chapter eleven

Adverse effects of naturally occurring nonnutritive substances

H. van Genderen

11.1 Introduction

By far the majority of the non-nutritive components are harmless. However, a number of naturally occurring substances have been identified that induce adverse effects. These originate mainly from plants and microorganisms.

In this chapter, such substances occurring in common foods are discussed. Cases of adverse effects following the intake of unusual foods, particularly in tropical regions, are not included. Their occurrence is too incidental. The following categories are discussed:

– (low-molecular) endogenous toxins of plant origin;

– toxic contaminants of microbial origin;
– plant proteins that interfere with the digestion of the absorption of nutrients.

11.2 Endogenous toxins of plant origin

Low-molecular endogenous toxins of plant origin are products from the so-called secondary metabolism in plants. In phytochemistry, a distinction is made between primary and secondary metabolism. Primary metabolism includes processes involved in energy metabolism such as photosynthesis, growth, and reproduction. Macro- and micronutrients are products of primary metabolism. Secondary metabolism is more or less species-, genus- and family-dependent. Each plant contains a large variety of secondary metabolites that function as pigments, flavors, protecting agents, or otherwise. The number of identified secondary metabolites involved in plant–animal interactions is estimated at 18,000.

Relatively few secondary metabolites in food plants have been shown to be toxic. They may induce a wide variety of effects, including growth inhibition and neurotoxicity, but also mutagenicity, carcinogenicity, and teratogenicity. For the majority of these substances, the information on their toxicity is limited, and often completely lacking. The studies involved were usually concerned with cases, and not with underlying mechanisms.

Testing of plant substances for toxicity is not provided for by official food safety regulation. Isolation and purification of amounts needed for toxicity testing are expensive. In general, industry has no interest in giving such studies financial support. Flavors of plant origin are an exception. Many of these are used in the production of food additives. As such, they come under the regulation of additives. The toxicity of some plant flavors has been examined by modern methods. Important additional information comes from experiences acquired with farm animals. These animals very often ingest one edible plant species or relatively simple and homogeneous feed mixtures over long periods of time, which may be comparable to chronic toxicity testing in experimental animals.

From a food safety point of view, two groups of non-nutritive natural food components can be distinguished:

– those that have given or still give rise to concern, but at present do not pose actual hazards;
– those that are of important toxicological relevance. The dietary intake of some of these substances has led to mass poisoning.

Table 11.1 lists a number of examples of the first group.

The second group is dealt with in the next subsection. It includes α-aminopropionic acid derivatives (N-oxalyl-diaminopropionic acid and β-cyano-L-alanine), agaritine, biogenic amines (serotonin, tryptamine and tyramine), cyanogenic glycosides (amygdalin, primasin, dhurrin, linamarin and lotaustralin), glucosinolates (sinigrin, progoitrin and glucobrassicin), glycoalkaloids (solanine, chaconine and tomatidine), and pyrimidine glycosides (vicine and convicine).

11.2.1 Nonnutritive natural food components of important toxicological relevance

11.2.1.1 α-Aminopropionic acid derivatives

α-Aminopropionic acid derivatives occur in peas of certain *Lathyrus* species. These substances are known to cause skeletal malformations (osteolathyrism) and neurotoxic effects

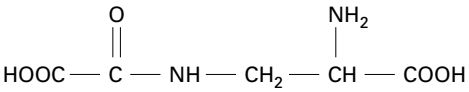
Table 11.1 Nonnutritive natural food components that have given or still give rise to concern

Substance/origin	Main toxic effect(s)	Comments
Erucic acid (fatty acid)/rapeseed	fibrotic myocardial lesions (in the rat)	varieties free from erucic acid have been bred
Cyclopropane and cyclopropene fatty acids/cottonseed oil	promotion of aflatoxin-induced carcinogenesis (in the trout)	the acids are largely removed during food processing; the main problem is their presence in feed
Carotatoxin (polyacetylene)/carrots	neurotoxicity	low levels in edible carrots
Thujone (α– and β–) (monoterpene)/spices (component of absinthe liqueur)	neurotoxicity	absinthe is prohibited; for use as food additive, not more than 10 ppm is allowed.
D-limonene (monoterpene)/citrus oil	nephrotoxicity (in male rats, not in female rats and other animals)	intake is considered to pose no toxicological risks to man
Cucurbitacin E (triterpene)/squash or zuchini (as glucoside)	irritation gastrointestinal tract; vomiting, diarrhea	rarely present in bitter summer squash; may originate from cross-breeding with a wild species
Safrole (phenol derivative, occurring in plants)/spices (mainly sassafras oil)	liver cancer	allowed for use as food additive in the EU, prohibited in the US.
Coumarin/various plants (e.g., woodruff) and spices	hepatotoxicity (in rats)	see above under safrole
Quercetin/many plants (free and as glycosides)	mutagenicity (in Ames'test; in mammalian test systems mainly negative)	evaluation needs further research
β-aminopropionitrile/seed of *Lathyrus odoratus*	skeletal malformations (osteolathyrism) and neurotoxicity (neurolathyrism)	

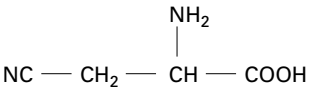
(neurolathyrism). The peas are easily grown on poor soil and are often used as feed. Both diseases have occurred as epidemic in Northern India in years with a poor harvest. At present, osteolathyrism has largely disappeared. Neurolathyrism still poses a serious health problem.

Neurolathyrism is associated with the long-term intake of the peas of *L. sativus*. The disease is characterized by muscular weakness, degeneration of spinal motor nerves, and paralysis. The peas have been found to contain a neurotoxin: N-oxalyl-diaminopropionic acid (ODAP). In addition, the peas may be contaminated with a vetch species (*Vicia sativa*), also containing a neurotoxic α-aminopropionic acid derivative, β-cyano-L-alanine.

