1 Introduction

Foodborne illness mediated by natural toxins of microbial, plant or animal sources is an important global public health problem. Foodborne illness has been defined by the World Health Organization (WHO) as ‘a disease of infectious or toxic nature caused by, or thought to be caused by, the consumption of food or water’ (WHO, 1997). Globally, the WHO has estimated that approximately 1.5 billion cases of foodborne illness and more than 3 million deaths occur in children under 5 years of age, and a significant proportion of these result from consumption of food contaminated with pathogenic microorganisms or natural intoxicants (WHO, 1997). These estimates of foodborne illnesses are probably 100–300 times less than the actual occurrence (Bryan et al., 1997; Lund et al., 2000). The annual incidence of foodborne illness in industrialized countries has been estimated to affect 5–10% of the population annually, and in many developing countries the incidence is probably considerably higher due to underlying morbidity, unsanitary conditions, inadequate food processing and handling, and a variety of other reasons (see Johnson, 2003).

Acute foodborne illnesses and intoxications are commonly classified into two main categories: (1) infections of the gastrointestinal tract by microbial pathogens, and (2) poisonings or intoxications resulting from consumption of preformed toxins or toxin precursors in foods. This classification, however, is overly simplistic, and does not take into account related aspects including chronic disease syndromes that

1 deceased
can develop following acute foodborne infections, and other factors inherent in the nature and chemical complexity of foods. In natural intoxications, consumption of the toxin alone or its subsequent metabolism is responsible for the illness. Although believed to be of diminished importance compared to gastrointestinal infections, exposure to oral toxins also has a large impact on the health and mortality of humans and animals (CAST, 1994; WHO, 1997; Dabrowski and Sikorski, 2005).

In the US and in many other parts of the world, the large majority of foodborne illnesses are caused by viral and bacterial infections (Bean and Griffin, 1990; Bean et al., 1996; Mead et al., 1999; CDC, 2000; Taylor and Hefle, 2002), while only a relatively small subset (5–10 %) is documented to result from the consumption of natural intoxicants. This relatively low frequency of chemical intoxications compared to pathogens reflects in part the greater emphasis and resources that have been allocated to the study of the epidemiology and virulence of microbial pathogens. Although foodborne illnesses caused by natural intoxicants are an important public health problem, particularly in developing and poorer countries, this area of research has not received the attention given to foodborne diseases caused by microbial pathogens. This is probably due to the relatively low incidence of intoxications in industrialized countries and consequently limited resources for such research, as well as to the difficulties in studying foodborne intoxications by chemicals. In most cases of foodborne disease caused by pathogens, the causative microbial agents can be cultured from foods on artificial media, thus enabling investigation by classic means such as proof of Koch’s postulates (see, for example, Johnson, 2003). In contrast, intoxications involve non-replicating chemical substances in the diet, and their detection often requires sensitive and sophisticated chemical methods. The involvement of natural intoxicants in dietary disease can be very difficult to demonstrate. Certain chemical intoxications also result from metabolic transformation in the host. Most food intoxications from natural compounds occur in poorer countries, and resources may not be available for adequate surveillance and epidemiological studies. Toxins from fish, shellfish and plants are the most common cause of chemical intoxications. Poisoning by natural intoxicants has also taken on a new dimension with the increasing cultivation of transgenic plants and other genetically engineered foods (Engeseth, 2001; Stewart, 2003; Toke, 2004), and the potential for biowarfare using plant toxins such as ricin (Khan et al., 2001; Franz and Zajtchuk, 2002).

This chapter focuses on intoxications with an emphasis on naturally occurring organic toxins mainly of plant, algal, microbial and animal origins. Several treatises have extensively covered intoxications caused by inorganic compounds such as lead and mercury, man-made industrial chemicals, and pollutants including PCBs, food additives, herbicides and many other classes of compounds (Hayes, 2001; Klaassen, 2001; Kotsonis et al., 2001; D’Mello, 2003), and these are not covered here. This chapter also does not include allergic responses, food intolerance, metabolic food reactions that occur due to excess intake and metabolism, and methods for analysis of food intoxicants. Excellent reviews of these latter topics are available elsewhere (FDA, 1995; Downes and Ito, 2001; Hui et al., 2001a, 2001b; Kotsonis et al., 2001; Taylor and Hefle, 2002).
1.1 Background and historical aspects of natural foodborne intoxications

Foodborne illnesses resulting from consumption of natural toxicants are distinct from microbial infections in several ways (Concon, 1988; Kotsonis et al., 2001). Naturally occurring toxicants are products of the biosynthesis and metabolism of plants, algae, animals and microorganisms (National Academy of Sciences, 1973; Watson, 1998; Coulombe, 2000; Park et al., 2000; Hui et al., 2001a, 2001b; Dabrowski and Sikorski, 2005). Certain intoxicants are present in these biological materials constitutively, while others are formed in response to infections in plants or other metabolic processes. Improper food production practices and processing can affect the levels of toxicants (Rahman, 1999). Natural intoxicants that have been documented to cause food poisoning are produced by various species of bacteria, fungi, plants, insects and animals (Lund et al., 2000; Klaassen, 2001; Reddy and Hayes, 2001). Unlike pathogens as etiologic agents, natural toxins are not able to reproduce in foods or in the gastrointestinal tract of humans and animals. In certain cases, the formation of toxicants such as nitrite can be mediated by microbial transformation of precursor molecules in foods or in the gut. In this chapter, intoxications due to natural toxicants are considered to be distinct from allergic and anaphylactic responses mediated by food substances. The toxic response is not immune-mediated, although its toxic mechanism may involve the release of chemical mediators. As defined by Kotsonis et al. (2001), food toxicity (poisoning) is ‘A term used to imply an adverse effect caused by the direct action of a food or food additive on the host recipient without the involvement of immune mechanisms.’

Through the centuries, humans probably learned to avoid consuming natural products that caused adverse reactions, and there is a rich historical record regarding human awareness and avoidance of specific foods (McNeil, 1976; Concon, 1988; Borzelleca, 2001). The ancient recognition of poisons in foods has been recorded in writings and illustrations from Egyptian, Chinese, Hindu, Roman, Arab, Greek and other civilizations (Borzelleca, 2001). Recognition of associations between food and chemical intoxications came about long before an understanding of toxicology, and some of the seminal events have been traced through history into the modern era (McNeil, 1976; Borzelleca, 2001).

The recognition and observations of poisons and poisoners was followed by eras of experimental, mechanistic and analytical toxicology (Borzelleca, 2001). Chemists and pharmacologists demonstrated that compounds in plants, insects, animals and macroscopic fungi could be poisonous when ingested. Knowledge of the microbial causes of foodborne disease began when Pasteur and Koch founded the science of microbiology, allowing microbiologists to isolate, characterize and systematically describe microorganisms associated with spoiled or poisonous foods (Brock, 1961; Tannahill, 1973; McNeil, 1976). In contrast, understanding of food poisoning by natural intoxicants came about through causal associations between consumption and illness, and from advances in analytical methods to identify the compounds and in pharmacology to understand the basis for their poisonous action in animal models. The age of safety, evaluation, quantification and prognostication followed (Borzelleca, 2001).
Landmark legislation occurred in the US with the 1906 Pure Food and Drugs Act and its successor, the 1938 Federal Food, Drug, and Cosmetic Act, to provide a safe and wholesome food supply (Hutt and Hutt, 1984; Middlekauf and Shubik, 1989; Miller and Taylor, 1989). Assurance of the safety and wholesomeness of foods is an important discipline fulfilled by legislators, industry and researchers. The US Food and Drug Administration published the *Redbooks I* and *II*, and the Organization for Economic Cooperation and Development (OECD) issued similar information, providing guidelines for sound science and data resources for determining safe exposure limits for consumers. The WHO Joint Expert Committee on Food Additives (JECFA) and other international organizations have applied sound toxicological thinking for establishing the safety of food chemicals and toxicants. The fields of surveillance and epidemiology have demonstrated the enormous impact that foodborne disease has on morbidity, mortality and economic losses throughout the world. In developing countries, foodborne disease is among the leading causes of morbidity and mortality in humans and animals, and has been a leading factor impeding technological progress (Miller and Taylor, 1989).

Food products can become contaminated by toxicants through a variety of means. Fish and shellfish can absorb toxins through feeding on toxic algae or bacteria. Certain other fish poisonings result from bacterial growth under non-refrigerated storage conditions. Some plants inherently produce toxins to high levels, while others form toxicants in response to microbial infections. Toxins can also accumulate during harvesting and processing of food commodities, such as by the mixing of toxic plants with edible crops or the formation of toxicants during processing. Paradoxically, a major source of food toxicants has resulted from advances in food technology and agricultural practices (Richards and Hefle, 2003). For example, enhanced agricultural productivity through the use of fertilizers, insecticides, pesticides, microbiocides, growth stimulants and antibiotics can sometimes lead to toxic levels of contaminants in a food through alterations in specific as well as global ecological systems. In the classic book *Silent Spring*, Rachel Carson lamented the presumed excessive use of pesticides with disregard for the ecological system, and its potential effect on global vitality.

Despite the importance of chemical intoxications, the study of foodborne illnesses has centered on viral, bacterial and fungal pathogens. Natural poisons and hazards in foods have focused mainly on pathogenic microorganisms and fungal toxins (National Research Council, 1985). Currently, the CDC limits chemical etiologies to certain seafood toxins, and most other countries also do not have thorough surveillance and preventive programs for natural toxicants. Persons suffering from chemical intoxications are generally referred to ‘poison centers’ (Spoerke, 2001a, 2001b), and systematic reporting programs for foodborne illness caused by most natural intoxicants have not been adequately implemented (National Research Council, 1985). The International Programme on Chemical Safety (IPCS) was established in 1980 to implement activities related to chemical safety (see www.who.int/ipcs/en/). This has established a world directory of poison centers (YellowTox). Although the IPCS collects and maintains data regarding some natural intoxicants, its emphasis has mainly been on inorganic minerals and synthetic chemicals.
1.2 Major principles of acute toxicology

What are natural toxins or poisons? According to Borzelleca (2001):

‘A poison is any substance (chemical, physical or biological) that is harmful or destructive to a biological (living) system. A poison derived from a natural source is a toxin, and the study of toxins is toxicology.’

It is a well known paradigm that all chemicals can be toxic depending on the dose:

‘What is there that is not a poison?
All things are poison and nothing [is] without poison. Solely the dose determines the thing that is a poison.’

(Paracelsus, 1493–1541, cited in Klaassen, 2001.)

Although this paradigm is fundamentally true, it is impractical to consider natural food toxicants in this manner (Johnson and Pariza, 1989; Taylor and Hefle, 2002). The vast majority of components in foods are either present in too low a concentration or have a very low intrinsic toxicity and do not present a hazard under normal conditions of food production and consumption (Kotsonis et al., 2001; Taylor and Hefle, 2002). The hazard to human health from natural toxicants results from their potency and capacity to cause illness on exposure. The potency of a toxin is often expressed as the median lethal dose (LD₅₀), a concept introduced by Trevan for the standardization of digitalis extracts, insulin, and diphtheria toxin (Trevan, 1927; DiPasquale and Hayes, 2001). The LD₅₀ represents the dose that causes a toxic response (i.e. lethality) in 50 % of a population of test animals in a designated period of time (Johnson and Pariza, 1989; Hayes, 2001; Klaassen, 2001). The LD₅₀ is chosen to quantify virulence or toxicity because of the nature of the dose–response relationship (Concon, 1988; Johnson and Pariza, 1989). In a typical sigmoid lethality curve, the rate of change in mortality (slope of the curve) as a function of dose reaches a maximum at the point of about 50 % survival. Curves with steeper slopes give a more accurate estimate of toxin concentration or infectious dose. The sigmoid shape of the LD₅₀ curve results primarily from the chance distributions of lethal events in any given animal (Concon, 1988; Johnson and Pariza, 1989). Various factors influence the LD₅₀ curve, including the type, strain, health and heterogeneity of the animal population; the route of administration; and the rate of metabolic detoxification. For these reasons, the LD₅₀ determination is usually combined with other methods for determining the level of natural intoxicant required for illness in experimental animals. Furthermore, LD₅₀ determines acute toxicity, and some compounds with low acute toxicity may have carcinogenic or teratogenic effects at doses that do not produce evidence of acute toxicity (Eaton and Klaassen, 2001). Compounds also may be toxic only when in combination with other substances, or may manifest toxicity on repeated exposure. Nonetheless, the dose–response curve using animal models is an essential and central tool for evaluating toxicity.

Natural toxins and poisons show tremendous variation in LD₅₀ (see examples in Table 17.1). For a large population the lethal responses to a given dose of a
A compound may follow a normal distribution pattern (Gaussian), which presents difficulty in predicting the response of a given individual in the population because the response may be unique and fall at any place on the normal distribution curve, or outside it (Concon, 1988). Related to the LD50 are the No Effect Dose (NED), analogous to the No Observable Adverse Effective Level (NOAEL) and No Effective Level (NEL). For toxicants, various compounds can share the same LD50 but have substantially different NED values (Concon, 1988). For many compounds, the determination of the LD50 and NED is difficult owing to several factors, such as the time for the toxic response to occur, the variations among test animals and between species, interaction with other compounds, metabolism, the intestinal barrier and other factors (Concon, 1988).

