The Food Safety Hazard Guidebook
2nd Edition

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Preface

Food safety is important. Consumers have a right to expect that those who supply the food that they buy have taken every care to manufacture products that will do them no harm. Those with a responsibility for the regulation of the global food industry recognise this principle and legislate accordingly. This confers a legal and a moral duty, as well as an economic incentive, on all food businesses to ensure that the food they supply is as free from food safety hazards as is practically possible. The food business that tries to evade its responsibilities in this regard will not remain in business for very long.

The business of managing and regulating the safety of the food supply chain has come a long way in the last 30 years or so. Prompted by the emergence of new food safety hazards, such as the bacterial pathogens *Listeria monocytogenes* and *E. coli* O157, powerful new techniques for evaluating and managing the risks presented by these threats have been developed. For example, hazard analysis critical control point, or HACCP, has now become the food safety management system of choice worldwide. Similarly, the technique of risk assessment has been developed to the point where it can be applied to almost anything. There now exists a comprehensive toolbox of techniques for managing the safety of food, and a plethora of training and guidance options for learning how to use the tools. As a result, there is now little to excuse any food business that fails to protect its customers from known food safety hazards.

Although food safety management tools are now widely available, they are still virtually useless unless they are supported by adequate and accurate information. HACCP does not work unless its practitioners have access to enough data and scientific knowledge to enable them to understand hazards and how to control them effectively. For example, there is little point in deciding that pasteurisation is the best way to control a bacterial pathogen unless its heat resistance is known. There is plenty of information available,

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in countless excellent books and other publications, and increasingly online. Unfortunately, accessing that information can be problematic, especially for smaller food businesses.

The *Food Safety Hazard Guidebook* is an attempt to address that problem by distilling the key facts about a wide range of individual food safety hazards into a single text. We have tried to adopt a clear format and to keep the information included as concise as possible so that it is easy to find the important facts. We would not claim for one moment that the book is a comprehensive or exhaustive reference work on food safety hazards, and it is not meant to be. As the title suggests, it is intended as a guidebook rather than an encyclopaedia, and has been conceived as a portal for the immense and ever expanding body of scientific knowledge that exists for food safety. To that end, we have included "Sources of Further Information" in every chapter for those needing more detail. As authors, we have drawn on our experience of supplying the technical and scientific information that food safety professionals require to address a real need for accessible knowledge.

This second edition of the guidebook retains the layout and structure of the first, but updates and extends the content to keep the book as relevant as possible to current food safety issues. Our objective for this edition remains the same, to produce a book that is accurate and reliable, as up-to-date as possible, and above all, useful.

Disclaimer

The material contained in this book is presented after the exercise of every possible care in its compilation, preparation and issue. However, the authors can accept no liability whatsoever in connection with its application and use.

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Food Safety Hazards

Food Safety

The term *food safety* has no universally accepted definition. In fact, it is sometimes used, wrongly, in relation to defects in food commodities that are much more to do with food quality than with safety. For example, microbial spoilage of food may make it unattractive, or even inedible, but if neither the micro-organisms concerned, nor the by-products of their growth and metabolism have any adverse effect on health, then it is not strictly a food safety issue, but one of acceptability. For the purposes of this book, food safety can usefully be defined as the practice of ensuring that foods cause no harm to the consumer. This simple definition covers a broad range of topics, from basic domestic and personal hygiene, to highly complex technical procedures designed to remove contaminants from sophisticated processed foods and ingredients.

Essentially, the practice of food safety can be distilled down to three basic operations:

- Protection of the food supply from harmful contamination.
- Prevention of the development and spread of harmful contamination.
- Effective removal of contamination and contaminants.

Most food safety procedures fall into one, or more than one, of these categories. For example, good food hygiene practice is concerned with the protection of food against contamination, effective temperature control is designed to prevent the development and spread of contamination, and pasteurisation is a measure developed to remove contaminants.

Food Safety Hazards

A *food safety hazard* can be defined as any factor present in food that has the potential to cause harm to the consumer, either by causing illness or injury.

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Food safety hazards may be biological, such as pathogenic bacteria, chemical, such as a toxin produced during processing, or a physical object, like a stone or piece of metal. In other words, hazards are the factors that food safety practice seeks to protect against, contain and eliminate from foods. In order to be effective, food safety practice must be informed about the nature of these hazards, and food safety procedures must be science-based. A thorough understanding of biological and chemical hazards is the first essential step in their control. This is less important for physical hazards, which also tend to have a much lower potential impact on public health. Physical hazards are not considered further here.