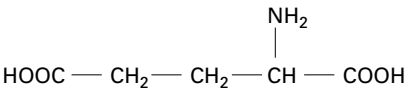
The neurotoxicity of the amino acids is attributed to their structural relationship with the neurotransmitter glutamic acid. ODAP and β-cyano-L-alanine are believed to bind irreversibly to the glutamate receptors on specific nerve cells. Long occupation of the glutaminergic receptors has been reported to result in neurodamage.



N – oxalyl – diamino – propionic acid



β – Cyano – L – alanine



Glutamic acid

11.2.1.2 Agaritine

Agaritine is a member of a series of hydrazine derivatives, occurring in mushrooms, including the common edible mushroom *Agaricus bisporus*. It is the most important derivative.

Agaritine undergoes degradation on cooking. It is partly left intact when heated in oil. In the body, it is hydrolyzed by glutamyltransferase into glutamic acid and 4-(hydroxymethyl)phenylhydrazine (Figure 11.1). Agaritine has proved to be mutagenic in the *Salmonella*/mammalian microsome assay. If glutamyltransferase is added, the mutagenicity increases, suggesting that 4-(hydroxymethyl)phenylhydrazine is a more potent mutagen. Since the 4-(hydroxymethyl) phenyldiazonium ion is highly mutagenic, it is assumed to be the ultimate mutagen. 4-(Hydroxymethyl)phenylhydrazine induces tumors in soft mouse tissues at the injection site. Recently, also in mice, tumors have been found after mushroom feeding. Further studies are needed to confirm this.

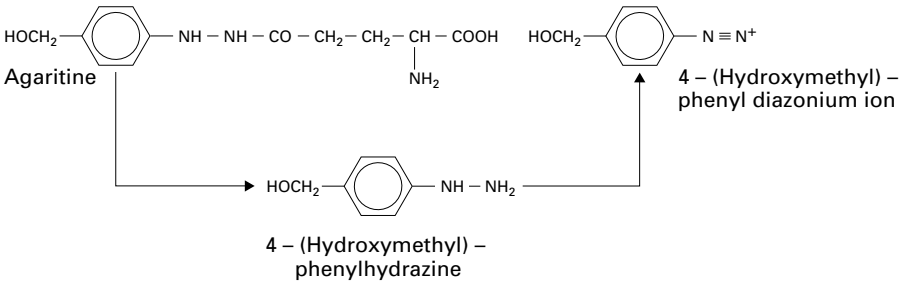


Figure 11.1 Hydrolysis of agaritine, followed by its activation.

11.2.1.3 Biogenic amines

Biogenic amines are formed by decarboxylation of amino acids. The term biogenic amines usually refers to the catecholamine neurotransmitters dopamine, norepinephrine, and epinephrine, the indoleamine neurotransmitter serotonin, and the mediator of inflammation histamine.

Examples of biogenic amines as food components are serotonin in bananas and pineapple, tryptamine in tomatoes, and tyramine in certain kinds of fully mature cheese. The

precursor of serotonin is 5-hydroxytryptophan, that of tryptamine, tryptophan, and that of tyramine, tyrosine. Biogenic amines in food can also originate from fermentation (beer, wine, cheese) or bacterial contamination (meat). Tyramine in fermentation products results from the bacterial decarboxylation of tyrosine.

Well-known toxic effects include hypertension, palpitations and severe headache. Under normal conditions, tyramine is detoxicated by monoamine oxidase (MAO). Patients taking MAO-inhibitors as antidepressants may suffer from headache, and attacks of palpitation and hypertension, if they consume foods containing considerable amounts of tyramine.

11.2.1.4 Cyanogenic glycosides

Cyanogenic glycosides are monosaccharide or disaccharide conjugates of cyanohydrins. There is evidence that the cyanohydrins are derived from amino acids. Cyanogenic glycosides are widely present in plants where they are the principal precursors of hydrocyanic acid. Their presence is believed to provide protection against herbivores.

Representatives of importance identified in edible plants are:

- amygdalin, the gentiobiose conjugate of mandelonitrile. It is present in bitter almonds, apple pips, and kernels of cherries, apricots, and peaches;
- primasin, the D-glucose conjugate of mandelonitrile. It is also found in bitter almonds and other fruit kernels;
- dhurrin, the D-glucose conjugate of p-hydroxybenzaldehyde cyanohydrin. It occurs in sorghum and related grasses;
- linamarin, the D-glucose conjugate of acetone cyanohydrin. It occurs in pulses, linseed, and cassava;
- lotaustralin, the D-glucose conjugate of 2-butanone cyanohydrin. See for its occurrence under linamarin.

Many cases of cyanide poisoning in man after dietary intake have been reported.

The formation of hydrogen cyanide from cyanogenic glycosides in plants takes place via a sequence of enzymic hydrolyses. In a first step, the glycosides are hydrolyzed by β-glycosidases to the cyanohydrins and mono- or disaccharides (see Figure 11.2). The cyanohydrins undergo further hydrolysis by lyases to hydrogen cyanide and the carbonyl compounds involved.

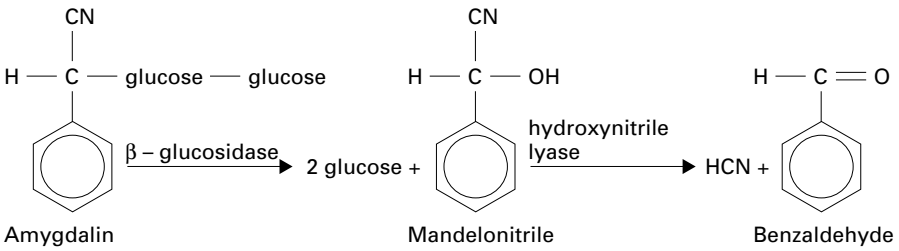


Figure 11.2 Hydrolysis of amygdalin.