An interesting aspect of toxicity requirements is the number of molecules needed to cause intoxication. It was proposed that toxic biological activity cannot occur for any single substance in a single cell below 10,000 molecules (Hutchinson, 1964; Dinman, 1972). However, many target-specific substances may show a lower threshold number in terms of total body cells. For example, when considered in terms of total body cells, it has been estimated that 2 µg or 8 × 10^{12} molecules of botulinum neurotoxin (molecular mass = 150,000 Da) is sufficient to produce lethality in an adult human (Lamanna, 1959). In reference to total body cells, this toxic dose is less

### Table 17.1 Potencies of selected natural toxins

<table>
<thead>
<tr>
<th>Estimated minimum lethal dose (µg/kg)</th>
<th>Toxin</th>
<th>Source or nature</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00003</td>
<td>Botulinum neurotoxin type A</td>
<td>Bacterium: Clostridium botulinum</td>
</tr>
<tr>
<td>0.00010</td>
<td>Tetanus neurotoxin</td>
<td>Bacterium: Clostridium tetani</td>
</tr>
<tr>
<td>0.020</td>
<td>Ricin</td>
<td>Plant: castor bean, Ricinus communis</td>
</tr>
<tr>
<td>0.15</td>
<td>Palytoxin</td>
<td>Zoanthid: Palythoa spp.</td>
</tr>
<tr>
<td>0.20</td>
<td>Crotalus toxin</td>
<td>Rattlesnake: Crotalus atrox</td>
</tr>
<tr>
<td>0.30</td>
<td>Diphtheria toxin</td>
<td>Bacterium: Corynebacterium diphtheriae</td>
</tr>
<tr>
<td>0.30</td>
<td>Cobra neurotoxin</td>
<td>Snake: Naja naja</td>
</tr>
<tr>
<td>2.7</td>
<td>Kokov venom</td>
<td>Frog: Phyllobates bicolor</td>
</tr>
<tr>
<td>8</td>
<td>Tarichatoxin</td>
<td>Newt: Taricha torosa</td>
</tr>
<tr>
<td>8</td>
<td>Tetrodotoxin</td>
<td>Fish: Sphoeroides rubripes</td>
</tr>
<tr>
<td>3.4-9</td>
<td>Saxitoxin</td>
<td>Dinoflagellate: Gonyaulax catenella</td>
</tr>
<tr>
<td>390</td>
<td>Bufotoxin</td>
<td>Toad: Bufo vulgaris</td>
</tr>
<tr>
<td>500</td>
<td>Curare</td>
<td>Plant: Chondophyton tomentosum</td>
</tr>
<tr>
<td>500</td>
<td>Strychnine</td>
<td>Plant: Strychnus nux-vomica</td>
</tr>
<tr>
<td>1200</td>
<td>Muscarin</td>
<td>Mushroom: Amanita muscarina</td>
</tr>
<tr>
<td>1500</td>
<td>Samandarin</td>
<td>Salamander: Salamandra maculosa</td>
</tr>
<tr>
<td>3000</td>
<td>Disopropylfluorophosphat</td>
<td>Synthetic nerve gas</td>
</tr>
<tr>
<td>10,000</td>
<td>Sodium cyanide</td>
<td>Inorganic poison</td>
</tr>
</tbody>
</table>

From: Mosher et al., 1964.

Minimal lethal dose refers to mouse except in the cases of ricin, where it refers to guinea pig, and bufotoxin and muscarin, where it refers to cat. In cat, administration was intravenous; in all other cases it was intraperitoneal. Since the survival times are variable and the experiments are not direct comparisons, these values are approximate and indicative only of relative toxicity by the designated route of administration.
than the 10 000-molecule threshold, but is exceeded when considered in terms of the
total number of motor neurons. Thus, the 10 000-molecule threshold appears reason-
able even for the most poisonous substance known.

The toxicity of natural poisons is also related to the frequency of exposure, since some
compounds are more effective toxicants with repeated exposures. Repeated exposures
may also have cumulative effects. The route of exposure is also an important variable,
since (with few exceptions) compounds are least toxic by the oral route (Concon, 1988).
Exceptions are those compounds that are activated during passage through the intestinal
tract (Concon, 1988). Dietary factors and endogenous resistance, including detoxifying
gut bacteria, will influence toxicity. Lastly, most toxicants show different binding
affinities to plasma proteins (Concon, 1988), and their displacement from plasma pro-
teins by compounds of greater affinity will increase the potency and effective dose
(Concon, 1988). Other physiologic mechanisms of poisoning (such as organ specificity,
 systemic and organ detoxification, stability and clearance) are important, but are
beyond the scope of this chapter; there are excellent treatises on this (see, for example,
Kotsonis et al., 2001).

1.3 Recognition, surveillance and epidemiology of foodborne
intoxications

The recognition of a toxin as an etiologic agent of foodborne disease usually results
first from associative epidemiological evidence, in which the occurrence of an illness in
a human epidemic is examined and found to correlate with the consumption of a food
(Evans and Brachman, 1992). The epidemiological association is ideally established by
demonstration of suspected toxins from clinical samples and the causative food. In the
case of miscellaneous chemical intoxications, metabolic reactions occurring in a food
or by microorganisms may transform the naturally occurring form of the chemical to
the toxicant. In practice, foodborne disease outbreaks caused by natural intoxicants are
diagnosed by first examining the onset time of illness and the symptoms, and then iso-
lating the toxin from the food and clinical samples (e.g. vomitus, feces, blood, organs) of
the victim(s). The successful epidemiological investigation coupled with the etiological
diagnosis can also help to facilitate both short-term and long-term control measures.

2 Bacterial toxins

2.1 Bongkreic toxin and toxoflavin

Bongkreic (BK) toxin can be produced during certain food fermentations, particu-
larly during production of bongkrek, an Indonesian food produced by fermentation
with the fungus *Rhizopus oligosporus* of coconut presscake or coconut milk wrapped
in banana leaves. In certain fermentations the bacterium *Burkholderia cocovenenans*
(formerly *Pseudomonas cocovenenans*) grows and produces two toxins: toxoflavin and
bongkreic acid (Van Veen, 1967; Garcia et al., 1999; Jiao et al., 2003). Consumption
of tempe bongkrek has led to numerous human fatalities (Van Veen, 1967; Concon,
The high mortality of bongrek poisoning led Van Veen and Mertens to conduct extensive research on the nature of the toxicity in the 1920s and 1930s (Van Veen, 1967), and the two toxins, bongkreke acid (BK) and toxoflavin, were found to be produced by the bacterium *B. cocovenenans* during the fermentation. *B. cocovenenans* has been isolated from foods including corn flour, edible fungi and soil (Hu et al., 1984; Jiao et al., 2003). The production of toxin is influenced by pH, fatty acids, and other intrinsic and extrinsic factors (Garcia et al., 1999). The production of BK can be prevented by using oxalis leaves for wrapping the presscake, which promotes a rapid drop in pH and thus prevention of growth and toxin formation by *B. cocovenenans*. BK is heat-stable and survives food processing.

The structures and toxicities of toxoflavin (oral LD₅₀ ~ 8 mg per kg bodyweight) and bongkreke acid have been elucidated (Van Veen, 1967). Toxoflavin has the empirical chemical formula C₇H₇N₅O₂ and contains two six-membered rings, a pyrimidine and a triazine system, each with one methyl group. When presented orally to a 1–2 kg monkey, 1–1.5 mg of bongkreke acid was fatal (Van Veen, 1967). BK shows strong antibiotic activity, particularly against molds and yeasts. BK (C₂₈H₅₀O₇) is a branched, unsaturated tricarboxylic acid. Since BK is much more toxic than toxoflavin, it is probably most responsible for illnesses and fatalities in humans. Following consumption of food containing BK, the onset of symptoms occurs within a few hours; the symptoms include abdominal pains, dizziness, excessive sweating, malaise, and eventually coma and death within 24 hours (Hu et al., 1984). The mechanism of toxicity appears to involve interaction with the electron transport system and a decrease in carbohydrate metabolism (Van Veen, 1967).

Bongrek poisoning and associated illnesses and deaths have also been reported in the People's Republic of China following the consumption of fermented corn flour or deteriorated *Tremella faciformis* (white fungi), with a fatality rate of more than 40% during the 1970s (Meng et al., 1988; Jiao et al., 2003). Production of BK in this fermentation is prevented by keeping the pH above neutral, which discourages the formation of the toxin. The toxin responsible was reported to be BK produced by *Flavobacterium farinofermentans* (Meng et al., 1988). *Flavobacterium farinofermentans* was later shown to be identical to *Pseudomonas cocovenenans* (Jiao et al., 2003). In 1995, *P. cocovenenans* was transferred to the genus *Burkholderia* as *B. cocovenenans* (Jiao et al., 2003).

### 2.2 Bacillus toxins

*Bacillus cereus* has long been known to produce two toxins – a diarrheal protein toxin and an emetic toxin (Granum and Baird-Parker, 2000) – whose structure remained elusive for several years (see Chapter 15). The prototype toxin is now known to comprise a cyclic peptide \[\text{D-O-Leu-D-Ala-L-O-Val-L-Val}_3\] with a molecular mass of 1.2 kDa (Granum and Baird-Parker, 2000). The quantity of cereulide to cause an emetic reaction in monkeys is approximately 12–32 µg per kg, corresponding to \(10^5\text{–}10^8\) cells per gram food (Granum and Baird-Parker, 2000). The emetic toxin has remarkable heat stability, retaining toxicity after treatment for 90 minutes at 121 °C. Cereulide binds to 5-HT3 receptors, and the binding stimulates the vagus afferent...
nerve. In common with many preformed foodborne toxins, the onset of symptoms is rapid (1–5 hours) and the duration of the vomiting illness is relatively short (6–24 hours) (Granum and Baird-Parker, 2000). Subsequent studies have shown that various *Bacillus* spp., including *B. licheniformis*, *B. pumilis*, *B. sphaericus*, *B. brevis* and possibly other species, also produce cereulide or other cyclic toxic peptides (Granum and Baird-Parker, 2000). It is becoming apparent that there is variation in structures depending on the species, and further characterization is required. Conditions influencing cereulide production in foods have been investigated (Granum and Baird-Parker, 2000).

### 2.3 *Clostridium* toxins

The genus *Clostridium* produces more protein toxins than other genera of bacteria (van Heyningen, 1950; Johnson, 1999). Most of the clostridial protein toxins causing foodborne illness are well-known, and include botulinum neurotoxin (see Chapter 13) and *C. perfringens* enterotoxin (see Chapter 4). Many species of *Clostridium* are common flora in the human and animal gastrointestinal tracts (Finegold *et al.*, 2002a). Recent evidence suggests that clostridia may produce toxins that are absorbed in the gastrointestinal tract and cause disease. For example, an association of *Clostridium bolteae* and other *Clostridium* spp. with late-onset autism has been suggested by microbiological and antimicrobial studies (Finegold *et al.*, 2002b). This is a very intriguing hypothesis – that neurotoxic clostridia in the gut could contribute to CNS-related behavioral diseases – but further research to evaluate this hypothesis is required. Nonetheless, these results suggest that relations between gut microbial flora and human disease may be more prevalent than previously realized.

### 2.4 Intoxications caused by bacterial formation of *N*-nitroso compounds (NOCs)

Although there are several dietary sources of NOCs (Henderson and Raskin, 1972; Keating *et al.*, 1973; Fassett, 1973; Bryan, 1982; Kotsonis *et al.*, 2001; Mensinga *et al.*, 2003), poisoning caused by nitrite (nitrite poisoning; methemoglobinemia) is usually related to consumption of foods high in nitrates and their subsequent reduction in the food or by the intestinal microbial flora. NOCs such as nitrosamines and nitrosamides can also be formed during drying or cooking of foods, or by migration from food contact materials (Henderson and Raskin, 1972; Fassett, 1973; Walley and Flanagan, 1987; Kotsonis *et al.*, 2001, Mensinga *et al.*, 2003). The environmental formation of nitrates in foods generally results from reduction of nitrogen precursors, particularly nitrate, and intoxications due to bacterial reduction of nitrate to nitrite have been reported in various foods. Certain plants, such as beets, broccoli, celery, brussels sprouts, corn, carrots, radish, rhubarb, spinach, turnip greens and others, may contain high levels of nitrite, depending on inherent and environmental factors (Keating *et al.*, 1973; Walley and Flanagan, 1987; Kotsonis *et al.*, 2001). Excessive fertilization of crops with nitrogen fertilizers, use of certain insecticides and herbicides, and other cultivation practices can increase plant nitrate levels. Various other
factors also have an impact on the level of nitrate in foods, including which part of the plant is utilized as food; the environmental conditions, such as drought and harvest conditions; and the physiological state of the plant, including nutrient deficiencies and stage of maturity.

Nitrite poisoning has also resulted from \textit{in vivo} bacterial activity in the oral cavity and stomach. Many species of bacteria, including pseudomonads, \textit{Enterobacteriaceae}, staphylococci, clostridia and others, readily reduce nitrate to nitrite, which can result in methemoglobinemia. Storage of foods at cold temperatures will reduce microbial metabolism and diminish the reduction of nitrate to nitrite. Several outbreaks of methemoglobinemia have occurred from inadvertent addition of excess levels of curing salts to meats, fish and cheeses (Fassett, 1973; Kotsonis \textit{et al.}, 2001, Mensinga \textit{et al.}, 2003). The use of well-water containing high nitrate levels has caused methemoglobinemia in home-dialysis patients (Carlson and Shapiro, 1970; Kotsonis \textit{et al.}, 2001). Methemoglobinemia initially manifests as darkened blood and a slate-gray cyanosis, which may occur mainly in the lips and mucous membranes in mild cases. Other symptoms may include nausea, vomiting, headache, shortness of breath and, occasionally, death. The cyanotic patient should receive respiratory support and airway management. In severe cases, adjunctive therapies may be required.

2.5 \textbf{Bacterial endotoxins}

Bacterial endotoxins are heat-stable lipopolysaccharides (LPS) that are associated with the outer membrane of Gram-negative bacteria. In itself, LPS is not considered to be a potent oral toxin. When injected into an animal, endotoxin rapidly causes shock and sepsis, and is often accompanied by severe diarrhea (Danner and Natanson, 1995; Klaassen, 2001). The absorption of endotoxin from the bowel is a major contributor to lethality in hemorrhagic shock. The importance of endotoxin does not appear to be limited to currently recognized infections. Certain Gram-negative bacteria are commonly found in the gut, and those causing enterotoxic lesions could facilitate the entry of endotoxin, leading to increased morbidity or death, depending on the exposure and host defense. Although not usually considered a foodborne oral toxin, endotoxin could be absorbed into circulation through intestinal lesions and cause intoxication to a number of organs (Danner and Natanson, 1995; Klaassen, 2001).