Biological Hazards

It is generally biological hazards that pose the greatest immediate food safety threat to the consumer. For example, the ability of food-poisoning bacteria to cause large outbreaks of acute illness within a short time is a threat with which most food businesses are likely to have to contend. There are few foods that are not vulnerable to biological hazards at some point in their manufacture, storage and distribution.

Technically, biological hazards may include larger organisms, such as insects and rodents. However, these rarely present a direct threat to health and are not considered further here. It is microorganisms and certain food-borne parasites that are of most concern as food safety hazards.

Bacteria

A significant number of bacterial species can be classified as food safety hazards. Some of these, such as *Salmonella* and *Listeria monocytogenes*, are very well known and familiar to consumers, whereas others are much less common and less well understood. Examples include *Vibrio parahaemolyticus*, a comparatively rare cause of food poisoning associated with seafood, and *Yersinia enterocolitica*, a cause of gastroenteritis that predominantly affects young children. *Campylobacter* is another example of a less well known cause of food-borne illness. Few consumers have heard of this organism, yet it is now the cause of more reported cases of food poisoning than any other agent, including *Salmonella*. *Campylobacter* is also less familiar to the food industry and there are still many unknowns surrounding its transmission to humans. This underlines the importance of continued research and scientific investigation for increasing our understanding of biological hazards.

Bacterial food safety hazards fall into one of two categories according to the mechanism by which they cause illness.

Infection

Most food-borne bacterial pathogens cause illness by multiplying in the gut after ingestion of contaminated food. They may then provoke symptoms by invading the cells lining the intestine, or in some cases, invading other parts

of the body and causing more serious illnesses. *Salmonella*, *Campylobacter* and *E. coli* O157 are all examples of bacteria that cause infective food poisoning. This type of food poisoning is usually characterised by a delay, or incubation time, of at least 8–12 hours (sometimes much longer) before symptoms develop.

This category also includes some bacteria that produce symptoms by multiplying in the gut and producing toxins, rather than by actively invading the tissues. An example of this type is *Clostridium perfringens*, a food-poisoning bacterium usually associated with cooked meat products.

Intoxication

There are a few food-borne pathogenic bacteria that produce illness not by infection, but by intoxication. These organisms are able to grow in certain foods under favourable conditions and produce toxins as a by-product of growth. The toxin is thus pre-formed in the food before ingestion and in some cases toxin may still be present even after all the bacterial cells have been destroyed by cooking. *Bacillus cereus* and *Staphylococcus aureus* are examples of bacteria able to cause intoxication, but the most important and potentially serious cause of intoxication is *Clostridium botulinum*. Intoxications usually have much shorter incubations times than infections, because the toxins are pre-formed in the food.

Viruses

Viral gastroenteritis is very common worldwide. There are a number of viruses that are capable of causing food-borne infections, although in most cases, other forms of transmission are more common. Perhaps the best known are noroviruses and hepatitis A, which has been responsible for a number of serious food-borne disease outbreaks, often as a result of poor personal hygiene by infected food handlers.

'New' viruses may also pose a threat to food safety. For example, highly pathogenic avian influenza viruses primarily affect birds, but in some cases may be transmitted to humans and cause serious disease. So far, there is no direct evidence that this transmission can be food borne, but these viruses are a source of great concern to the poultry industry and there is still much to learn about them.

Parasites

A wide range of intestinal parasites can be transmitted to humans *via* contaminated foods, although for most, faecal–oral, or water-borne transmission are more common. These organisms are much more prevalent in developing countries with poor sanitation, but the increasingly global nature of the food supply chain may increase their importance in the developed world. Currently, protozoan parasites are the most important, but other types also need to be considered as food safety hazards.

Protozoans

The protozoan parasites that can cause food-borne illness in humans include several well known species, such as *Entamoeba histolytica*, the cause of amoebic dysentery, and *Cryptosporidium parvum*. However, in recent years, some unfamiliar species have emerged as threats to food safety, especially as contaminants in imported produce. An example is *Cyclospora cayetanensis*, the cause of several outbreaks of gastroenteritis in the USA associated with imported fruit.

Other Types of Parasite

Other types of food-borne parasite include nematode worms, such as *Trichinella spiralis* and the anisakid worms found in fish, and cestodes (tapeworms), such as *Taenia solium*. Although many of these are far less prevalent in developed countries than was once the case, thanks to improved sanitation, they are still significant causes of illness worldwide.