Hydrolysis of the cyanogens requires tissue disruption, such as crushing of the wet, unheated tissues. The destruction of the compartmental organization of the cells brings the glycosides in contact with the hydrolytic enzymes.

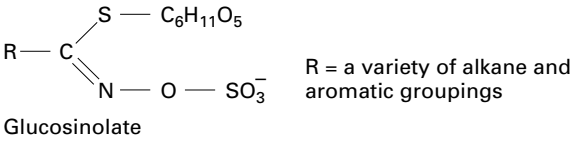
The occasional intake of small amounts of cyanogenic glycosides does not involve danger. The cyanide formed is generally detoxicated by conversion to thiocyanate. This reaction is catalyzed by the sulfurtransferase rhodanase.

Overloading of the detoxication route by taking in large amounts of cyanogenic glycosides can lead to cyanide intoxication. Fatal poisonings of children have been reported as a result of eating 7 to 10 bitter almonds.

In addition, there are the toxicological risks due to chronic consumption of improperly prepared cassava. Damage to the nervous system after chronic intake of cassava in a number of African countries is believed to be a long-term effect of cyanide or, perhaps, of thiocyanate, resulting from insufficient removal of the cyanogen.

11.2.1.5 Glucosinolates

Glucosinolates are thioglucosides. They have a sulfur atom between the glucosyl group and the aglycon. Glucosinolates also derive from amino acids.



Glucosinolates are thyroid agents. Their main effects are hypothyroidism and thyroid enlargement.

The glucosinolates themselves are not the active agents. They need activation by hydrolysis. All thioglucosides of natural origin are associated with enzymes that can hydrolyze them to an aglycone, glucose and bisulfate. The aglycone can undergo intramolecular rearrangements to yield isothiocyanate, nitrile, or thiocyanate (Figure 11.3).

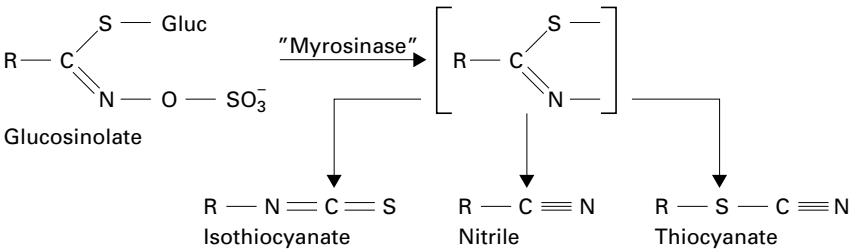


Figure 11.3 Hydrolysis of glucosinolates.

Thiocyanates contribute to the antithyroid activity, isothiocyanates are alkylating agents, and the nitriles have also been found to be toxic. Glucosinolates occur in plants belonging to the Cruciferae. The main food sources are cabbage, broccoli, turnips, rutabaga, and mustard greens. Each cruciferous plant may contain up to 10 different glucosinolates. Major representavies are sinigrin (in the above general structure of glucosinolates, R = allyl), progoitrin (R = 2-hydroxy-3-butenyl) and glucobrassicin (R = 3-indolylmethyl).

Sinigrin occurs in cabbage species and black mustard. Its hydrolysis product, allylisothiocyanate, has been shown to be a mutagen in the Ames' test. Swelling of the throat in rats fed on a diet containing Ethiopian rapeseed has also been attributed to the formation of the reactive metabolite. At high concentrations, allylisothiocyanate acts as lachrymator and vesicant. There are no indications that the present consumption of mustards can lead to the induction of adverse effects.

Progoitrin is a major component of rutabaga and a minor one of cabbage, kale, Brussels sprouts, and cauliflower. As its name indicates, progoitrin is a goitrogen or antithyroid. Two types of reactive metabolites are believed to be responsible for the goitrogenic activity: (2-hydroxy-3-butenyl) isothiocyanate and 5-vinyloxazolidine-2-thione (goitrin). Goitrin is formed from the isothiocyanate in a cyclization reaction (Figure 11.4).

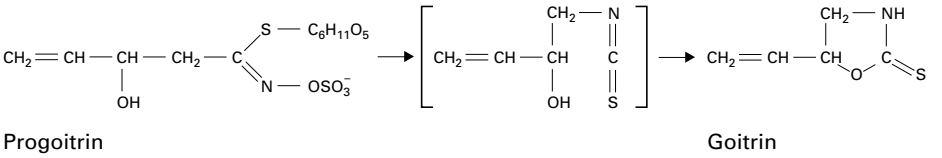
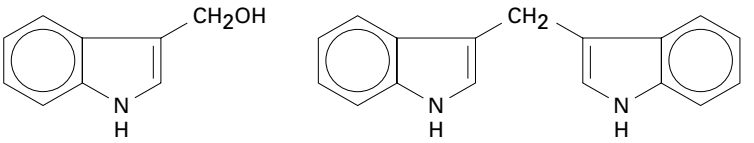


Figure 11.4 Formation of 5-vinyloxazolidine-2-thione.

Oxazolidine-2-thiones inhibit the production of thyroid hormones by preventing the incorporation of iodine in tyrosine.

Glucobrassicin occurs in a variety of cabbage species. Hydrolysis of this glucosinolate results in the formation of a number of products: indole-3-acetonitrile, indole-3-carbinol (I3C) and indole. I3C can cause sedation, ataxia, and sleep. Further, given orally, it is a potent inducer of hepatic as well as intestinal phase I and phase II drug-metabolizing enzymes. On parenteral administration and in isolated hepatocytes, however, it does not induce enzymes. Under the acidic conditions of the stomach, I3C undergoes oligomerization to yield products such as diindolylmethane. Diindolylmethane is also an enzyme inducer.