2.6 \textbf{Miscellaneous bacterial toxins}

A number of species of bacteria have occasionally been associated with foodborne disease through the production of toxic compounds. In particular, enterococci, group B streptococci and a variety of species of \textit{Enterobacteriaceae} have been presumptively associated with foodborne illness (Bryan, 1979; Stiles, 2000). Streptococci and enterococci are known to produce a variety of protein toxins; these organisms occur commonly in foods and are also found in the gastric tracts of humans and animals, and it is possible that the toxins cause foodborne intoxications. Organisms transmitted as zoonoses have also been implicated in foodborne diseases. Conclusive evidence
for illness association would ideally involve the solving of Koch’s postulates (see, for example, Bryan, 1979; Stiles, 2000; Johnson, 2003), but there can be limitations to meeting the criteria, and molecular techniques are increasingly being used to identify new toxigenic organisms. Infrequent microbial infections and intoxications are discussed elsewhere in this book (see Chapter 10).

3 Seafood toxins

With the exception of fish and shellfish, most animals do not produce toxins that are known to cause illness on consumption – apart from the formation of prions by certain livestock. On the other hand, many fish and shellfish produce oral toxins that can cause human disease, and these are discussed in this section (reviewed in Halstead, 1967; Ahmed, 1991; Falconer, 1993; Yasumoto and Murata, 1993; Anderson, 2000; Fleming et al., 2001; Llewellyn, 2001; Johnson and Schantz, 2002; Backer et al., 2005).

Finfish and shellfish are nutritious food sources that contribute to a healthy and delicious human diet (Brown et al., 1999; McGinn, 1999). Seafood consumption has increased in many countries during the past two decades. Currently, it is estimated that people throughout the world receive about 6% of their total protein and 16% of their animal protein from fish. Although seafoods have positive nutritious and health attributes, they can also serve as vehicles for a variety of foodborne illnesses (Halstead, 1967; Ahmed, 1991; Falconer, 1993; Yasumoto and Murata, 1993; Lipp and Rose, 1997; Anderson, 2000; Fleming et al., 2001, Llewellyn, 2001; Johnson and Schantz, 2002; Backer et al., 2005). They have been associated with the transmission of bacterial and viral gastroenteritis, and have also supported outbreaks of bacterial intoxications – including botulism and staphylococcal poisoning (see Chapters 13 and 14 of this book, respectively).

Seafoods are intriguing in also transmitting diseases mediated by non-protein, heat-stable, small molecular-weight toxins produced mainly by microalgae and bacteria (Ahmed, 1991; Yasumoto and Murata, 1993; Yasumoto and Murata, 1993; Plumley, 1997; Okada and Niwa, 1998; Llewellyn, 2001; Backer et al., 2005). Seafood toxins cause a variety of illnesses of humans and animals in many areas of the world (Meyer et al., 1928; Sommer and Meyer, 1937; Halstead, 1967; Ahmed, 1991; Falconer, 1993; Anderson, 2000; Fleming et al., 2001; Johnson and Schantz, 2002; Backer et al., 2005). There has also been concern that toxins are formed during intensive aquaculture of fish and shellfish (Jensen and Greenlees, 1997). Several of these illnesses – such as paralytic shellfish poisoning (PSP), puffer fish poisoning (PFP) and neurotoxic shellfish poisoning (NSP) – have been known for centuries, whereas others – such as amnesic shellfish poisoning (ASP), diarrhetic shellfish poisoning (DSP) and azaspiracid shellfish poisoning (AZP) – have been recognized more recently. Scombroid fish poisoning is one of the more common seafood diseases in many parts of the world, and is caused by histamine formed by bacteria in poorly refrigerated fish. Ciguatera is also a relatively common form of seafood poisoning that is transmitted in certain species of fish in tropical waters which are contaminated with ciguatoxin. Toxins produced by Pfiesteria and cyanobacteria are also recognized
waterborne causes of diseases in fish, and could potentially cause human foodborne illnesses. Contaminated shellfish, including mussels, clams, cockles, oysters, scallops and other varieties feeding on toxic microalgae, are a main cause of seafood intoxications. Most toxic finfish and shellfish accumulate the toxins from water, are not visibly spoiled and cannot be distinguished from non-toxic seafoods on harvest. For some toxin-mediated illnesses, a single clam or mussel contains enough poison to kill a human, but without noticeable health or organoleptic effects to the shellfish (Fleming et al., 2001; Johnson and Schantz, 2002; Backer et al., 2005).

Preventive measures mainly rely on sampling algal blooms and foods, quantitative detection of the causative toxins, and warning the seafood industry and consumer (Halstead, 1967; Ahmed, 1991; Price et al., 1991; Falconer, 1993; Smayda and Shimizu, 1993; Anderson, 2000; Fleming et al., 2001, Johnson and Schantz, 2002; Backer et al., 2005). In the US, Canada and certain other countries, governmental monitoring of coastal waters for toxic algae and toxins in seafoods and cautionary warnings have reduced the risk of consumption of poisonous seafood. Algal blooms and associated shellfish contamination have become more common during the past two decades, and it is anticipated that seafood illnesses will correspondingly increase in their incidence. Currently, the Centers for Disease Control and Prevention (CDC) conducts surveillance and reports the incidence of ciguatera, scombroid fish poisoning, and paralytic shellfish poisoning. Other seafood intoxications are prospective risks that may warrant enhanced surveillance and reporting. Mortality for the majority of seafood diseases is generally low, and treatment is mainly supportive (Rosen et al., 1988; CDC, 2001; Fleming et al., 2001; Johnson and Schantz, 2002; Backer et al., 2005).

### 3.1 Overview of the causes of seafood intoxications

The main recognized human intoxications from fish and shellfish include ciguatera fish poisoning (CFP), paralytic shellfish poisoning (PSP), puffer fish poisoning (PFP), diarrhetic shellfish poisoning (DSP), neurotoxic shellfish poisoning (NSP), amnesic shellfish poisoning (ASP), scombroid fish intoxication (CFP) and certain other rare intoxications (Halstead, 1967; Ahmed, 1991; Falconer, 1993; Anderson, 2000; Fleming et al., 2001; Hui et al., 2001a; Johnson and Schantz, 2002; Backer et al., 2005; Table 17.2). Except for scombroid toxin (histamine), which is produced by bacterial spoilage of improperly refrigerated fish, most of the toxins are produced by marine unicellular algae or phytoplankton. Of the more than 5000 known species of phytoplankton, about 60–80 are known to produce harmful toxins. Fewer than 25 toxic species were recognized only a decade ago. Occasionally, the algae grow to large numbers and form ‘blooms’ that are visible as patches near the water surface (Smayda and Shimizu, 1993; Anderson, 2000). ‘Red tide’ is a common name for a harmful algal bloom (HAB) in which the red pigments of the algal species give the ocean patch its characteristic color. Blooms vary in color depending on the algae, and appear as red, brown or green. Early records and folklore indicate that toxic algal blooms have occurred for hundreds of years, but their actual incidence and the associated causative algae were not accurately identified until relatively recently. Reports of toxic algal blooms are increasing worldwide in frequency, magnitude and
Table 17.2  Seafood intoxications (adapted from Johnson and Schantz, 2002)

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Geographic areas</th>
<th>Source of toxin</th>
<th>Major toxin</th>
<th>Onset time; duration</th>
<th>Major symptoms*</th>
<th>Foods involved</th>
<th>Treatment</th>
<th>Prevention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paralytic shellfish poisoning (PSP)</td>
<td>Worldwide</td>
<td>Toxic dinoflagellates: <em>Alexandrium</em> spp., <em>Gymnodinium catenatum</em>, <em>Pyrodinium bahamense</em></td>
<td>Saxitoxin</td>
<td>5–30 min; occasionally few hours–few days</td>
<td>n, n, d, p, r</td>
<td>Mussels, clams, bay scallops, some fin fish</td>
<td>Supportive (respiratory)</td>
<td>Seafood surveillance; quarantine of seafood region; rapid reporting</td>
</tr>
<tr>
<td>Puffer fish poisoning</td>
<td>Pacific regions near Japan and China; rare in US</td>
<td>Puffer fish: poison in liver, gonads, and roe; possibly produced by bacteria</td>
<td>Tetrodotoxin</td>
<td>Similar to PSP</td>
<td>n, v, d, p, r, bp</td>
<td>Puffer or globe fish</td>
<td>Supportive (respiratory)</td>
<td>Regulated food source; preparation; rapid reporting</td>
</tr>
<tr>
<td>Ciguatera</td>
<td>Tropical areas around world; in US, mainly near Florida</td>
<td>Toxic dinoflagellates: <em>Gambierdiscus toxicus</em>, <em>Prorocentrum</em> spp., <em>Ostreopsis</em> spp., <em>Coolia monotis</em>, <em>Thecadinium</em> spp., <em>Amphidinium carterae</em></td>
<td>Ciguatoxin</td>
<td>Hours; months–years</td>
<td>n, v, d, t, p</td>
<td>Edible tropical fish, commonly barracuda, kahala, snapper, grouper</td>
<td>Supportive</td>
<td>Seafood surveillance; quarantine of region; rapid reporting</td>
</tr>
<tr>
<td>Diarrhetic shellfish poisoning (DSP)</td>
<td>Mainly in Europe, Japan; rare cases in Chile, Southeast Asia, New Zealand</td>
<td>Toxic dinoflagellates: <em>Dinophysis sp Prorocentrum</em> spp.p</td>
<td>Okadaic acid</td>
<td>Hours; days</td>
<td>d, n, v</td>
<td>Mussels, clams, scallops</td>
<td>Supportive</td>
<td>Seafood and water surveillance; quarantine of seafood, region; rapid reporting</td>
</tr>
</tbody>
</table>

Symptoms: a, allergic-like; b, bronchoconstriction; bp, decrease in blood pressure; d, diarrhea; n, nausea; p, paresthesias; r, respiratory distress; t, reversal of temperature sensation; v, vomiting.

*(Continued)*
<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Geographic areas</th>
<th>Source of toxin</th>
<th>Major toxin</th>
<th>Onset time; duration</th>
<th>Major symptoms*</th>
<th>Foods involved</th>
<th>Treatment</th>
<th>Prevention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurotoxic shellfish poisoning (NSP)</td>
<td>Gulf of Mexico, South Atlantic Bight, New Zealand</td>
<td><em>Gymnodinium breve</em>; possibly other species</td>
<td>Brevetoxin</td>
<td>30 min–few hours; few hours</td>
<td>n, v, d, b, t, p</td>
<td>Bay scallops, clams, oysters, quahogs, cohinas</td>
<td>Supportive</td>
<td>Seafood and water surveillance; quarantine of seafood, region; rapid reporting</td>
</tr>
<tr>
<td>Amnesic shellfish poisoning (ASP)</td>
<td>NE Canada; rare or affects animals in NW US, Europe, Japan, Australia, New Zealand</td>
<td>Diatom: <em>Pseudonitzschia</em> spp.</td>
<td>Domoic acid</td>
<td>Hours; months–years</td>
<td>n, v, d, p, r</td>
<td>Mussels, clams, crabs, scallops, anchovies</td>
<td>Supportive (respiratory)</td>
<td>Seafood surveillance; quarantine of seafood; rapid reporting</td>
</tr>
<tr>
<td>Scombroid fish poisoning</td>
<td>Worldwide</td>
<td>Bacterial decomposition of fish at elevated temperatures (&gt; 5˚C)</td>
<td>Histamine</td>
<td>10–90 min; hours</td>
<td>a, d, h, v</td>
<td>Various fish: common in mahimahi, tuna, bluefish, mackerel, skipjack</td>
<td>Supportive; antihistamine</td>
<td>Regulated food handling; keep temperature &lt; 5˚C</td>
</tr>
</tbody>
</table>

Symptoms: a, allergic-like; b, bronchoconstriction; bp, decrease in blood pressure; d, diarrhea; n, nausea; p, paresthesias; r, respiratory distress; t, reversal of temperature sensation; v, vomiting.
geographical extent (Smayda and Shimizu, 1993; Anderson, 2000). They have a marked detrimental effect on fisheries, aquaculture and human health. The ecological factors contributing to this increased occurrence of toxic algal blooms are not completely understood, but recognized contributing factors include the increased availability of nutrients through pollution and oceanic currents and upwelling. Changes in climate have been proposed to contribute to blooms (Smayda and Shimizu, 1993; Anderson, 2000; Fleming et al., 2001; Johnson and Schantz, 2002; Backer et al., 2005).

Microalgal toxins are produced as secondary metabolites and are transferred through the food web, where they accumulate in shellfish and finfish. Since the toxins accumulate in the seafood through the food chain, usually the toxic seafoods appear unspoiled and seemingly harmless. Most of the toxins are tasteless and colorless at poisonous levels. This mode of toxin contamination combined with the heat-stability of the toxins presents considerable difficulty in the prevention of seafood intoxications. Currently, prevention of seafood intoxications depends mainly on surveillance and detection of toxins in the commodities at the point of harvest (Fleming et al., 2001; Johnson and Schantz, 2002; Backer et al., 2005).

3.2 Incidence of seafood intoxications
Globally, about 60 000 seafood intoxications and at least 100 deaths are reported worldwide each year. Like most other foodborne illnesses, this is certainly an underestimation by at least 100–300-fold, since many cases of seafood intoxications are mild, are not reported or are misdiagnosed. The most common intoxications worldwide are SFP, CFP, PSP, NTP and ASP. Globally, PSP is probably the most widespread geographically of seafood intoxications, while most seafood intoxications are clustered in geographic locations near to the area of harvest. Only CFP, SFP and PSP are reported in the Morbidity and Mortality Weekly Report by the CDC in the category of ‘Chemical Poisonings’. In the US, SFP and CFP are responsible for more than 80% of seafood intoxications. In the latest published statistics from the CDC for chemical poisonings, this category accounted for ~14% of the outbreaks and ~2% of the total foodborne illness cases for the period 1988–1992. In the US, seafood ranked third on the list of products that caused foodborne disease between 1983 and 1992 (Fleming et al., 2001; Johnson and Schantz, 2002; Backer et al., 2005).

