures of mycotoxins ergotamine and ochratoxin A.

Figure 13. Major carcinogenic heterocyclic amines in cooked foods.

Figure 14. Mechanism of metabolic activation of IQ.


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See also MYCOXINS.

TOXICOLOGY AND RISK ASSESSMENT

Risk assessment is the process used to determine the probability, type, and magnitude of human toxic effects anticipated from exposure to specific levels of chemicals. All chemicals have the potential to produce adverse effects on health under some conditions of exposure. According to a sixteenth-century principle expressed by Paracelsus (ca. 1492-1541), it is the right dose that differentiates a poison and a remedy. There is, however, a great variation of potency among chemicals in regard to their ability to cause adverse health effects; some chemicals may cause toxicological effects at very low doses, whereas others require much higher doses before effects are produced. All our food is comprised of chemicals, and there are numerous types of chemicals that have been subject to risk assessment and/or regulation (Table 1).

The U.S. Food and Drug Administration (FDA) manages risks from food additives and veterinary drugs that may persist as residues in foods of animal origin (milk, meat, and eggs). The FDA has published comprehensive guidelines for safety assessment of direct food and color additives (1). The U.S. Environmental Protection Agency (EPA) is responsible for the control of several chemicals that may contaminate food, including pesticides and water pollutants. The EPA has developed a variety of guidelines for the toxicological testing of pesticides. At the international level, the regulatory authorities include different committees of the Food and Agriculture Organization of the World Health Organization (FAO/WHO), such as the Joint FAO/WHO Expert Committee on Food Additives, the Joint FAO/WHO Meeting on Pesticide Residues, and the Codex Alimentarius.

TOXICITY: TYPES AND TARGETS

Based on the duration of exposure to chemicals, toxicity is often classified as acute (involving a single dose), chronic (generally involving exposure over a lifetime), or sub-chronic (repeated exposures).

Different organs can be damaged by chemicals, including the liver, kidney, skin, bone, lungs, stomach, spleen, intestines, bladder, eyes, blood, and blood vessels. Different systems can also be affected, including the cardiovascular, nervous, immune, and reproductive systems, as well as the developing embryo or fetus. Although some toxicological effects can be reversed, others are irreversible. Carcinogens are chemicals capable of producing tumors, teratogens cause birth defects (2).

Toxicity Testing of Food Chemicals

In early times, trial-and-error methods were required to allow the distinction between safe and unsafe foods. In