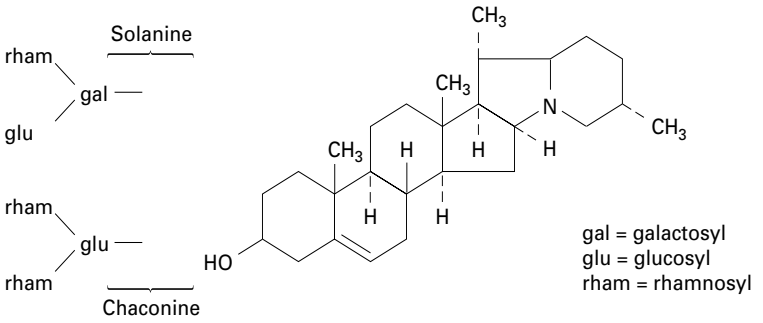


In some cases, the prevention of cancer has been related to the intake of glucosinolates. The formation of I3C is believed to decrease tumor induction by a variety of carcinogens. Further, feeding of cabbage to experimental animals prior or during the treatment with carcinogens was found to result in inhibition of tumor induction. Cabbage-feeding after administration of the carcinogens led to promotion of carcinogenesis. Probably, the protection against carcinogens is related to a more effective detoxication resulting from enzyme induction.

11.2.1.6 Glycoalkaloids

Steroidal alkaloids are mainly present as glycosides in the family of the Solanaceae, including the potato and the tomato. The major glycoalkaloids in potatoes are α-solanine and α-chaconine, both glycosides of solanidine.

Solanine and chaconine are potent irritants of the intestinal mucosa and cholinesterase inhibitors, the first being the most active. Poisoning with either substance results in gastrointestinal and neurological symptoms. The gastrointestinal symptoms can include vomiting and diarrhea, and the neurological symptoms include irritability, confusion, delirium, and respiratory failure, which may ultimately result in death. Further, poisoning is often accompanied by high fever.

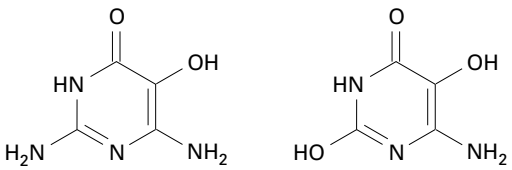


In general, the glycoalkaloid contents of potato tubers do not pose adverse effects in humans. Serious poisonings have been reported following the consumption of potatoes with high glycoalkaloid contents (≥200 mg/kg). Potatoes that have been exposed to light, and those that are diseased by fungal infection or have been mechanically bruised may contain toxic levels of glycoalkaloids.

Results of epidemiological studies on birth defects in regions with fungous potato disease suggested a relationship between the severity of the fungal infection and the occurrence of spina bifida and anencephaly. In animal studies, teratogenic effects and fetal mortality have been observed at dose levels that caused maternal mortality, either on administration of the pure alkaloids or on feeding with diseased potatoes. As far as testing for teratogenicity is concerned, the WHO Expert Task Group on Updating the Principles for the Safety Assessment of Food Additives and Contaminants in Food stated: "If the test substance injures reproduction or development at levels comparable with levels that cause toxicity in adults, then no special concern should be attached to the results of the reproduction/development toxicity studies." Recently, a Dutch expert, however, added to this statement: "It is advisable that for the selection of new varieties the guideline of about 60–70 mg glycoalkaloids/kg is followed in potato breeding, until an appropriate acceptable level has been set." The major glycoalkoloid in tomatoes is α-tomatidine, with tomatidenol as the aglycone. It is present in all parts of the plant. In the fruit, the concentration decreases during ripening. Poisonings in humans due to the consumption of tomatoes have not been reported.

11.2.1.7 Pyrimidine glycosides

For more than a century, a disease caused by the ingestion of fava beans has attracted the attention of toxicologists. The disease, called favism, is characterized by acute hemolysis, in serious cases accompanied by jaundice and hemoglobinuria. It is mainly found in Mediterranean populations with a congenital deficiency of NADPH-dependent glucose-6-phosphate dehydrogenase (G6PD). Fava beans contain two pyrimidine glycosides that have been shown to induce hemolysis: vicine and convicine. The aglycons are divicine and isouramil, respectively.



Divicine and isouramil are powerful reducing agents. In red cells, they are readily oxidized by oxyhemoglobin under the formation of methemoglobin, H_2O_2, and Heinz bodies (thought to consist of denaturated hemoglobin). The oxidation products undergo reduction by glutathione, and H_2O_2 is reduced by glutathione peroxidase. The oxidized

glutathione produced by these reactions is reduced by NADPH, generated from glucose-6-phosphate and G6PD.

The defect leading to hemolysis lies in the red cells which have insufficient G6PD, i.e., diminished levels of reduced glutathione, to protect them against oxidative attack.

11.3 Toxic contaminants of microbial origin

In addition to the naturally occurring food components discussed in the preceding section, several important groups of toxic contaminants of microbial origin may enter the food production chain. These may be produced by fungi (mycotoxins), marine algae, or bacteria.

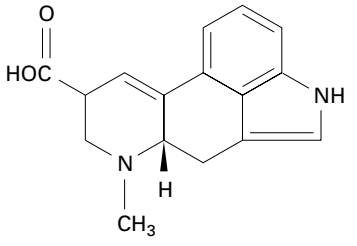
11.3.1 Mycotoxins

Mycotoxins are secondary fungal metabolites. They induce toxic effects upon inhalation or consumption by humans or animals. Mass-poisonings by mycotoxins are unusual in humans.