3.3 Ciguatera fish poisoning (CFP)
Ciguatera fish poisoning (CFP) is one of the most common seafood illnesses, and is caused by eating finfish from tropical reef and island habitats that have accumulated ciguatera toxins (CTXs) from epibenthic dinoflagellates in the food chain. Approximately 20 000 cases have been estimated to occur worldwide annually. The CDC generally reports 10–20 outbreaks per year, with 50–100 cases in the US. The outbreaks usually occur in Hawaii, Puerto Rico, the Virgin Islands and Florida, but can occur in other regions to which the fish are shipped.

As many as 400 species of fish have been implicated in ciguatera poisoning in the Caribbean and Pacific regions, and the fish most commonly involved are amberjack,
snapper, grouper, barracuda, goatfish, and reef fish in the *Carrangidae*. Ciguatoxins reach particularly high concentrations in large predatory reef fishes, and these fish (such as barracuda) are frequently sought by sport fisherman on reefs of Hawaii, Guam and other South Pacific Islands. Various dinoflagellates are known to produce CTXs, including *Gambierdiscus toxicus*, *Procentrum* spp., *Ostreopsis* spp., *Coolia monotis*, *Thecadinium* spp. and *Amphinidium carterae*.

Ciguatera poisoning can involve gastrointestinal, neurological and cardiovascular symptoms. Gastrointestinal symptoms include diarrhea, abdominal pain, nausea and vomiting; their onset is a few hours after ingestion of the fish, and they last for only a few hours. Neurological symptoms usually begin 12–18 hours after consumption, and vary in severity. Neurological signs include reversal of temperature sensation (for example, ice cream tastes hot, hot coffee tastes cold); muscle aches; dizziness; tingling and numbness of the lips, tongue and digits; a metallic taste; dryness of the mouth; anxiety; sweating; and dilated eyes, blurred vision and temporary blindness. Paralysis and death have been documented, but these are rare. There is considerable variation of symptoms and recovery time in individual patients. Recovery may require weeks, months or even years, and the chronic effects of CFP have not been elucidated. Intravenous administration of mannitol can help to relieve acute symptoms, and amitryptiline or tocainide have been suggested for the treatment of chronic symptoms. The most common treatment is supportive, with attention to respiratory and cardiovascular functions. The fatality rate for CFP overall is less than 1%, but has ranged from 0–12% in various fish outbreaks (Fleming *et al*., 2001; Johnson and Schantz, 2002; Backer *et al*., 2005).

Several CTXs have been isolated from toxic fish and algae. They consist of a family of lipophilic, brevitoxin-type polyether compounds, and the prototype compound – gambiertoxin-4 – was isolated and characterized from *Gambierdiscus toxicus*. The total synthesis of brevetoxin A and ciguatoxin CTX3C have been accomplished (Hirami *et al*., 2001). A family of CTXs has been found, since different algal species produce variant toxin structures, and the toxins may be modified by animal or human metabolism. The structures of at least 8 ‘native’ CTXs have been determined, and 11 CTXs formed by oxidative metabolism have been detected. The toxic mechanism of CTXs involves the binding to and opening of sodium and calcium channels in excitable membranes. Like most other seafood toxins, CTXs are commonly detected by mouse bioassay. Immunoassays are available, including ‘dipstick’ tests and commercialized kits (Backer *et al*., 2005).

Fish containing toxic levels of CTXs usually do not appear spoiled. Prevention of intoxications depends on surveillance and detection of toxins in fish and algae from endemic areas, and rapid reporting and treatment of cluster outbreaks. For the majority of US consumers, the illness is contracted from fish imported from endemic areas (Fleming *et al*., 2001; Johnson and Schantz, 2002; Backer *et al*., 2005).

### 3.4 Neurotoxic shellfish poisoning (NSP)

NSP was noticed centuries ago by Spanish explorers and Tampa Bay Indians to cause massive fish kills during certain seasons, when coastal waters became red in color. NSP occasionally accumulates in oysters, clams and mussels from the Gulf of
Mexico and the Atlantic coast and southern US states. Shellfish poisonings of
humans were reported in the late 1800s and in 1946. NSP is usually confined to these
regions, but a bloom was spread by the Gulf Stream leading to an outbreak in North
Carolina, and outbreaks have occurred in New Zealand.

The symptoms of NSP mimic those of ciguatera, in which gastrointestinal and
neurologic symptoms predominate. The onset of symptoms occurs 30 minutes to
3 hours after ingestion of the fish, and include nausea and vomiting; diarrhea;
numbness and tingling in the mouth, arms and legs; incoordination; bronchorestric-
tion; and paresthesias. The illness usually subsides within 2 days. The symptoms are
usually less severe than in ciguatera, and no deaths have been reported, but the illness
is still debilitating. Unlike ciguatera, which can persist for weeks, NSP generally
subsides within a few days. Treatment is supportive, and there is no antidote. NSP
blooms can become aerosolized in the surf and cause respiratory and asthma-like
problems to people on the beach who breathe them. NSP appears to be rare through-
out the world, with documented outbreaks mainly in the US and New Zealand.
Algae related to **Gymnodinium breve** have been detected in Spain and Japan, and it is
possible that intoxications could occur from shellfish harvested from these regions.

**Gymnodinium breve** scavenged from toxic blooms was found to produce polyether
brevetoxins with structures related to certain ciguatoxins. Brevetoxin causes opening
of the sodium channels in nerves and other tissues. Brevetoxin is detected by mouse
bioassay or by ELISA tests. Prevention of poisoning from shellfish depends on
surveillance of waters for toxic algae and rapid reporting. Coastal waters have been
monitored for **G. breve** cell counts, and this has successfully prevented illnesses
(Fleming *et al.*, 2001; Johnson and Schantz, 2002; Backer *et al.*, 2005).

### 3.5 Paralytic shellfish poisoning (PSP)

PSP is a serious and life-threatening intoxication that occurs by eating shellfish
contaminated with saxitoxin (STX) and related toxins (Meyer *et al.*, 1928; Sommer
and Meyer, 1937). PSP has a wider worldwide geographic distribution than other
seafood intoxications caused by microalgal toxins. PSP was first reported in 1793,
after five members of Captain George Vancouver’s ship crew became ill and one
sailor died after eating mussels from Poison Cove in central British Columbia (Kao,
1993). PSP from toxic mussels and clams was also recognized on the Pacific coast in
the 1700s by Native Americans, who associated the poisoning with red (brownish-
red) tides and accompanying bioluminescence. PSP occurs through ingestion of toxic
bivalve mollusks (mainly mussels, clams, oysters and scallops) that have fed on toxic
dinoflagellates including *Alexandrium* spp., *Gymnodinium catenatum* and *Pyrodinium
bahamense* (Kao and Levinson, 1986; Ahmed, 1991). In the United States PSP has a
wider geographical distribution than other dinoflagellate poisonings, and occurs in
the Pacific Northwest Coast and Alaska, and in New England from Massachusetts to
Maine. Toxic algal blooms of *Alexandrium* spp. and other PSP-producing microalgal
species in northern California and other cold temperate regions are seasonal, occur
mainly during the spring, and may be sustained through the summer in upwelling
waters. Owing to current testing and control procedures, outbreaks are rare in
commercial shellfish harvested from coastal regions. Most PSP outbreaks involve recreational collectors of bivalves, often from quarantined areas. The incidence of PSP appears to have increased since the 1970s (Fleming et al., 2001; Johnson and Schantz, 2002; Backer et al., 2005).

The symptoms of PSP generally begin within minutes after eating toxic shellfish, and initially affect the peripheral nervous system. The first signs of intoxication are a prickly or tingling feeling in the lips, tongue and fingertips, followed by numbness in the extremities and face. The intoxication continues with an ataxic gait and muscular incoordination followed by ascending paralysis. Death from respiratory failure may occur within 2–24 hours, depending on the quantity of toxin consumed (2–4 mg is considered to be the lethal dose for a human; Kao and Levinson, 1986). If a sufferer survives the first 24 hours, the prognosis for complete recovery is good and no chronic effects of the poisoning generally occur. There is no effective antidote; poisoned individuals should receive artificial respiration and supportive medical care as soon as these can be administered. Emergency treatment and first aid for victims of PSP have been described. In particular, attention should be given to cardiopulmonary resuscitation (CPR) and respiratory ability, and the victim should rapidly be transported to a hospital emergency facility (Rosen et al., 1988).

STX was the first toxin recognized in shellfish, and it has been extensively characterized (Kao and Levinson, 1986). The nature of the poison responsible for PSP was elusive until it was discovered in the 1930s that culture supernatants lethal to mice were produced by phytoplankton and attributed to the genus Gonyaulax. A lethal substance was extracted from dinoflagellates harvested from blooms and from toxic shellfish. PSP or toxic mussel poison, now called saxitoxin (STX), was purified and identified by E. J. Schantz and colleagues (Schantz, 1992). Toxic extracts were prepared from large quantities of harvested poisonous California mussels (Mytilus californius) and butter clams (Saxidomus giganteus) from Alaska. The toxic substances were purified and found to consist of tetrahydropurine derivatives. Good-quality crystals of STX were obtained and the three-dimensional structure was resolved. The availability of purified toxin allowed the elucidation of the pharmacological mechanism of saxitoxin, and it was demonstrated to bind selectively and with high affinity to sodium channels of excitable membranes and to block completely the inward flux of sodium, in a manner very similar to tetrodotoxin. These toxins have become important neurobiological tools because of their selective and high-affinity blockade of the voltage-gated sodium channels of excitable membranes of neurons and skeletal muscle. Like most other marine microalgal toxins, PSP exists as a family of related compounds, called saxitoxins, neosaxitoxins or gonyautoxins, and more than 20 distinct structures have been elucidated (Kao and Levinson, 1986; Hui et al., 2001a). Current taxonomic understanding is that PSP is produced by various dinoflagellate species of the genus Alexandrium, Gymnodinium catenatum and Pyrodinium bahamense, although reports have indicated that certain bacteria, including Moraxella sp., can produce low quantities of STX or inactive precursors and derivatives in culture.

The prevention of PSP occurs primarily by proactive monitoring of coastal algal blooms and seafoods for the presence of saxitoxin, and rapidly alerting the shellfish industry and public of a health hazard from eating clams, mussels and certain other
shellfish from a designated region. Early investigators in California were instrumental in instigating a prevention program in the 1920s, which consisted mainly of posting warning placards on the beaches with instructions to not eat clams, mussels and certain other shellfish during the high season. The mouse bioassay is currently used by governmental personnel in the US and Canada to detect PSP and to determine whether dangerous levels are present in shellfish (Kao and Levinson, 1986; Hui et al., 2001a). High-performance liquid chromatography (HPLC) is used as an alternative to the mouse bioassay, and capillary electrophoresis and ELISA methods are also being developed (Backer et al., 2005). Industry personnel or recreational consumers of shellfish who plan to gather shellfish should contact their local, state or national health authorities to obtain information regarding the safety of these foods. There are no uniform tolerance levels for PSP, but most countries apply a level of 0.8 mg saxitoxin (equivalent to 400 mouse units) per kg mussel meat. If it is assumed that a person consumes 100 g of mussels, this level would afford a safety factor of two to four for an adult, and a minimum safety factor of seven to nine for severe intoxication or death (Kao and Levinson, 1986).

### 3.6 Puffer fish poisoning (PFP)

Puffer fish poisoning has traditionally been associated with eating certain species of fish belonging to the Tetraodontiformes. These fish are commonly referred to as *fugu*, pufferfish, globefish or swellfish, because they can inflate themselves. It has been recognized for centuries that eating these fish can result in a paralytic poisoning. Puffer fish poisoning most commonly occurs in countries that consume *fugu* as a delicacy, such as China and Japan. Puffer fish poisoning can be fatal, and it has been estimated that about 1800 Japanese have died in the past 40 years following consumption of PFP-tainted and improperly prepared *fugu*. The toxicity of puffer fish varies according to its source, the variety and species of fish, and whether it is wild-caught and grown or kept alive in aquaculture facilities.

The symptoms of pufferfish poisoning are similar to those of PSP, including an initial tingling and prickling sensation of the lips, tongue and fingers within a few minutes of eating poisonous fish. Nausea, vomiting and gastrointestinal pain may follow in some cases. Depending on the quantity of toxin consumed, the pupillary and corneal reflexes are lost and respiratory distress ensues. No antidote is currently available; treatment is supportive, with particular attention to maintaining respiration.

Puffer fish poison was first isolated in 1909 and named tetrodotoxin (TTX). The structure of TTX and its derivatives was reported from Japan and the US in 1964. TTX is an amino perhydroquinazoline compound with a molecular weight of about 400, depending on the form. The chemical structure is distinct from STX, although the symptoms are analogous. It is one of the most poisonous non-protein substances known; the lethal dose is about 0.2 µg for a mouse, 4 µg for a 1-kg rabbit and 1–4 mg for a human. Like STX, it has a highly specific action on sodium channels within excitable membranes.

Tetrodotoxin was long assumed to be produced by the *fugu*; but its detection in certain newts, frogs, marine snails, octopuses, squids, crabs, starfish and other creatures has indicated that it is formed lower in the food chain, possibly by bacteria.
including species of *Alteromonas*, *Vibrio*, and other bacterial genera. These and possibly other bacteria produce various forms of TTXs that vary in potency, including non-toxigenic precursors or derivatives (Fleming *et al.*, 2001; Johnson and Schantz, 2002; Backer *et al.*, 2005).