Prions

Prions are a relatively recent threat to food safety and are still not fully understood, but their probable involvement in potentially food-borne new variant Creutzfeldt–Jakob disease (vCJD), an invariably fatal brain disease, has lead to considerable concern.

Chemical Hazards

The presence of chemical hazards in food is usually less immediately apparent than that of bacteria and other biological hazards. Acute toxicity caused by food-borne chemical contaminants is now very rare in developed countries. Of much more concern is the potentially insidious effect of exposure to low levels of toxic chemicals in the diet over long periods. In some cases this can lead to chronic illness and there is also the risk that some contaminants may be carcinogenic.

There is potential for an enormous range of chemical contaminants to enter the food chain at any stage in production. For example, agricultural chemicals, such as herbicides and insecticides, may contaminate fresh produce during primary production, some commodities may contain ‘natural’ biological toxins, and chemicals such as detergents and lubricants may enter food during processing. It is also possible for chemical contaminants to leach out of packaging into foods during storage.

Some of the main classes of chemical contaminant important in food safety are as follows:

- Agricultural chemicals, pesticides *etc*.
- Veterinary drugs

- Natural biological toxins
  - Fungal toxins
  - Plant toxins
  - Fish toxins
- Environmental contaminants (*e.g.* dioxins and heavy metals)
- Contaminants produced during processing (*e.g.* acrylamide)
- Contaminants from food contact materials (*e.g.* plasticisers)
- Cleaning and sanitising chemicals
- Adulterants (*e.g.* illegal food dyes)

The total number of potentially harmful chemicals that may contaminate food is very large. For example, UK legislation contains maximum residue levels (MRLs) for over 28 000 pesticide/commodity combinations. It is therefore not practical to cover pesticides here in anything but the most general terms. Fortunately, the use of pesticides is very strictly controlled in many countries and residues in imported foods are regularly monitored. Links are provided in the "Sources of Further Information" section for readers needing specific information on pesticides.

The list of potential adulterants is also an extensive one. Almost by definition, adulterants are often compounds that would not be expected to be present in foods and little may be known about their health significance if present in the diet. Recent examples include synthetic Sudan dyes found in imported spices and other commodities in the EU. These are illegal for food use, but the health effects of low levels in foods are uncertain, and there has been some discussion over their food safety significance. For these reasons, it is not practical to cover potential adulterants here, with one notable exception. The industrial chemical melamine has been found in food commodities and ingredients, especially from China. Its presence in foods has been found to cause potentially serious kidney damage in animals and humans and it was responsible for a very large outbreak of illness among Chinese infants, which lead to at least six deaths. Because of the seriousness of this incident, the widespread nature of potential contamination and the known health hazard, melamine is included here.

The Chemical Hazards section focuses on contaminants that are known to be food safety hazards, and which have received some attention from food safety researchers and regulators to establish the level of risk they carry.

Allergens

In recent years, the problem of food allergy has been growing in importance for the food industry as the number of people, particularly children, affected by allergy symptoms has increased. Food manufacturers have been encouraged to respond to this development, particularly in terms of labelling foods clearly. Along with clear allergen labelling comes a responsibility to ensure that such labels are accurate. When foods are labelled as not containing specific allergens, it is extremely important that they do not become contaminated with those allergens during production. This is vital for allergens such as peanuts, which

may cause life-threatening anaphylactic reactions in sensitive individuals. The presence of undeclared allergens in foods is a growing cause of product recalls in the EU, North America and elsewhere.

The control of allergens in food is now a rapidly developing aspect of food safety, which many manufacturers will need to be concerned with. Fourteen specific major food allergens are currently recognised by EU legislation, although many more foods are likely to be capable of causing allergic reactions in sensitive individuals.

These are:

- Celery
- Crustaceans
- Egg
- Fish
- Lupin
- Milk
- Molluscs
- Mustard
- Peanuts
- Soya
- Sesame
- Sulphur dioxide and sulfites
- Tree nuts
- Wheat

It is probable that food allergies will continue to grow in importance in the coming years, and that further allergens will be recognised in legislation.

The Obligations of Food Businesses

In most countries, the safety of the food supply is regulated by national and local authorities. Food businesses are required to meet the demands of food safety regulations, at the very least, in order to protect consumers from hazards in food. These are likely to include the setting up of an effective food safety management system, such as hazard analysis critical control point (HACCP). In addition, many food businesses will need to meet the requirements of their customers, such as large retail chains, or will need to comply with the food safety provisions of third party audit schemes. Most of these will expect more extensive food safety measures than are required by relevant legislation.