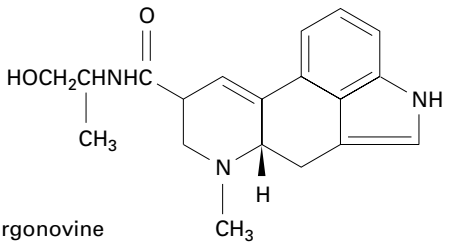
The history of human intoxications by mycotoxins (mycotoxicoses) dates back to the Middle Ages, when epidemics of hallucinations, delirium, convulsions, and gangrene were not uncommon. By the 1850s, the *ergot alkaloids* (products of the fungus *Claviceps purpurea*) were identified as the causative agents of the disease. Renewed interest in mycotoxin-caused diseases resulted from the death of thousands of turkeys and ducks (Turkey X disease) in England in the early 1960s. The animals were fed diets containing peanut meal contaminated by so-called *aflatoxins*, products of the fungus *Aspergillus flavus*. In the last three decades, more than 100 mycotoxins have been identified throughout the world. Two more classes of mycotoxins posing health hazards are the *ochratoxins* and *trichothecenes*.

11.3.1.1 Ergot alkaloids

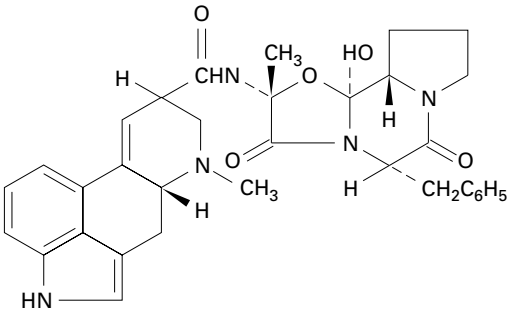
Recurrent poisonings by ergot alkaloids (ergotism) in the past resulted from the consumption of *Claviceps purpurea*-infected rye as bread. All ergot alkaloids are derivatives of lysergic acid, with ergonovine (ergometrine) and ergotamine as the most important ones.



Lysergic acid



Ergonovine



Ergotamine

Ergotism can manifest itself in two ways: a gangrenous type and a convulsive type. The first syndrome is characterized by intense tingling of and hot and cold sensations in the limbs, followed by progression to gangrene and mummification of the extremities. Gangrenous ergotism is largely due to long and intense peripheral vasoconstriction. Ergot alkaloids are partial α-adrenergic agonists. They promote vasoconstriction.

The convulsive syndrome includes central nervous system symptoms such as vomiting, headache, numbness, muscle spasm, and convulsions. Ergonovine has been reported to increase uterine motility, which may cause abortion.

Epidemic ergotism has almost been eliminated. In 1977 and 1978, cases were reported in Ethiopia. In rye, low levels of contamination with ergot alkaloids may still occur.

11.3.1.2 Aflatoxins

Aflatoxins are highly substituted coumarin derivatives that contain a fused dihydrofuran moiety. They are divided into two major groups: the B-group (with a cyclopentanone ring) and the G-group (with a lactone ring), based on blue and green fluorescence (Figure 11.5).

Foodstuffs most likely to become contaminated by aflatoxins are peanuts, various other nuts, cottonseed, corn, and figs. Human exposure can also occur from intake of aflatoxins from tissues and milk (in particular aflatoxin M1, a metabolite of aflatoxin B1) from animals that have eaten contaminated feeds.

The group of aflatoxins includes hepatotoxicants and carcinogens. Hepatotoxicity seen in experimental animals is characterized by bile duct epithelium proliferation, fatty infiltration, and centrilobular necrosis. Aflatoxin B1 is highly hepatotoxic and one of the most potent hepatocarcinogens in rats. In many cell systems, it has also been demonstrated to be a mutagen. The hepatotoxicity as well as the mutagenicity and carcinogenicity are believed to depend on its activation by cytochrome P-450 to the 2,3-epoxide. This potent electrophile can covalently bind to proteins and form adducts with DNA.

Epidemiological data indicate that there is a difference in risk of liver cancer between populations in Asia and Africa on the one hand and in North America on the other. Recent studies on the occurrence of hepatitis B virus suggest that chronic infection may contribute to a higher incidence of liver cancer in aflatoxin-exposed populations. In experimental animals, aflatoxin B1 has been shown to suppress cell-mediated immunity.

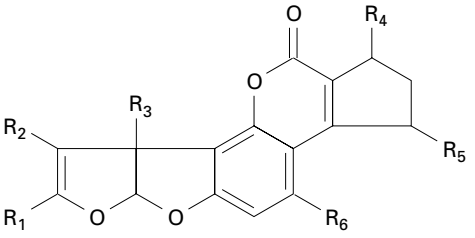
11.3.1.3 Ochratoxins

Ochratoxins are a group of seven dihydroisocoumarin derivatives. The isocoumarin moiety is linked to phenylalanine by an amide bond. Further, some of the ochratoxins distinguish themselves from other mycotoxins by possessing a chlorine atom (Figure 11.6). Ochratoxins have been identified in grains, soybeans, peanuts, and cheese.

Epidemiological studies on the cause of nephropathy in several areas of Yugoslavia, Rumania, and Bulgaria in the late 1950s presented evidence implicating ochratoxin A, present in foodstuffs infected by *Aspergillus ochraceus* and a number of other *Aspergillus* and *Penicillium* species. A similar nephropathy was observed in swine in Denmark and the US. Symptoms include necrosis, fibrosis, and decreased glomerular filtration. In cattle, the ochratoxins undergo degradation by ruminal microorganisms. In addition to nephropathy, ochratoxins have been reported to induce teratogenic effects, renal adenomas, and hepatomas in mice. Tests for mutagenicity, however, gave negative results.