3.7 **Amnesic shellfish poisoning (domoic acid)**

Amnesic shellfish poisoning (ASP) is a life-threatening shellfish intoxication. It results from eating shellfish contaminated with domoic acid, which is produced by diatoms in the species *Pseudo-nitzschia*. It is a newly recognized seafood intoxication that was first described in 1987 from persons who ate poisonous blue mussels from Prince Edward Island, Canada (Quilliam and Wright, 1989). Until this outbreak, the diatom *Pseudo-nitzschia* was not thought to produce toxins poisonous to humans or animals. Most of the persons in the Canadian outbreak experienced gastroenteritis including vomiting (75%), diarrhea (42%) and abdominal cramps (49%), while some older persons with underlying chronic diseases developed neurological symptoms including memory loss, confusion, disorientation, seizure, coma or cranial nerve palsies within 48 hours. Certain patients experienced short-term memory loss for at least 5 years, and some patients were unable to recognize family members or perform simple tasks. Interestingly, memory loss was more common in patients greater than 70 years of age than in the young. Evidence suggests that persons with impaired renal function may be at greater risk of domoic-acid neurotoxicity because of an impaired ability to inactivate and excrete the toxin. Of the 107 persons affected in the Canadian outbreak, 3 patients died within 3 weeks of eating the mussels. Currently there is no medical treatment for ASP other than supportive care. Medications have been administered to control seizures and potentially reduce the extent of brain lesions.

Investigators were unable to find infectious levels of pathogenic bacteria or viruses, or toxic levels of substances such as heavy metals or organophosphorous pesticides, in the poisonous Canadian mussels. Using the mouse assay designed to detect saxitoxin, it was found that mussel extracts caused death of the mice, usually within 15–45 minutes of intraperitoneal injection. However, the symptoms were distinct from those of PSP, with a unique scratching syndrome of the shoulders and hind leg, followed by convulsions and death. Upon examination of the mussels, it was found that the digestive glands of poisonous animals contained green phytoplankton. Investigators were able to purify domoic acid, and show that it caused similar unique symptoms in mice. The toxic mussels contained up to 900 mg of domoic acid per kg of tissue. Domoic acid was initially characterized in 1958 by Japanese researchers, and its ingestion in algae has been responsible for deaths of pelicans and cormorants in Monterey Bay, California. Although several algae can produce domoic acid, it was found that a bloom of the pennate diatom *Nitzschia pungens f. multiseries* that was occurring at the time of the outbreak contained domoic acid. Isolates of this *Nitzschia* strain produced domoic acid in culture, demonstrating that the diatom was the causative agent and not just a vehicle for the toxin. Domoic acid was produced as a secondary metabolite in axenic cultures of the alga *N. pungens f. multiseries*. Evidence indicated that domoic acid was produced by a limited number
of species in the genera Nitzschia, Digenea, Vidalia, Amansia and Chondriaarmata. Anderson (2000) indicated that species of the genus Pseudo-nitzschia is the current taxon primarily responsible for domoic acid production.

Domoic acid is water-soluble, with a molecular weight of 311 Da. It contains a glutamate-like moiety, and is an analogue of kainic acid. Kainic acid binds to certain CNS receptors and stimulates the release of glutamate in the manner of excitotoxins (Llewellyn, 2001; Backer et al., 2005). Evidence suggests that domoic acid affects calcium transport and stimulates a calcium-dependent process that regulates release of glutamate from presynaptic nerve endings. Domoic acid has been shown to be excitotoxic in a number of animal models, including rodents and primates, and induces seizures at high doses. Domoic acid produces a loss of neurons in various brain regions, particularly the hippocampus, in rodents. On autopsy, brain tissue from the victims of the 1987 Canadian outbreak had lesions in several regions, including the hippocampus, amygdala, thalamus and cerebral cortex (Todd, 1993). In mice, the IP LD₅₀ was estimated to be 3.6 mg domoic acid per kg body weight. Human intoxication in ASP cases occurred after ingestion of an estimated 1–5 mg of domoic acid per kg of body weight. Domoic acid has also been shown to cause deaths among various marine animals, including birds and sea mammals such as sea lions and humpback whales.

Initially the IP mouse assay for PSP detection was used to survey seafood for domoic acid. However, this assay is not consistently sensitive enough to detect the toxin at the Canadian regulatory level of 20 µg/g tissue. A non-destructive extraction method, combined with reverse-phase chromatographic separation and UV detection at 242 nm, was employed and adopted as an official first action by the AOAC in 1990. The detection limit of this method is about 1 µg domoic acid per gram of tissue. Other biochemical methods have also been investigated for sensitive and accurate detection of domoic acid. The current Canadian and UK guideline for the limit of PSP in seafood is 20 mg per kg edible meat.

Although toxic Pseudo-nitzschia species occur in oceans worldwide, only two outbreaks of ASP affecting humans or animals have been reported. The first was the Canadian outbreak in 1987, followed by a large outbreak in seabirds in September 1991 in Monterey, California. High levels of domoic acid were found in Pseudo-nitzschia australis harvested from the area. Since anchovies are a major food source for seabirds in the area, it is possible that the intoxication could be transmitted in herbivorous finfish such as anchovies. An ASP outbreak affected 24 people who consumed razor clams and became ill with gastrointestinal symptoms; 2 people developed mild neurological symptoms. Surveys showed that razor clams and Dungeness crabs in Washington and Oregon contained domoic acid.

Monitoring of phytoplankton blooms for domoic acid, in response to the 1987 outbreak, has contributed to the prevention of ASP. It has been recommended that shellfish from suspect regions be tested and those that contain ≥20 mg/kg not be harvested for human consumption. If shellfish are found with levels above 5 mg/kg but below 20 mg/kg, then the harvest area is monitored closely. Domoic acid is permitted in the US in bivalve shellfish and cooked crab viscera at levels of 20 mg/kg and 30 mg/kg tissue, respectively. In 1988 the US started the National Shellfish Sanitation
Program, which is a cooperative program designed to reduce risks from the consumption of toxic shellfish. The program includes contingency plans in case of an outbreak; the certification of harvesters, processors and distributors; and tracking of the shipping of shellfish. The most extensive shellfish monitoring is directed at PSP, but domoic acid is also monitored to a lesser extent. As of 1998, monitoring programs in Canada and the US have found domoic acid in seafood products in Washington, California, Oregon, Alaska, the Bay of Fundy, British Columbia and Prince Edward Island. Domoic acid has also been detected at low concentrations in regions of Australia, Europe, Japan and New Zealand. In addition to monitoring for domoic acid, depuration has been considered as a detoxification method for shellfish. However, depuration rates vary greatly depending on the animal species and type of toxin, and overall this is not a consistent method of detoxification.

3.8 Diarrhetic shellfish poisoning (DSP)

Diarrhetic shellfish poisoning (DSP) was observed in the 1960s and 1970s, caused by toxic mussels, scallops or clams. Consumption of toxic shellfish causes gastrointestinal disturbances and diarrhea. DSP occurs mainly in Japan and northern Europe, but also in various other regions of the world – there have been outbreaks in South America, South Africa, southeastern Asia, and New Zealand. DSP is caused by toxins produced by *Dinophysis* spp. DSP is not usually fatal, but shellfish may become toxic in the presence of dinoflagellates at low cell densities (≥ 200 cells/ml). DSP is characterized by gastrointestinal symptoms such as severe diarrhea, nausea, vomiting, abdominal cramps and chills, which start 30 minutes to a few hours after eating toxic shellfish. Complete recovery usually occurs within 3 days. In more severe cases, hospitalization and administration of an electrolyte solution may be necessary.

Various toxins are produced by *Dinophysis* spp., including okadaic acid, pectenotoxins and yessotoxin. The parenteral toxicity of yessotoxins has been estimated to be approximately one order of magnitude greater than that of the oral dose. It has been estimated that symptoms from OA or DTX consumption begin at about 40–50 µg for an adult. Certain of the *Dinophysis* toxins appear to have mutagenic, cancer-inducing, hepatotoxic or immunogenic properties; but the chronic toxic effects in humans are not known. The mouse bioassay is most commonly used to detect the presence of DSP toxins, although HPLC combined with mass spectrometry, immunoassays and cytotoxicity based assays has also been evaluated. Limits for diarrheal shellfish toxins (usually undetectable by mouse assay) have been proposed in Japan and certain European countries. The European Union applies a tolerance level of 0.16 OA equivalents per kg of mussel meat, which generally provides a safety factor of ≥ 2 before symptoms are noticed.

3.9 *Pfiesteria* intoxications

*Pfiesteria* heterotrophic dinoflagellates were proposed in the early 1990s to cause massive fish kills of millions to billions of finfish and shellfish in the coastal waters of the mid-Atlantic and southeastern US (Morris, 1999; Berry et al., 2002; Collier and
Burke, 2002). The predatory organism, mainly the species *P. piscicida*, had also been reported from the Mediterranean Sea, the Gulf of Mexico and the western Atlantic. The organism exists in several life stages. It remains in river and coastal bottoms for years as cysts and, when induced by unknown factors in fish feces, the cysts bloom into a motile form of the organism. These then swarm to the upper waters and produce very potent toxins, resulting in a 'feeding frenzy'. After this, the organism transforms to an ameba state that feeds on microorganisms and fish remains, followed by reformation of cysts, which settle in the coastal sediment to complete the cycle.

Recently, considerable controversy has arisen regarding whether the organism dubbed the 'cell from hell' (Morris, 1999) is predatory to fish, and whether it also produces toxins that can cause illness in humans. Despite several documented fish kills, human illness due to environmental exposure has not been shown definitively (Morris, 1999, 2001; Berry et al., 2002; Collier and Burke, 2002). It had been reported (Glasgow et al., 1995) that researchers were intoxicated by chronic exposure to *P. piscicida* in aquarium water. Three workers reported symptoms of asthenia, skin lesions, emotional lability and memory dysfunction. Other symptoms included gastrointestinal pain, nausea, headache, spatial disorientation, impaired concentration and severe loss of short-term memory (Collier and Burke, 2002). However, neurologic examinations were normal. The speculation that humans could contract disease from *P. piscicida* was also questioned, since 254 crabbers who work in infested waters have not been known to contract the disease. After much debate, it has been concluded that exposure to the alga may cause human illness under the right conditions (Collier and Burke, 2002). This would not be surprising, since other dinoflagellates cause diseases that are mainly mediated by toxins. Despite preliminary evidence presented by Burkholder and colleagues (Glasgow et al., 1995), the production of toxins has not been shown definitively for *P. piscicida* (Berry et al., 2002; Collier and Burke, 2002). Experiments are also in progress to determine whether the toxic effects in humans are due to *Pfiesteria* solely, or to *Pfiesteria* together with associated microorganisms. Currently, *Pfiesteria* should be considered as a cause of human illness from contaminated waters, as well as an occupational and laboratory hazard. There have been no definitive reports of foodborne illness, but these may be forthcoming with effective investigation and diagnosis.

Recommendations have been suggested for the closing and reopening of waters affected by *Pfiesteria* or *Pfiesteria*-like events. Closure is recommended when a significant fish kill is reported and fish are found that contain sores and lesions consistent with the toxic activity of *Pfiesteria*, or when a significant number of fish exhibit erratic behavior that cannot be attributed to other factors such as low oxygen levels in the water. Waters may be reopened for recreational and commercial activities when these signs have not been apparent for 14 days.

### 3.10 Cyanobacterial intoxications

Unlike dinoflagellates and diatoms, which cause human food poisoning from marine finfish and shellfish, cyanobacteria (sometimes called blue-green algae) have caused severe animal illnesses from consumption of drinking water (Falconer, 1993; Chorus,
2001). The vast majority of illnesses due to cyanobacteria are waterborne and affect animals. The main toxic genera of prokaryotic cyanobacteria are the filamentous species *Anabaena*, *Aphanizomenon*, *Nodularia* and *Oscillatoria*, and the unicellular species *Microcystis*. Like the marine eukaryotic microalgae, they form blooms under appropriate conditions in freshwater. Blooms usually occur in the summer and autumn during warm days, and are promoted by nutrient availability (especially nitrogen and phosphorous) that often derives from water runoff containing fertilizers, or from livestock or human waste. Toxic blooms occur in many lakes, ponds and rivers throughout the world. The primary toxicoses include gastrointestinal disturbances, acute hepatotoxicosis, neurotoxicoses, respiratory distress and allergic reactions.

Most of the poisonings by cyanobacteria involve acute hepatotoxicosis and death mediated by microcystins and nodularin, which are heat-stable, small peptides (Shimizu, 2003). Certain cyanobacteria also produce neurotoxicoses due to the alkaloidal anatoxins and anaphatoxins. These toxins can cause death within minutes to a few hours, depending on the animal species and the quantity of toxin consumed. Cyanobacteria also produce a cholinesterase inhibitor called anatoxin-α(s), which has an organophosphate structure. Certain cyanobacteria have been reported to produce paralytic shellfish-like toxins, including saxitoxin and neosaxitoxin.

Cyanobacterial toxins have sporadically caused human intoxications from drinking water (Chorus, 2001). In most cases, water treatment systems are adequate to remove cyanobacteria by coagulation and filtration, and microcystins can be adsorbed by charcoal filters and are degraded by chlorine. However, extracellular cyanobacterial toxins may survive water treatment and are resistant to boiling. Cyanobacterial toxins in human drinking water have been documented to cause hepatic toxicity, gastroenteritis, contact dermatitis and allergic responses, and neuronal and brain damage. Some cyanobacteria, e.g. *Spirulina*, have been marketed as health foods. Although studies in rodents have shown no toxicity, it is important that *Spirulina* and other cyanobacteria food supplements are produced under hygienic conditions and do not contain cells or toxins from toxic species.

Diagnostic procedures for human and animal illnesses include association of a bloom of a toxigenic cyanobacterial species with consumption of water, the presence of characteristic symptoms in the animal or human, microscopic identification of the toxic species of cyanobacteria in the suspect water, and verification of the presence of toxin in the water by chemical and bioassays. Procedures for prevention of cyanobacterial intoxications from water rely on monitoring programs and quarantine measures when toxic algae reach a certain concentration in the bloom water. Methods with increased sensitivity compared to microscopic identification are being developed to detect toxin-producing cyanobacteria rapidly. Chemicals, particularly copper sulfate, have been added to lakes to kill cyanobacteria. The reduction of agricultural runoff and animal or human fecal contamination of water will also reduce bloom formation. Obviously, if a bloom occurs, animals and humans should avoid consumption of the water.