Most businesses will find it necessary to adopt a risk assessment and HACCP-based approach to addressing food safety, and there is considerable assistance and support available to help with this. Nevertheless, it is important that every food business develops at least a basic understanding of the specific food safety hazards that may be relevant to their products and processes. Only then can food safety management systems operate effectively. The following pages are designed to help provide that basic understanding.

Section 1: Biological Hazards

CHAPTER 1.1

Bacteria

1.1.1 *AEROMONAS*

Hazard Identification

What are Aeromonas*?*

Aeromonas species are gram-negative, non-spore-forming, bacteria, many of which are psychrotrophic (*i.e.* able to grow at low temperatures). Older references may state that these organisms are in the family *Vibrionaceae*, but they have recently been classified in a new family, the *Aeromonadaceae*, and this family now includes at least 14 described *Aeromonas* species.

Although a number of these species have been associated with human disease, the role of *Aeromonas* species as food-borne pathogens has yet to be confirmed. *Aeromonas hydrophila*, *Aeromonas caviae*, *Aeromonas veronii* biovar sobria and *Aeromonas trota* are the main species that are thought to cause gastrointestinal disease in man and it is considered that the main vehicle for these organisms is drinking water. Many *Aeromonas* species can be divided into two groups based on the temperature range at which strains are able to grow, and within a specific species some strains are psychrotophic, while others are mesophilic (not able to grow below 10 °C). For *A. hydrophila*, evidence suggests that those strains that are pathogenic to humans are mesophilic, whereas psychrotrophic strains are pathogenic to fish.

Occurrence in Foods

Aeromonas species are common contaminants in unprocessed foods and on occasions numbers can be high, exceeding 10^6 cfu g^{-1}. Because of their widespread occurrence it is thought likely that not all strains of *Aeromonas* species

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are pathogenic. *Aeromonas* species have been isolated from the following food commodities: fresh vegetables; salads; fish; seafood; raw meats including beef, lamb, pork and poultry; and raw milk as well as high-pH cheeses produced from raw milk. *Aeromonas* species have also, on occasions, been isolated from some processed foods including pasteurised milk, whipped cream, ice cream and ready-to-eat animal products.

Possible gastroenteritis-causing species have been isolated from most of the above food groups. However, *A. caviae* is more commonly isolated from vegetables and salad while *A. hydrophilia* is more commonly isolated from meat, fish and poultry.

Hazard Characterisation

Effects on Health

Although there is increasing evidence to suggest that *A. hydrophila*, *A. caviae* and *A. veronii* biovar sobria are causative agents of food-borne gastroenteritis in humans, this is still a subject of debate. However, aeromonads are often detected in gastrointestinal infections.

The infectious dose is unknown, although data suggests that it is probably high, probably $>10^6$ cells. Volunteer feeding studies involving ingesting high numbers of *A. hydrophila* cells ($>10^7$) have been inconclusive, whereas the organism has been isolated from the stools of divers who became ill after taking in small amounts of contaminated water. Gastroenteritis associated with *Aeromonas* species is most frequently reported in young children, although it can occur in individuals of any age with the number of cases peaking in the summer months.

It is thought that when ingested, these organisms can cause gastrointestinal disease in healthy individuals, chronic enterocolitis in the elderly and septicaemia in the immunocompromised. Symptoms are thought to start to occur within 24–48 hours of ingestion of cells. Infection can manifest itself in one of two distinct forms. The more common form is a cholera-like illness (watery diarrhoea accompanied by a mild fever), sometimes accompanied by vomiting in children less than two years old. The less common form is a dysentery-like illness (diarrhoea with blood and mucus in the stools). The disease is usually self-limiting, lasting 1–7 days. Occasionally however, the diarrhoea can last for several months, or even longer (12 months plus). Rare cases of haemolytic uremic syndrome, following infection with *Aeromonas* species, have been linked to verocytotoxin-producing aeromonads.

Incidence and Outbreaks

Most *Aeromonas* infections are thought to be caused by contaminated water and there are few reported outbreaks of *Aeromonas*-associated gastroenteritis where food is the suspected vehicle of infection. These few incidents are mostly associated with seafood products such as raw oysters and clams, sashimi,

cooked prawns, shrimp cocktail and raw fermented fish. The literature suggests that other food groups such as edible land snails, egg salad and smorgasbord (comprising shrimp and various ready-to-eat meat products) have also been involved.