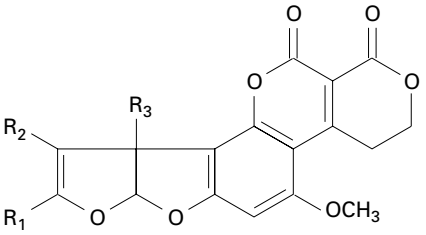
11.3.1.4 Trichothecenes

The trichothecene mycotoxins constitute a group of more than 80 sesquiterpenes, derivatives of 12,13-epoxytrichothecene. In the first half of this century, outbreaks of a mycotoxicosis associated with the consumption of contaminated food were reported in Russia. The disease, called alimentary toxic aleukia (ATA), caused atrophy of bone marrow, agranulocytosis, necrotic angina, sepsis, and death. Later, it was related to the infection of grains



Aflatoxin B1
and its derivatives

Aflatoxin	R_1	R_2	R_3	R_4	R_5	R_6
B1	H	H	H	= O	H	OCH_3
B2	H_2	H_2	H	= O	H	OCH_3
B2a	HOH	H_2	H	= O	H	OCH_3
M1	H	H	OH	= O	H	OCH_3
M2	H_2	H_2	OH	= O	H	OCH_3
P1	H	H	H	= O	H	OH
Q1	H	H	H	= O	OH	OCH_3
R_0	H	H	H	OH	H	OCH_3

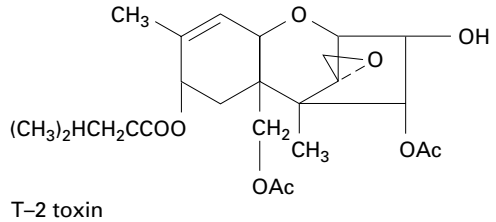


Aflatoxin G1
and its derivatives

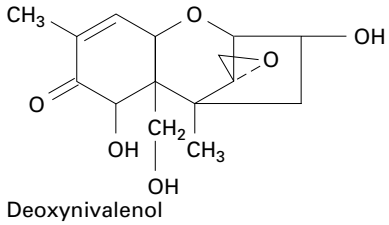
Aflatoxin	R_1	R_2	R_3
G1	H	H	H
G2	H_2	H_2	H
G2a	OH	H_2	H
GM1	H	H	OH

Figure 11.5 Structures of aflatoxins.

with *Fusarium* species. The most important source of trichothecenes is the fungus genus *Fusarium*. The main contaminants of grains are T-2 toxin and 4-deoxynivalenol (vomitoxin).

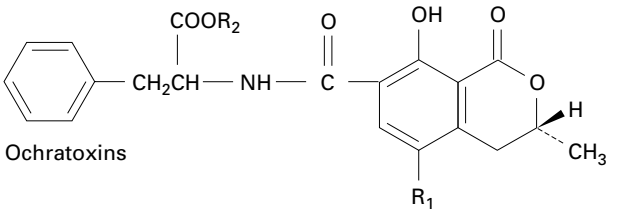


T–2 toxin



Deoxynivalenol

There are two forms of trichothecene-caused toxicosis: an acute form, characterized by neurological signs, and a chronic form, characterized by signs of dermanecrosis, leukope-



Ochratoxins

Ochratoxin	R_1	R_2
A	Cl	H
B	H	H
C	Cl	C_2H_5
methylester A	Cl	CH_3
methyl or ethylester B	H	CH_3 or C_2H_5

Figure 11.6 Structures of ochratoxins.

nia, and gastrointestinal inflammation, and hemorrhages.

Many toxic effects of trichothecenes are believed to originate from inhibition of protein synthesis. Trichothecenes are generally recognized as the most potent inhibitors of protein synthesis in eukaryotic cells. The inhibition can take place at the initiation, elongation as well as termination phases.

In animals, the trichothecenes are rapidly metabolized to nontoxic compounds. They undergo deacetylation, hydroxylation, and glucuronidation in the liver and kidneys. This detoxication mechanism may contribute to the reduction of the risks in humans from dietary intake of trichothecenes.

11.3.2 Toxins originating from marine algae or plankton

Only a few of the large number of marine organisms capable of producing toxins are involved in food poisoning. Poisonings following the ingestion of toxins produced by algae or plankton form significant public health problems in seafood consumption.

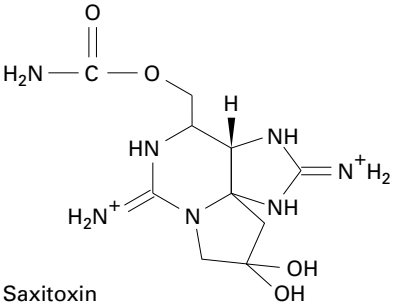
Here, two types of marine illnesses will be discussed: shellfish poisoning, a disease resulting from the consumption of shellfish that have ingested toxic algae, and ciguatera poisoning. The latter is caused by ingestion of contaminated fish, in which the toxin has accumulated via a food chain. The alga involved is consumed by a small herbivorous fish. Larger fish feeding on the smaller fish, concentrate the toxin further in the chain. *Shellfish poisoning* manifests itself in two forms: paralytic shellfish poisoning and diarrheic shellfish poisoning.

Paralytic shellfish poisoning is a neurological syndrome. It is characterized by a sequence of events. Within a few minutes after consumption, signs such as numbness of the lips, tongue, and fingers manifest themselves. After extension of the numbness to the limbs, this is followed by muscular incoordination, paralysis, and death.

Paralytic shellfish poisoning is caused by a mixture of several toxins variously termed *paralytic shellfish poison* (PSP) and saxitoxin (in fact, a component of the mixture).

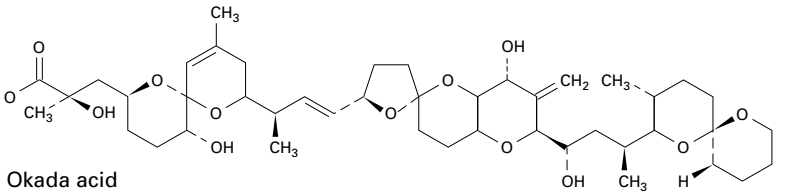
PSP is produced by toxic species of the dinoflagellate genus *Gonyaulax*. Bivalve shellfish (clams and mussels) concentrate the toxin ingested with these organisms. The shellfish are toxic during seasons of algae bloom (so-called "red tide"), i.e., when the concentration of algae is high. Mussels pose the greatest hazard. Paralytic shellfish poisoning is believed

to be due to interference of the toxins with ion transport. Saxitoxin is known to block sodium conductance.