Although there are no documented outbreaks of food poisoning caused by cyanobacterial toxins, shellfish can filter cyanobacteria from water and may accumulate their toxins. Mussels (*Mytilus edulis*) fed *Microcystis* accumulated microcystins
that persisted for several days after transfer to freshwater. Fresh fruits and vegetables washed with contaminated water could also potentially acquire these toxins. Since scenarios exist by which food could transmit cyanobacterial toxins, it may be important to monitor water from suspect sources that will have contact with foods. The UK and the State of Oregon have set limits of 1 ppm for microcystins in drinking water and dietary supplements. It is anticipated that cyanobacterial poisonings of humans will persist until we can prevent the blooms.

3.11 Scombroid (histamine) fish poisoning

Scombroid poisoning is probably the most prevalent of the seafood-transmitted illnesses worldwide (Taylor, 1986; Fleming et al., 2001; Johnson and Schantz, 2002; Backer et al., 2005). Scombroid poisonings have been commonly reported in Japan, Canada, the US, the UK and other countries that have a high dietary intake of fish. Scombroid poisoning symptoms mimic those of an Ig E-mediated food allergy, with flushing of the face, neck and upper arms, nausea, vomiting, diarrhea, abdominal pain, headache, dizziness, blurred vision, faintness, itching, rash, hives, and an oral burning sensation in the mouth. Hypotension, tachycardia, palpitations, respiratory distress and shock may occur in severe cases. The symptoms of scombroid illness usually occur within 10–90 minutes of eating contaminated fish. Individuals exposed to scombroid poison will usually experience only a few of these symptoms. The duration of the illness is usually less than 12 hours. Diagnosis of scombroid poisoning can generally be made by the short onset time, the non-specific yet characteristic symptoms, and a history of consumption of fish. The diagnosis can be confirmed by detection of histamine in the spoiled fish (scombroid poisoning has been diagnosed by measurement of plasma histamine). Corticosteroids and H1 and H2 antihistamines can be used to treat the symptoms.

Due to the variety of symptoms and their similarity to allergic responses, the illness is frequently misdiagnosed and is often confused with an allergic reaction. Many of the symptoms of allergic reactions mimic those apparent in scombroid illness, since histamine is a primary mediator of allergic disease. Normally treatment is unnecessary, as the vast majority of cases are mild and self-limiting, but antihistamine therapy can provide relief and rapid recovery. Hydration and electrolyte replacement may also be beneficial. Scombroid poisoning can be severe in persons with a history of allergic disease or with pre-existing cardiac or respiratory conditions, or in people being treated with certain drugs such as isoniazid or monoamine oxidase inhibitors. Antihistamines should be administered only under close medical supervision in these special situations.

Nearly all cases of scombroid poisoning have been associated with marine fish, particularly of the scombroid (dark flesh) variety, such as tuna, bonito and mackerel. Non-scombroid fish and shellfish have also been implicated in scombroid poisoning, including mahi-mahi, swordfish, salmon, dolphin, marlin, sardines, bluefish, amberjack, anchovy and abalone. Other foods have also transmitted scombroid poisoning, including Swiss cheese and some fermented foods and extracts. Scombroid poisoning is caused by certain bacterial species that grow in fish stored at inappropriate elevated
temperatures, where the bacteria decarboxylate histidine to histamine. Histamine is heat-stable and withstands cooking. Since orally-administered histamine generally does not elicit symptoms, it is believed that potentiators such as the diamines putrescine and cadaverine promote the illness.

Several species of bacteria produce histamine through the action of the enzyme histidine decarboxylase. Bacterial species associated with scombroid poisoning include *Morganella morganii*, *Klebsiella pneumoniae*, *Vibrio* sp., *Enterobacter aerogenes*, *Clostridium perfringens*, *Hafnia alvei*, *Lactobacillus buchneri* and *Lactobacillus delbrueckii*. Other enteric *Enterobacteriaceae*, clostridia and vibrios have been associated with scombroid poisoning, but *M. morganii* and *K. pneumoniae* are the most common species implicated. These organisms are not frequently associated with living fish, and must contaminate the fish during handling and storage. Since the organisms forming scombroid toxin are not psychrophiles, temperatures above 15˚C are generally required to permit adequate growth and histamine formation. Histamine production on skipjack tuna was optimal at 30˚C, but once a large population of bacteria has been formed the enzyme histidine decarboxylase can stay active even under refrigeration conditions. Most of the histamine is produced near the intestines and then diffuses into the flesh.

The standard analytical method for detection of histamine and other biogenic amines is high-performance liquid chromatography, although other methods (including radioimmunoassay kits) are commercially available. The generally accepted toxic level of histamine in fish is 100 mg/100 g of flesh, the amounts in ingested fish actually causing illness have not been accurately defined. Histamine can be used to judge the freshness of certain raw fish. The FDA considers 20 mg of histamine per 100 g of gless, or 200 ppm, indicative of spoilage in tuna, and 50 mg/100g (500 ppm) an indication of a hazard. Since other finfish and shellfish intoxications show similar signs to scombroid poisoning, the final diagnosis may depend on detection of the toxins in the foods (Fleming *et al.*, 2001; Johnson and Schantz, 2002; Backer *et al.*, 2005).

The most important contributing factor to scombroid poisoning is improper refrigeration of the harvested fish, allowing bacterial proliferation. Fish should be chilled as rapidly as possible, and be brought below 15˚C and preferably below 10˚C within 4 hours; lengthier storage of fish should be at 0˚C (32 ˚F) or below, using ice, brine or mechanical refrigeration. Maintaining sanitary conditions during handling, processing and distribution will help to prevent bacterial contamination. Histamine is heat-stable and will withstand cooking. Improved reporting of scombroid incidences to public health agencies will increase awareness of the disease and its prevention.

### 3.12 Other finfish and shellfish toxins

Various substances have been implicated in toxic fish kills and potentially caused human disease. Food poisoning from eating parrot fish has been reported in Japan, and the causative toxin was identified as palytoxin (PTX). PTXs occur in various marine organisms such as seaweeds and crabs, and they also appear to be synthesized by microalgae. Tetramine occurs in the salivary gland of a few whelk species, and has occasionally caused human intoxications. New and emerging toxins posing hazards
in seafoods have been proposed, including pinnatoxins, azaspiracids, gymmodimine and spirolides (Backer et al., 2005). Azaspiracids are polyether toxins produced by the dinoflagellate Protoperidinium, formerly thought to be benign. Other substances have been suggested to cause seafood illnesses or toxic blooms with resulting fish kills, including unique hemagglutinins, and reactive oxygen metabolites such as superoxide anions and hydroxyl radicals. Sardine poisoning associated with high mortality (~40 %) and hallucinatory fish poisoning have been described, but the causative toxins are not known.

3.13 Treatment and prevention of seafood intoxications

The structures of seafood toxins, as well as the signs, symptoms and pharmacologic and therapeutic treatments, have been published in several reviews (Fleming et al., 2001; Johnson and Schantz, 2002; Backer et al., 2005). The Centers for Disease Control and Prevention website (www.cdc.gov) also contains valuable information on the incidence, symptoms and treatment of seafood illnesses. Therapy for most shellfish intoxications depends on rapid supportive care, with particular attention given to cardiopulmonary sufficiency, respiratory distress and shock. Antidotes are not available for most of the shellfish toxins, although certain low molecular-weight compounds and monoclonal antibodies have been proposed to alleviate symptoms for some seafood intoxications. Scombroid poisoning can be treated with corticosteroids and H₁ and H₂ antihistamines, but physicians should refer to patient medication status and authoritative guidelines before administration of these.

Most seafood health risks originate in the environment, primarily from harmful algal blooms, and prevention depends on control at harvest. With few exceptions, risks cannot be detected by organoleptic inspection. Surveillance and sensitive detection of the causative algae and toxins by inspection and sampling provide the cornerstone for prevention of seafood intoxications caused by algal toxins. In contrast, prevention of scombroid poisoning requires prompt refrigeration and maintaining the temperature of the fish near to 0˚C. Reducing the incidence of seafood intoxications will require coordinated efforts of regulatory agencies and the seafood industries. A comprehensive surveillance and identification program of the etiologic agents and toxins responsible for seafood intoxications can provide valuable information for handling, processing and instituting programs such as hazard analysis critical control points (HACCP) to identify research needs and prevention strategies (Todd, 1997; Williams and Zorn, 1997; National Research Council, 1985).

3.14 Safety precautions for handling toxic seafoods and algae

Working with toxic seafoods, toxin-producing algae in culture, and extracts or purified toxins requires care and adequate safety precautions. Protective clothing, including lab coats, face and eye protection and impervious gloves, as well as air handling requirements, are recommended to prevent exposure to toxins or aerosols. Chlorine can be used to kill the organisms, but spills of toxins may require additional chemical treatment. The US Army has developed procedures for the chemical inactivation of
various toxins. Brevetoxins, microcystins, tetrodotoxins, saxitoxins and palytoxins can be inactivated by 30-minute exposure to 2.5 % NaOCl or, more effectively, by 2.5 % NaOCl + 0.35 N NaOH. Algal seafood toxins are resistant to autoclaving at 121˚C or 10 minutes exposure to dry heat at 200˚F, and chemical decontamination is usually required.

4    Plant toxins

4.1 Overview and importance of plant toxins

In most countries of the world, foods of plant origin supply most (~70 %) of the protein consumed by humans. Many food plants produce specific natural toxicants (Liener, 1980; Liener and Kadade, 1980; Hui et al., 2001a; Norton, 2001; Panter, 2005). These toxicants can produce acute or chronic illness, or developmental perturbations (Table 17.3). The public perception of plant toxicants, relative to other

<table>
<thead>
<tr>
<th>Toxin</th>
<th>Chemical nature</th>
<th>Main food sources</th>
<th>Major toxicity symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protease inhibitors</td>
<td>Proteins (4000–24 000 kDa)</td>
<td>Beans (soy, kidney, mung, lima, navy); chick-peas; peas; potatoes (sweet, white); cereals</td>
<td>Impaired growth and food utilization; pancreatic hypertrophy</td>
</tr>
<tr>
<td>Hemagglutinins</td>
<td>Proteins (10 000–124 000 kDa)</td>
<td>Beans (castor, soy, kidney, black, yellow, jack), lentils, peas</td>
<td>Impaired growth and food utilization; agglutination of erythrocytes in vitro; mitogenic activity to cell cultures in vitro</td>
</tr>
<tr>
<td>Saponins</td>
<td>Glycosides</td>
<td>Soybeans, sugar beets, peanuts, spinach, asparagus</td>
<td>Hemolysis of erythrocytes in vitro</td>
</tr>
<tr>
<td>Glucosinolates</td>
<td>Thioglycosides</td>
<td>Cabbage and related species, turnips; rutabaga, radish; rapeseed; mustard</td>
<td>Hyperthyroidism and thyroid enlargement</td>
</tr>
<tr>
<td>Cyanogens</td>
<td>Cyanogenic glycosides</td>
<td>Peas and beans; pulses; linseed; flax; fruit kernels, cassava</td>
<td>HCN poisoning</td>
</tr>
<tr>
<td>Gossypol</td>
<td>Gossypol pigments (β-aminoproponitrile and derivatives)</td>
<td>Cottonseed</td>
<td>Liver damage; hemorrhage; edema</td>
</tr>
<tr>
<td>Lathyrogens</td>
<td>(β-aminoproponitrile and derivatives)</td>
<td>Chick pea; vetch</td>
<td>Neurolathyism (CNS damage)</td>
</tr>
<tr>
<td>Cycasin</td>
<td>Methylazoxymethanol</td>
<td>Nuts of Cycas genus</td>
<td>Cancer of liver and other organs</td>
</tr>
<tr>
<td>Favism</td>
<td>Vicine and convicine (pyrimidine-β-glucosides)</td>
<td>Fava beans</td>
<td>Acute hemolytic anemia</td>
</tr>
<tr>
<td>Phytoalexins</td>
<td>Simple furans (ipomeamarone)</td>
<td>Sweet potatoes</td>
<td>Pulmonary edema; liver and kidney damage</td>
</tr>
</tbody>
</table>
sources of foodborne illness, is rudimentary compared to other sources, such as salmonellosis. It is often perceived by the public that certain plants are toxic and should not be consumed, but that other plants are nutritious and non-toxic. Although many herbal medicines are perceived as not causing toxic effects because they derive from natural substances, several over-the-counter herbal preparations can have severe toxicity. The toxicity of herbal medicines is covered in some excellent reviews (Schilter et al., 2003; Zhou et al., 2004).

Populations in poor countries may be more susceptible to plant toxicants; this derives from the necessity to rely on plants as main sources of nutrition and is often related to poor cultivation practices and quality aspects of the foods. Environmental occurrences such as drought and flood, as well as war and civil unrest, can increase the dependence on poor-quality plants as foods. For example, about 400 million people in Africa rely on the root crop cassava (Manihot esculenta) for subsistence, but this plant contains natural toxins and can cause severe disease. Resources are often not available to remove potent toxins from cassava. The slowly developing and chronic diseases of many plants are also often not considered as hazards in the food supply. Moreover, livestock losses from toxic plants can be substantial (Hui et al., 2001a; Panter, 2005).

The ability of plants to cause toxicity and illness depends on many factors, such as the disease state of the plant (many plants produce toxicants in response to bacterial or fungal infections), its maturity, the environmental conditions and soil characteristics, and processing (Hui et al., 2001a; Wittstock and Gershenzon, 2002). As with other foodborne intoxications, risk increases in the very young or elderly, in those with underlying diseases or immunodeficiency, and in those suffering from malnutrition. Most plant toxicity occurs through preformed toxicants, while certain illnesses occur through metabolism, or by postharvest treatments and food processing (Rahmann, 1999). β-Carbolines such as norharman and harman, are formed during the cooking of foods from a reaction between tryptophan and aldehyde components.