Sources

Aeromonas species are ubiquitous, although the main source of the organisms is generally accepted as water. The organisms are found in flowing and stagnant fresh water, in water supplies (including chlorinated water), sewage and in marine waters, particularly those that border with fresh water such as in estuaries. *Aeromonas* species are also often found in household environments such as drains and sinks, and can be isolated from soil.

Aeromonads are found in aquatic animals such as frogs, fish and leeches, in reptiles and in domestic animals such as pigs, sheep, poultry and cows. They can also be carried by humans without symptoms on occasions, although carriage rates are higher in tropical or developing regions.

Growth and Survival Characteristics

The growth temperature range for *Aeromonas* species is variable, but is reported to be between <5 °C and 45 °C. Within a particular species there can be psychrotrophic strains (capable of growth at chill temperatures) and mesophilic strains (cannot grow below 10 °C). Although the optimum temperature for growth is generally reported as 28 °C, this figure is likely to vary depending on strain. Although environmental strains may not grow at 37 °C, many clinical strains can grow at 5–7 °C. *A. hydrophila* is reported to grow from 1–42 °C, with an optimum temperature of 28 °C.

Aeromonads are reported to survive freezing temperatures and have been isolated from frozen foods after storage for approximately two years.

The optimum pH range for the growth of aeromonads is between 6.5 and 7.5. The organisms are tolerant of pH values of up to 10 and many strains will grow down to pH 5.5 or less (under otherwise ideal conditions), but this characteristic is uncommon at chill temperatures. Aeromonads are inactivated at pH values <4.5.

Many aeromonads will not grow at salt levels >4%, although there are reports of some strains growing at concentrations of 6%. Studies have shown that when foods are stored at chill temperatures, *Aeromonas* species are unlikely to grow when the salt levels are more than 3–3.5% and pH values are below 6.0.

Aeromonas species are facultative anaerobes (capable of growth with or without oxygen). At chill temperatures however, it has been reported that growth rate is either unaffected, or possibly reduced, when fish is modified atmosphere/vacuum packaged. Modified atmospheres containing high levels of oxygen (>70%) have been shown to retard the growth of *A. caviae* on ready-to-eat vegetables at refrigeration temperatures.

Aeromonas species are not notably resistant to preservatives or sanitisers.

It is thought that their presence in chlorinated water is the result of post-treatment contamination or inefficiencies in the chlorination process.

Thermal Resistance

Aeromonads are not heat-resistant organisms and are readily inactivated by pasteurisation or equivalent processes. Decimal reduction times (*D*-values) of 3.20–6.23 min at 48 °C in raw milk have been recorded.

Control Options

Processing

At present, research suggests that if some *Aeromonas* strains are indeed food-borne pathogens, it is foods containing high numbers of the organisms that pose the greatest health risk.

Measures to reduce the likelihood of high numbers occurring should include: using treated water supplies in food processing; keeping foods chilled; and the thorough, frequent cleaning of equipment used to process foods, especially those that are not later cooked by the consumer, *e.g.* salads and vegetables.

Aeromonas species are easily inactivated by pasteurisation, or equivalent processes used by the food industry. Preventing the recontamination of heat-processed products, particularly those with a high water activity and neutral pH that are to be stored chilled, should ensure that aeromonads are not a potential health risk in these foods. Measures to reduce the risk of recontamination include keeping raw and cooked foods separate and implementing good handling and packaging practices.

Product Use

Aeromonas species should be considered as possible pathogens and it has been suggested that very young children, the elderly and the immunocompromised should avoid foods that could be contaminated with high numbers of these organisms.

Legislation

There is no specific legislation in the EU or the USA on levels of *Aeromonas* species in foods.

Sources of Further Information

Published

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1.1.2 *ARCOBACTER*

Hazard Identification

What is Arcobacter*?*

Arcobacters are potentially pathogenic, gram-negative, non-spore-forming bacteria, often described as aerotolerant *Campylobacter*-like organisms. Arcobacters are closely related to *Campylobacter*, and species in both genera share some similar morphological and metabolic characteristics. Both genera belong to the family *Campylobacteraceae*, however *Arcobacter* species can be differentiated from *Campylobacter* species by their ability to grow in air as well as at lower temperatures. There are currently nine described *Arcobacter* species, but it is *Arcobacter butzleri*, and more rarely *Arcobacter cryaerophilus*, that have been implicated in cases of human illness. On two occasions however, *Arcobacter skirrowi* has been linked to human infection, including in an individual suffering from chronic diarrhoea.