Saxitoxin

Diarrheic shellfish poisoning is characterized by gastrointestinal complaints, including diarrhea, vomiting, nausea, and abdominal spasms. Recently, toxins involved in this poisoning have been chemically identified. They constitute a group of derivatives of a C_{38} fatty acid, okada acid.



Okada acid

These diarrheic shellfish poisons (DSP) are produced by the dinoflagellate species *Dinophysis* and *Prorocentrum*.

Ciguatera fish poisoning results from the consumption of nondirect plankton feeders. Fish species constituting a food chain concentrate the ciguatera toxins. The fish acquire the toxins by ingestion of the photosynthetic dinoflagellate *Gambierdiscus toxicus*. This kind of intoxication is found in the South Pacific and the Caribbean.

The various ciguatera toxins do not all contribute to the poisoning to the same extent. The main cause is ciguatoxin. Symptoms of ciguatera poisoning are paresthesia in lips, fingers and toes, vomiting, nausea, abdominal pain, diarrhea, bradycardia, muscular weakness, and joint pain. The mechanism underlying these symptoms may be based on the neuroactivity of ciguatoxin. It increases sodium permeability, leading to depolarization of nerves.

11.3.3 Bacterial toxins

Food contaminated by bacteria forms a major source of human disease.
A distinction can be made between food-borne infections, caused by the pathogenic bacteria themselves, and food intoxications, resulting from toxin production by the bacteria. Here, two examples of the latter type of bacterial disease will be discussed.

A major concern of the food industry is contamination of food with *Clostridium botulinum*, not primarily because of a high incidence, but because of the extreme toxicity of the enterotoxin produced by this bacterium. Botulinum toxin is generally regarded as the most acutely toxic chemical known. The toxic syndrome it causes is known as botulism. Cases of botulism have mainly been associated with the consumption of inadequately processed home-canned meat and vegetables.

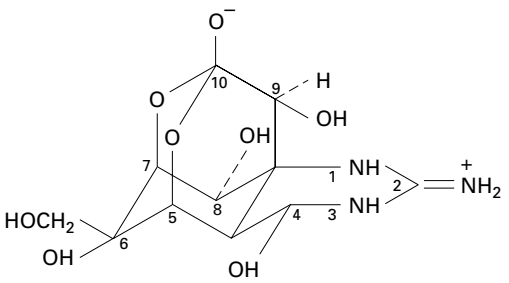
The bacterium in question has received much attention with respect to food-borne bacterial illnesses. However, there is increasing evidence that other bacteria are also important. For a long time a well-known type of fish poisoning, tetrodotoxin intoxication, was attributed to the production of the toxin by the fish itself, the pufferfish. Recently, however, it has been found that tetrodotoxin is formed by bacteria (*Schewanella putrefaciens*) in the intestines of the pufferfish.

Botulinum toxins consist of at least seven immunologically distinct types labeled A through G. All toxins are similar heat-labile proteins, varying in molecular mass from 128,000 (type F) to 170,000 (type B). Types A, B, and E are commonly associated with human botulism. *Botulinum* toxin is highly potent, with a mouse-LD_{50} of 2 ng/kg i.p. Signs and symptoms usually appear 12 to 36 hr after ingestion of the toxin. Initial symptoms include nausea, vomiting, and diarrhea. These are followed within 3 days by predominantly neurologic symptoms such as headache, dizziness, double vision, weakness of facial muscles, and difficulty with speech and swallowing. Progression of the toxicity leads to paralysis of the respiratory muscles and diaphragm, resulting in failure of respiration and death, usually in 3 to 10 days.

Botulinum toxins produce their toxic effects by blocking the release of acetylcholine at the endings of cholinergic nerves. They bind irreversibly to the neuromuscular junction and impair presynaptic release of the neurotransmitter. The toxic part of all types of botulium toxins consists of two subunits: one smaller (molecular mass 50,000, L) and the other larger (molecular mass 100,000, H). The subunits are linked by a disulfide bridge. The neurotoxicity results from the complementary action of the two subunits. The H subunit binds to receptors at the nerve endings, enabling the L subunit to interfere with the release of acetylcholine.

Treatment involves gut decontamination as well as the use of antitoxins. Gastric lavage, emesis, and activated charcoal may be used if consumption is recent. Neutralization of the toxins and prevention of progression of the toxicity may be achieved by administration of antitoxins.

Pufferfish poisoning is characterized by tingling of the lips and vomiting, followed by paralysis of the chest muscles and death. The toxin, *tetrodotoxin*, is extremely toxic. Toxic consumptions result in a mortality of about 60%.



Tetrodotoxin

The neuroactivity of tetrodotoxin is comparable to that of saxitoxin (see Section 11.3.2). However, it is more prolonged. Tetrodotoxin also disrupts sodium conductance. At the pH of the extracellular fluid, the guanidine group of tetrodotoxin is ionized. It is known that free guanidinim ions can compete with sodium ions for common receptors on the sodium channels of the nerve membranes.

11.4 *Antinutrional plant proteins*

Main groups of these proteins are protease inhibitors and lectins. The first group is constituted by substances that interfere with the digestion of proteins. Lectins may disturb the absorption of nutrients.