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**Table 17.3** Plant toxicants (adapted from Pariza, 1996)—cont’d

<table>
<thead>
<tr>
<th>Toxin</th>
<th>Chemical nature</th>
<th>Main food sources</th>
<th>Major toxicity symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrrolizidine alkaloids</td>
<td>Dihydropyrroles (psoralins)</td>
<td>Families Compositae and Boraginaceae; herbal teas; sassafras; black pepper</td>
<td>Liver and lung damage; carcinogenesis</td>
</tr>
<tr>
<td></td>
<td>Benzofurans (wterone)</td>
<td>Celery, parsnips</td>
<td>Skin photosensitivity</td>
</tr>
<tr>
<td></td>
<td>Acetylcycenic furans (wterone)</td>
<td>Broad beans</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Isoflavonoids (pisatin and phaseollin)</td>
<td>Peas, French beans</td>
<td>Cell lysis in vitro</td>
</tr>
<tr>
<td>α-amantin</td>
<td>Bicyclic octapeptides</td>
<td>Amanita phalloides mushrooms</td>
<td>Salivation; vomiting; convulsions; death</td>
</tr>
<tr>
<td>Attractyloside</td>
<td>Steroidal glycoside</td>
<td>Thistle (Atractylis gummifera)</td>
<td>Depletion of glycogen</td>
</tr>
</tbody>
</table>

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β-Carbolines such as norharman and harman, are formed during the cooking of foods from a reaction between tryptophan and aldehyde components.
with subsequent oxidation. Recently, acrylamide has been considered as an important toxicant, which is formed during cooking of certain foods – particularly fried foods such as potato snacks (Friedman, 2003).

Many diverse chemical classes of toxicants occur in plants, including glucosinolates, pyrrolizidine alkaloids, amino acid analogues, psoralens, lectins and others (Hui et al., 2001a; Panter, 2005). Certain food plants also may produce hormone-disrupting toxicants, and many are well known to produce allergens. Importantly, particular plant toxicants can elicit long-term or delayed disease syndromes, but, despite their importance, the compounds and the effects of the toxicants have been poorly characterized.

4.2 Pyrrolizidine alkaloids

Alkaloids comprise a diverse family of compounds produced by various fungi and plants (Smith and Culvenor, 1981; Mattocks, 1986; Rizk, 1991). Most alkaloids are basic nitrogenous compounds and characteristically contain a heterocyclic ring system. They are mainly derived from amino acids, but terpenoids also serve as precursors for certain classes. Several have beneficial actions as medicines, but some of them are toxic to humans and animals by the oral route. Alkaloids are among the most important plant toxicants for humans. They can elicit acute illnesses, but their ingestion can also cause or trigger delayed or long-term effects.

Pyrrolizidine alkaloids (PAs) are among the most important food toxicants for humans and animals (Smith and Culvenor, 1981; Mattocks, 1986; Rizk, 1991). Consumption of toxic levels of pyrrolizidine alkaloids has been responsible for massive outbreaks of diseases in livestock, causing severe economic damage. PAs have also caused serious illnesses and deaths in humans, particularly in less-developed countries. The intoxication can also be contracted by ingestion of herbal teas and other preparations (Hui et al., 2001a; Panter, 2005).

PAs have been found in flowering plants, but also are predominant in several groups of food plants (Smith and Culvenor, 1981; Mattocks, 1986; Rizk, 1991). They are primarily found in the plant families Compositae (Asteraceae), Boragineaceae and Leguminosae, but also have been detected in Apocyanacae, Ranunculacae and Scrophulariaceae. In certain flowering plants, PAs have been detected at high levels (~3–5%). They are most prevalent in buds, flowers and young leaves, compared to older leaves and stems. High levels have also been found in seeds of Crotalaria, and toxic levels in seeds of the grain for human consumption. Oral exposure in humans usually occurs through inadvertent contamination of foodstuffs or through consumption of herbal preparations. PAs, particularly comfrey, are consumed by humans in certain herbal medicines in the US and Europe. Since the PAs probably cause chronic effects, their poisoning of humans has been underestimated and the disease syndromes poorly characterized. Consumption of seeds of Heliotropium have caused liver pathology in persons in Afghanistan, and 28 patients died and 67 persons became ill in India following consumption of grain contaminated with seeds of Crotolaria. Contamination of milk from goats fed ragwort (Senecio jacobaea) has also led to human intoxications. Honey produced from S. jacobaea and Echium platagineum has been shown occasionally to contain high levels of PAs. Honey
produced in hives in areas of prolific ragwort growth can accumulate high levels of PAs (Hui et al., 2001a; Panter, 2005).

The chemistry and structure of PAs have been extensively reviewed (cited in Hui et al., 2001a; Panter, 2005). PAs are activated by cytochrome P-450s primarily of the 3A4 family. Certain cytochrome P450s convert PAs to less toxic forms, and animals such as sheep that possess this pathway suffer less severe toxic effects than animals that convert PAs to the toxic pyrrole form (such as rats and horses). The ingestion of PAs is well known to cause damage to the liver. PAs also affect other organs, particularly the lungs, but may cause chronic effects in humans and grazing animals after long-term consumption. Although PAs are well-recognized to cause impairment of human health, the control of intoxications has been difficult to achieve. The plant sources grow prolifically in many areas of the world. It has been suggested that the primary food source posing the highest risk of large-scale poisoning is cereals that become contaminated with PAs (cited in Hui et al., 2001a; Panter, 2005). PA consumption in herbal medicines also could pose a considerable risk for human intoxications and long-term harmful effects.

### 4.3 Psoralens

Psoralens comprise a group of phototoxic fucocoumarins present in many plant families, including the Apiaceae (formerly Umbelliferae; e.g. celery, parsnips), Leguminosae (certain legumes), Rutaceae (bergamot, limes, cloves) and Moraceae. When photoactivated by sunlight, compounds in this family can be mutagenic. Certain psoralens, such as xanthotoxin, methoxsalen and beghapten, have been studied for their potential by photoactivation to prevent skin diseases such as psoriasis and fungal infection.

Coumarin occurs widely in vegetables such as cabbage, radish and spinach, and in plants used as flavoring agents, such as lavender and sweet woodruff. Coumarin is widely found in herbal tea. At high concentrations, coumarin causes liver damage in test animals, and its former use as a food additive has been banned by the FDA. Coumarin is also a strong anticoagulant and is frequently used in rodent baits and in certain human medicines as a blood-thinning agent (Hui et al., 2001a; Panter, 2005).

### 4.4 Cyanogenic glycosides

Cyanogenic glycosides have been detected in more than 2000 plant species, as well as in certain bacteria, fungi, and even members of the animal kingdom (Hui et al., 2001a; Reddy and Hayes, 2001; Panter, 2005). Cyanogenic glycosides can release highly toxic hydrocyanic acid (HCN). Although found in many sources, they are mainly of concern in the seeds (kernels) of stone fruits including apples, apricots, cherries, peaches, pears, plums and quinces, and in almonds, sorghum, lima beans, cassava, corn, yams, chickpeas, cashews and kirsch. Most poisonings are associated with the consumption of cassava in Africa, Asia and Latin America (Reddy and Hayes, 2001). Although more than 20 cyanogenic glycosides have been identified, poisonings are typically associated with amygdalin, dhurrin, linamarin and lotaustralin. Cyanogenic lipids have also been detected in plants (Reddy and Hayes, 2001).
Accumulation of cyanogenic glycosides in plants is enhanced during stress conditions such as drought and frost, and availability of toxic precursors is stimulated by physical disruption – such as the trampling of plants during harvest, and food processing procedures including chopping. During ingestion, enzymes such as β-glucosidase, hydroxynitrile lyase and other enzymes found in the gastrointestinal tract of humans or in their gut flora release toxic HCN. Although HCN is quite toxic, the levels that occur in foods are relatively low and reports of poisoning are infrequent. The lethal dose has been estimated to be 0.5–3.5 mg HCN per kg body weight. Poisoning by cyanogenic glycosides is much more important in livestock, generally through the consumption of large quantities of forage sorghums, arrow grass and wild cherries. Cases of cyanosis from HCN poisoning have occurred following ingestion of lima beans, cassava and bitter almonds. Due to the high level of consumption of cassava in Africa and South America, cyanogenic glycosides have presented a substantial health risk in these areas. Processing of cassava, such as soaking, boiling, sun-drying and fermentation, can eliminate most of the cyanide. Cyanogenic glycosides have also been suspected to contribute to birth defects, diabetes, endemic goiter and ‘konzo’ (an upper myelopathic motorneuron disease endemic to East Africa). A number of glycosides occur in various plant species, but their risk as food toxicants is uncertain.

4.5 Allyl isothiocyanates

Allyl isothiocyanates are mainly responsible for the pungent flavor of certain foods including mustard and horseradish, where they are present at 50–150 ppm (Coulombe, 2000; Hui et al., 2001a; Panter, 2005). They are also present at much lower levels in broccoli, cabbage and cassava. Isothiocyanates occur in cruciferous vegetables as glucosinolate conjugates, and cyanide can be released during digestion. Isothiocyanates have been implicated in causing hyperthyroidism (goiter), particularly in geographical regions like India and Africa, where the consumption of minimally processed foods occurs together with iodine deficiency (Coulombe, 2000). As with cyanogenic glycosides, simple processing (chopping, rinsing, milling) can reduce the level of isothiocyanates.

4.6 Glycoalkaloids

Glycoalkaloids occur mainly in potatoes (Solanum tuberosum), particularly in green potatoes. They are also found in low levels in tomatoes. High levels of these glycoalkaloids can cause gastroenteritis, and can also impart a bitter taste to potatoes. They are of concern in new varieties of potatoes, including those derived by biotechnology. When injured, exposed to light or sprouted, potatoes can accumulate the glycoalkaloids α-solanine and α-chaconine. These compounds are potent inhibitors of acetylcholinesterase. Poisoning symptoms include gastric pain, weakness, nausea, vomiting and difficulty in breathing. Poisonings have occurred in animals fed damaged potatoes, greens or trim (Coulombe, 2000; Hui et al., 2001a; Panter, 2005).
4.7 Hydrazines and other toxins in edible mushrooms

Commonly cultivated mushrooms, including *Agaricus bisporus*, shiitake (*Cortinellus shitake*) and the false morel (*Gyromitra esculenta*), contain substantial quantities of hydrazines (up to 500 ppm). Certain hydrazines have been implicated as liver toxins and animal carcinogens. Shiitake and false morel mushrooms also contain substantial levels of agaritine and gyromitrin, which can be transformed to carcinogens. Many wild mushroom species are known to contain other toxicants that can cause a variety of foodborne syndromes (Lovenberg, 1973; Reddy and Hayes, 2001).

4.8 Caffeic acid and chlorogenic acid

Caffeic acid, quinic acid derivative and chlorogenic acid phenolic compounds, are prevalent in a wide variety of plants and vegetables. Chlorogenic acid is hydrolyzed to caffeic acid and quinic acid in the gastrointestinal tract. Caffeic acid is metabolically transformed in humans to o-methylated derivatives, including ferulic, dihydroferulic and vanillic acids, as well as meta-hydroxyphenyl derivatives, which are excreted in the urine (Coulombe, 2000; Hui *et al.*, 2001a; Panter, 2005). Caffeic acid and its derivatives are present at relatively high levels in certain spices and seasonings (thyme, basil, dill, anise, caraway, rosemary, sage, tarragon and marjoram), vegetables (lettuce, potatoes, radishes and celery), and fruits (grapes, berries and tomatoes). Coffee also contains significant quantities of chlorogenic acids. Caffeic acid is an inhibitor of 5-lipoxygenase, which is a central enzyme for the biosynthesis of eicosanoids including leukotrienes and thromboxanes. At high doses caffeic acid and chlorogenic acid have also been demonstrated to be carcinogenic, and their presence in the diet at significant levels may also enhance carcinogenesis by other compounds.

4.9 Toxicants in spices

Many spices contain a vast and interesting array of secondary metabolite compounds, some of which can be toxicants (Coulombe, 2000). Examples of potentially toxic spice components are saffrole, myristicin, β-asarone and isosafrole, which have analogous structures to certain carcinogens such as alkyl benzenes. Capsaicin is a prominent flavoring agent in red and yellow chili peppers, and can cause irritation to the eyes and mucous membranes. It also affects neurotransmission and causes depletion of substance P, which is involved in pain mediation. Other components of spices that can have toxic effects include glycyrrhizin, a saponin-like glycoside present in licorice. Licorice root extract was used as an expectorant in ancient times, and has been suspected of causing hypertension and other metabolic disorders. Certain other classes of compounds, such as terpenoids (δ-limonene), can be toxic to animals at high levels. The potential of spices for causing adverse chronic effects is evident, but it would be expected that prudent use would not affect normal human health.
4.10  **Biologically active amines**

Certain vaso- and psychoactive amines, including tyramine, octopamine, dopamine, epinephrine, norepinephrine, histamine, serotonin and others, are present in foods, particularly fermented products such as cheeses, yeast products, beer, wine and pickled herring. They also occur in coffee, chicken liver, broad beans, chocolate, pineapple, banana, plantain and avocado (Reddy and Hayes, 2001). Amines with vasoconstrictive properties are present in a variety of foods (Lovenberg, 1973; Reddy and Hayes, 2001). Pressor amines including tyramine and tryptamine, as well as certain related compounds (serotonin, adrenaline, noradrenaline, dopamine), affect central nervous system activity. Low levels of these compounds (10 mg) can cause severe hypertensive crisis in individuals treated with monoamine oxidase (MAO) inhibitors for depression and other mood disorders (Lovenberg, 1973; Reddy and Hayes, 2001).

4.11  **Protease inhibitors**

Plants contain inhibitors of difference classes of enzymes, including proteases, lipases, amylases and others (Kassell, 1970; Liener and Kadade, 1980). However, protease inhibitors are responsible for most toxic effects. The main protease inhibitor from soybean, commonly called Kunitz inhibitor, is capable of inhibiting trypsin and certain other proteases (Hui et al., 2001a; Panter, 2005). Ingestion of raw soybean decreases digestion of protein, causing increased secretion of pancreatic enzymes and reduction in body mass gain. Other protease inhibitors from food sources, including beans, peas, egg white, cereal grains, alfalfa and potatoes, have also shown toxic effects in humans and animals. The main effects in animals include pancreatic hypertrophy, adenomas, and nodular hyperplasia associated with growth depression (Hui et al., 2001a; Panter, 2005).

4.12  **Lectins (phytohemagglutinins) and glutens**

Lectins are high molecular weight (100–150 kDa), heat-labile proteins that have been detected in numerous edible plant species, particularly those belonging to the leguminoseae (beans, peas, etc.). Lectins have also been found in food animals, including crustaceans, mollusks, fish and even mammals (Reddy and Hayes, 2001). Their consumption can result in growth reduction in animals and humans, presumably due to their effects on the intestinal mucosa and disruption of nutrient transport. Necrosis of intestinal epithelia has also been observed. Systemic lectin exposure has caused fatalities due to liver damage. One of the most toxic lectins is ricin, present in the castor bean (see section 4.18 below). Ingestion can cause severe necrosis and eventually death from organ damage.

Gluten enteropathy (celiac sprue) has been associated with the consumption of wheat-germ agglutinin contaminating gluten in cereal foods. A strong genetic component contributes to the susceptibility to celiac sprue, but diet and probably environmental factors also participate in the etiology. Necrosis and loss of jejunal villi are characteristic of celiac sprue. The syndrome in children has been associated with vomiting, a bloated abdomen, behavioral changes (including irritability and
restlessness), speech impairment, impaired growth, chronic diarrhea, and myopathy characterized by weakness and fatigue. Celiac sprue has been estimated to have a prevalence of 0.1% in certain areas of Europe (Troncone et al., 1996). The majority of patients respond within weeks to a gluten-free diet. Susceptible individuals are advised to avoid food products prepared from wheat, rye, barley and oats. Interestingly, a gluten-free diet in certain children has been associated with a reduction in neuropsychological phenomena such as autism (Dohan, 1976). The mechanism of the neuropsychological manifestations is unclear, but one possibility is that neuroactive peptides produced during the digestion of food proteins cross the gut barriers that have been necrotized. This hypothesis has previously been proposed as contributing to schizophrenia (Dohan, 1976), and it has been reported that a gluten-free diet may reduce the symptoms of schizophrenia. However, increased permeability of the small intestine was not observed in schizophrenic patients (Lambert et al., 1989). Also, a link between celiac disease and childhood autism could not be demonstrated (Black et al., 2002). Nonetheless, these findings raise the intriguing possibility of a connection between diet, gut flora, and behavioral diseases.

4.13 Phytates

Phytates (hexaphosphate esters of myo-inositol) bind di- and tri-valent metals and can lead to mineral deficiencies in human and animal diets. Such deficiencies primarily occur in developing countries where cereals are consumed as major or exclusive source of proteins. Treatment of foods and feeds with phytase results in phosphate utilization and reduction of environmental pollution from phosphates. The sequestration of metals by phytates can be alleviated by supplementing diets with minerals and vitamin D (Kotsonis et al., 2001; Reddy and Hayes, 2001).

4.14 Estrogens

More than 200 species of plants contain estrogenic isoflavonoids (e.g. genistein) or their glycosides (e.g. genistin) (Reddy and Hayes, 2001). Coumestans and lignans are other important sources of plant estrogens. Phytoestrogens have been known to cause infertility in animals grazing in forages including subterranean clover and alfalfa. Genistin in soybeans has been associated with most human toxic effects and has reportedly interfered with steroid metabolism in infants fed soy-based formulas. In women, changes in menstrual cycles have been reported to occur owing to soy consumption. It is currently unclear whether consumption of phytoestrogens may lead to longer-term effects in humans (Reddy and Hayes, 2001).

4.15 Canavanine and other amino acid analogues

Canavanine is an arginine analogue (2-amino-4-(guanidinoxy)-butyric acid) that is widespread in seeds of Leguminosae. Alfalfa sprouts (Medicago sativa) and Jackbean (Canavalia ensiformis) contain high levels (up to 15 000 ppm) of canavanine (Coulombe, 2000; Hui et al., 2001a; Panter, 2005). Since canavanine is an analogue of arginine, it can
be incorporated into cellular proteins and partially disrupt function. It is suspected of causing autoimmune disorders such as lupus erythematosus (Coulombe, 2000).

Amino acid derivatives that disrupt essential metabolic processes, including neurotransmission and bone development in humans and animals, are formed by various plants. For example, consumption of β-N-oxalylamino-L-alanine (BOAA), which is found in legumes such as grass-pea, can trigger lathyrism, a form of spastic paraparesis characterized by muscle weakness, increased muscle tone, and hyper-reflexia in the lower limbs (Spencer and Berman, 2003). Long-term consumption can lead to permanent inability to move the legs. Lathyrism is primarily a problem in regions such as areas of India, where edible material other than grass pea is scarce. The amino acid analogue β-(γ-L-glutamyl)-aminopropionitrile (BAPN) inhibits lysyl oxidase, which is important in collagen and bone formation. Feeding of BAPN to rodents results in joint and skeletal deformities.

Other disease syndromes have been observed in humans and animals that consume plants containing non-protein amino acids. Vomiting sickness, hypoglycemia and hepatic encephalopathy have been observed in people in West Africa who consume the fruit from the ackee tree, which produces hypoglycin (γ-glutamyl dipeptide). The ackee tree was imported to the Caribbean islands, and ingestion of unripe fruit has caused severe disease in poorly nourished people there.

4.16 Miscellaneous flavonoids

Flavonoids are widespread in plant-derived foods, including fruits and fruit juices, certain vegetables, tea, cocoa, red wine, dill, soybeans and others (Coulombe, 2000). These have been suspected as being carcinogenic. Rutin can be metabolized by intestinal bacteria to form quercetin, which has anticarcinogenic properties.

4.17 Other plant toxicants

Other plant compounds considered to be intoxicants in human and animal diets, including saponins (steroidal compounds and certain terpenoids), lipids (erucic acid, phytanic acid, cyclopropene fatty acids), anti-vitamin factors and mushroom compounds (psilocybin, coprine and others), have also been suspected to cause growth depression, psychoabnormality or toxic effects, and have been discussed in more comprehensive reviews (see, for example, Reddy and Hayes, 2001). The large number of plant-derived compounds showing potential adverse effects on humans and animals illustrates the paucity of knowledge in this area and the need for further research to evaluate toxicity and other effects.

4.18 Plant toxicants of potential risk in bioterrorism

There has been a heightened awareness of the potential for toxins to be used as bioterrorist agents in foods or by other means of dispersal (Khan et al., 2001; Franz and Zajtchuk, 2002). Among the various pathogens and toxins, certain plant toxins have been considered as significant threat agents. In particular, ricin and other closely
related plant toxins such as abrin have been considered as poisoning agents. Ricin is a potent protein cytotoxin that is derived from castor beans. Ricin can be aerosolized, and poisoning can occur through inhalation or ingestion. The symptoms of ricin poisoning depend on the route of administration and the quantity ingested. Following oral ingestion, initial symptoms usually occur with 6 hours. Gastrointestinal symptoms include severe abdominal pain, vomiting, diarrhea, and ulcerations and hemorrhages of the gastric and small-intestinal mucosa (as detected by endoscopy). The toxin also affects liver function, and tests are abnormal. Hallucinations, seizures, and blood in the urine are also characteristic signs, and death has been documented. People who know or suspect that they have been poisoned by ricin should seek immediate medical care. Currently there is no antidote other than passive immunotherapy, but improved vaccines and therapeutics are being developed.

5 Other foodborne toxicants

5.1 Insect- and mite-derived toxins in foods

Insect infestation of foods is clearly undesirable, and certain insects can produce chemical toxicants that can result in foodborne illnesses. Flour beetles (Tribolium spp.) produce benzoquinones that are carcinogenic in animals and possibly in humans (Wirtz et al., 1978; Taylor and Hefle, 2002). No acute cases of human illness have been reported from flours infested with Tribolium spp., but the long-term effects of consumption are unknown. Human illnesses have been reported from foods contaminated with dust mites, which produce allergens that can cause allergic symptoms in humans on ingestion (Taylor and Hefle, 2002).

5.2 Intoxication of unknown etiology – bovine paraplegic syndrome

Bovine paraplegic syndrome (BPS) was first described in Venezuela in the mid-1950s, and has recently spread alarmingly in the cattle-growing areas of Venezuela and Paraguay (Sevcik et al., 1993). Since the animals affected are generally in seemingly good condition, the disease is also called enfermedad de las bonitas (‘disease of the pretty ones’) by the farmers. It is mainly pregnant or lactating cows that are affected, and the mortality rate has been estimated to range from 5% to 25% in the animals at risk. The disease is characterized by ventral or sternal decubitis, and animals are unable to stand when stimulated. The diagnosis is dependent on eliminating other possible causes, including botulism, paralytic rabies, and blood parasites. All cows die within a few days, and there is no known treatment. The clinical etiology has not been established. It has been proposed that ruminal bacteria produced a heat- and acid-stable toxin (Sevcik et al., 1993). The toxin blocked the sodium current in giant squid axons. Subsequent research has suggested that saxitoxin is produced by bacteria (the Enterobacter asburiae, E. cloacae and Klebsiella pneumoniae in the rumen) and that the characteristic sodium channel-blocking toxin is responsible for BPS (Sevcik et al., 2003). However, other investigators have proposed that BPS is caused by toxins
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produced by *Clostridium perfringens* type D (Muller *et al*., 1998) and *Lactobacillus vitulinum*, as well as several Gram-negative rumen isolates (Domínguez-Bello *et al*., 1993). A possible role of bovine immunodeficiency virus has been indicated by immunological, virological and seroprevalence studies (Walder *et al*., 1995). Thus the etiology remains unclear, although saxitoxin has also been isolated from bacteria and cyanobacteria (Mahmood and Carmichael, 1986; Carmichael *et al*., 1997; Gallacher and Smith, 1999). The production of saxitoxin during cyanobacterial blooms has been implicated in the death of animals drinking water from ponds undergoing blooms (Schantz and Johnson, 1992).

5.3 Intoxications from genetically modified foods

Plant and animal genomes have long been modified by traditional breeding practices, but, with the development of biotechnology, specific new genes have been incorporated into foods and feed substances. Depending on the commodity and country of production, substantial percentages of crops and animals contain genes introduced by biotechnology (Stewart, 2003; Toke, 2004). For example, ~25% of corn cultivated in 1999 in the US contained an anti-pest gene from *Bacillus thuringiensis* (Stewart, 2003). Considerable controversy and debate has been voiced regarding the potential for toxicants generated by the insertion of genes into plants using biotechnology.

The safety of genetically modified crops and other foods has been evaluated by a number of methods and regulated under international laws. It is beyond the scope of this chapter to discuss the safety assessments and regulations, and the reader is referred to authoritative reviews (Kotsonis *et al*., 2001; Taylor and Hefle, 2002).

5.4 Dietary supplements

The toxicology and safety of dietary supplements has also been a subject of considerable controversy. Although many consumers utilize dietary supplements, this class of foods has unique safety qualifications. Aspects of supplements' safety and toxicology are covered in recent authoritative reviews (Kotsonis *et al*., 2001; Schilter *et al*., 2003).

6 Laboratory practices, conclusions, and perspectives

Laboratory considerations regarding the handling of natural toxins have been reviewed and regulations implemented (Wannemacher, 1989; CDC, 1999; Fleming and Hunt, 2000), and these considerations have assumed increased importance with the implementation of Select Agent Regulations in the US Patriot Act. Intoxications by natural toxicants are illnesses resulting from exposure (usually by the oral route) to toxins produced by microorganisms, plants and, occasionally, animals. Comprehensive safety programs for laboratories working with toxins have been described (Wannemacher, 1989; CDC, 1999; Fleming and Hunt, 2000; Malizio *et al*., 2000).
The provision of a nutritious and safe food supply is an essential goal of society to ensure the health and survival of humankind throughout the world. Epidemiological evidence has indicated that certain food-associated bacteria and natural toxicants are the major causes of illness and mortality transmitted by foods. Although progress has been made in the identification and understanding of the toxicology of miscellaneous intoxicants present in microorganisms, plants and animals, the body of knowledge in this research area is meager compared to our understanding of foodborne diseases caused by microbial and fungal pathogens. Natural intoxicants comprise a vast array of compounds with a myriad of structures and modes of action. Unlike many microbial pathogens, their involvement in animal and human foodborne disease cannot be determined by classical methods of identifying disease, such as solving of the famous Koch’s postulates. Since natural intoxicants do not reproduce on their own, but depend on the host for production, they are often present in minute quantities in foodstuffs. Identification often depends on association of a plant or animal with disease, followed by sophisticated chemical tests to identify candidate compounds. Subsequently, in vitro and animal models are employed to evaluate toxicity. For many of these compounds minimum acceptable levels have not been established, and in certain cases it will not be possible to establish these. Since natural foodborne toxicants can be as deleterious as synthetic toxicants or even microbial pathogens, US and worldwide resources should be allocated to evaluate more thoroughly their impact on animal and human health. Natural foodborne intoxicants have an enormous medical and economic impact on societies, particularly in developing countries. Further surveillance, together with the identification and evaluation of health effects, could lead to a quantitative risk assessment. The education of producers, processors and consumers would have a tremendous benefit in improving the world’s food supply.

Although microbial food safety is a major public health issue of increasing importance, many public health authorities in certain countries throughout the world do not fully appreciate its importance for human health and economic development (WHO, 1997). National and international programs to enhance food safety are considered a low priority in many countries, partly because resources are not available to develop food safety programs. Many countries have not developed legislation and the public health infrastructure to control foodborne disease. Although consumers are integral to the prevention of foodborne disease, many are unaware of their importance in enhancing food safety and do not receive adequate education to prevent illnesses within the home or at community events. As emphasized by the WHO (1997), strategies for decreasing the incidence of foodborne disease, enhancing human well-being and facilitating technological developments will require a shared responsibility among governments, industry, scholarly institutions and consumers to accomplish these goals.

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