Protease inhibitors are proteins that inhibit proteolytic enzymes. They occur mainly in plants. Well-known are the trypsin inhibitors in legumes (e.g., soybeans), vegetables (e.g., alfalfa), cereals, and potatoes. Protease inhibitors have been reported to inhibit the growth of rats, chickens, and other monogastric animals. Apart from that, an important finding is enlargement of the pancreas in rats, chickens, mice, and young guinea pigs following the administration of trypsin inhibitors in feeding experiments. This effect is attributed to an increase in trypsin synthesis by the pancreas in response to enzyme inhibition. In the pancreas of rats fed on diets rich in trypsin inhibitors during long periods of time, nodular hyperplasia and adenomas have been observed. These symptoms did not show themselves in pigs, dogs, calves, and Cebus monkeys. Mechanistic studies revealed that the so-called Kunitz trypsin inhibitor forms stable one-to-one complexes with the protease. The inhibitor binds to the acitve site of the protein substrate, followed by hydrolysis of a peptide bond between two amino acids, viz. arginine and isoleucine. A disulfide bridge prevents dissociation of the inhibitor. As a result, it remains bound to the enzyme.

Lectins are proteins of plant origin. They especially occur in legumes such as peanuts, soybeans, kidney beans, and peas. Initially, they were called hemagglutinins, as they can agglutinate the red blood cells. After the finding that lectins can bind to specific receptors on a variety of cells including epithelial cells lining the small intestine and lymphocytes, the name lectins has found general acceptance.

Bean lectins have been shown to affect the morphology of the small intestine (proliferation of the intestinal epithelium, Figure 11.7), to inhibit absorption of nutrients and to disturb the immune function of the gut. These effects are attributed to a sequence of events: binding to receptors on epithelial cells of the small intestine, uptake by the cells through endocytosis, and stimulation of the protein synthesis. Some members of this group of proteins have been structurally identified. The lectin concanavalin A, occurring in jack beans, was found to be a lipoprotein, the protein part being composed of 4 identical polypeptides of 237 amino acids. Each subunit appeared to contain binding sites for Ca, Mn, and sugar moieties.

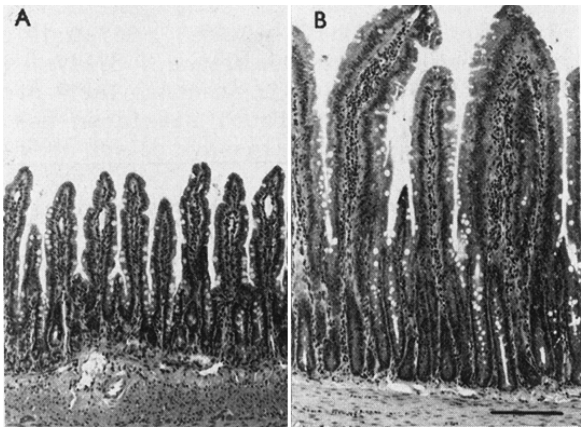


Figure 11.7 Section through jejunum from rats fed on a control (A) or a soybean lectin-containing diet (B). Source: Huisman et al., 1989.

Apart from inhibition of absorption, lectins can also be toxic. A highly toxic example is ricin, present in castor beans. Many poisonings leading to death have been reported, such as children dying after ingestion of raw castor beans. Feeding insufficiently heated raw material proved to be fatal for animals. Ricin consists of two polypeptide chains. One chain is used for binding to the cell, the other inactivates the ribosomal subunits involved in protein synthesis (after uptake through endocytosis). Inhibition of the protein synthesis results in the disappearance of essential enzymes, and ultimately in death.

11.5 Summary

This chapter described a selection of toxic food components of natural origin and their main effects in man and animals. With the exception of the toxic plant proteins, lectins, and protease inhibitors, all are secondary metabolites produced by higher plants, fungi, algae, or bacteria. As a threat to health, the toxins produced by marine algae are most important, followed by the mycotoxins. There is a need for more research in these fields.

Reference and reading list

Cheeke, P.R. and L.R. Shull, *Natural Toxicants in Feeds and Poisonous Plants.* Westport Conn., AVI Publ. Cy., 1985.

Cheeke, P.R., (Ed.), *Toxicants of Plant Origin,* 4 volumes. Boca Raton, Florida, CRC Press Inc., 1989.

Harborne, J.B., *Introduction to Ecological Biochemistry,* 3rd ed. Academic Press, 1988.

Hardegree, M.C. and A.T. Tu, (Eds.), Bacterial toxins, Vol. 4 in: Handbook of natural toxins. New York, Marcel Dekker Inc., 1988.

Hauschild, A.H.W., *Clostridium Botulinum,* in: *Foodborne Bacterial Pathogens,* M.P. Doyle (Ed.). New York, Marcel Dekker, 1989.

Huisman, J., T.F.B. van der Poel and I.E., Liener, *Recent Advances of Research in Antinutritional Factors in Legume Seeds.* Wageningen, Pudoc, 1989.

Keeler, R.F. and A.T. Tu, (Eds.), Plant and fungal toxins, Vol. 1 in: Handbook of natur*al toxins*. New York, Marcel Dekker Inc., 1983.

Keeler, R.F. and A.T. Tu, (Eds.), Toxicology of plant and fungal compounds, Vol. 6 in: *Handbook of natural toxins.* New York, Marcel Dekker Inc., 1991.

Moss, J. and A.T. Tu, (Eds.), Bacterial toxins and virulence factors in disease, Vol. 8 in: *Handbook of natural toxins*. New York, Marcel Dekker Inc., 1995.

Rechcigl, M., (Ed.), *Handbook of Naturally Occurring Food Toxicants.* Boca Raton, Florida, CRC Press Inc., 1983.

Smith, L.D.S. and H. Sugiyama, *Botulism: The Organism, its Toxins, the Disease,* 2nd ed. Springfield, IL, Charles C. Thomas, 1988.

Sugiyama, H. and J.N. Sofos, Botulism, in: *Developments in Food Microbiology* 4, R.K. Robinson (Ed.). London, Elsevier Applied Science, 1988.

Tu, A.T., (Ed.), Marine toxins and venoms, Vol. 3 in: *Handbook of natural toxins*. New York, Marcel Dekker Inc., 1988.

Tu, A.T., (Ed.), Food poisoning, Vol. 7 in: Handbook of natural to*xins*. New York, Marcel Dekker Inc., 1992.