It is thought that the consumption of food contaminated with *Arcobacter* species may play a role in the transmission of these pathogens, although this has not yet been conclusively demonstrated. The most significant source of the organisms is thought to be contaminated water sources, however the organisms are also considered possible zoonotic agents (*i.e.* direct transmission may occur from animals to humans).

Occurrence in Foods

Arcobacters are associated with foods of animal origin and have been detected in beef, poultry, pork and lamb, but are most frequently found in poultry and pork products. Chicken carcasses and poultry processing plants are often contaminated with *Arcobacter* species and the organisms have been isolated from retail chicken and turkey products. However, evidence suggests that eggs are not usually contaminated with these bacteria. They have also been found in raw milk and shellfish (clams and mussels). Arcobacters are not routinely examined for in foods, and so their prevalence in other food types is unknown.

Hazard Characterisation

Effects on Health

Arcobacter butzleri is the most common *Arcobacter* species implicated in human disease. Those most at risk from developing the symptoms associated with *Arcobacter* infection are very young children, although any age group is susceptible. Asymptomatic infections are reported to occur.

The infective dose and incubation time is unknown. Clinical symptoms include abdominal pain, nausea and acute watery diarrhoea, typically lasting 3–15 days, although this can persist or re-occur on occasions for up to two

months. Occasionally, vomiting, fever and chills are reported. Extra-intestinal disease such as septicaemia has also been documented occasionally.

Incidence and Outbreaks

The incidence of *Arcobacter* enteritis is unknown, and outbreaks caused by *Arcobacter* species have rarely been reported. One reason for this may be because these organisms are not routinely included in clinical screening. Three water-borne outbreaks linked to faecally-contaminated water sources are described in the literature: two occurred in the USA and one in Slovenia.

Sources

Humans suffering from *Arcobacter* infections can be a source of *Arcobacter* species and the faecal–oral route is one probable route of transmission.

Arcobacters are a cause of enteritis and abortion in animals, although the organisms can also be isolated from apparently healthy animals. Cattle, pigs, sheep, poultry and even horses are thought to be reservoirs for these bacteria. Although meat and associated products from all these animals could be contaminated with *Arcobacter* species, the organisms are most frequently associated with poultry and pork products. Unlike campylobacters, arcobacters are not considered to be normal inhabitants of the poultry intestine, and it is thought that poultry carcasses become contaminated with the organism after slaughter.

Animal faeces can lead to the contamination of soil and water with *Arcobacter* species. Arcobacters have been isolated from water sources, including drinking-water reservoirs, canals, rivers, lakes and seawater. They have also been found in raw sewage and disinfected effluent.

Growth and Survival in Foods

Arcobacters can be differentiated from the campylobacters in that they are aerotolerant and are able to grow at lower temperatures.

The temperature range, within which arcobacters are able to grow, is between 15 and 37 °C (although some isolates are reported to grow up to 42 °C). The organisms are tolerant of refrigerated storage, although numbers do decrease very gradually over time. Arcobacters survive well when frozen at –20 °C.

Arcobacters can grow or survive in both aerobic and microaerophilic atmospheres and under optimal laboratory conditions cells have survived for at least 250 days. The organisms can grow over a pH range of 5.5–8.5, possibly up to pH 9.0. Arcobacters do not grow at water activities below 0.980.

Thermal Resistance

Arcobacters are relatively heat sensitive and are readily inactivated at temperatures of 55 °C and above. For *A. butzleri*, *D*-values in phosphate-buffered saline at pH 7.3 have been reported as 0.07 to 0.12 min at 60 °C, 0.38 to

0.76 min at 55 °C, and 5.12 to 5.81 min at 50 °C. Reducing the pH has been found to increase the heat sensitivity of the organism. *D*-Values in pork have been reported as 18.51 min and 2.18 min, at 50 °C and 55 °C, respectively.

Control Options

Although there is no direct evidence linking *Arcobacter* to food-borne disease in humans, the presence of the organisms in foods suggests that contaminated foods may play a role in their transmission. Effective controls should therefore focus on prevention of contamination.

Processing

Research suggests that these organisms are not normal contaminants of the poultry gastrointestinal tract, and so concentrating on the prevention of contamination in the poultry processing environment and ensuring the rapid chilling of carcasses could reduce the prevalence of these organisms in associated products.

Product Use

Arcobacters are easily inactivated during normal cooking processes. Consumers should be advised to avoid the consumption of inadequately cooked meat products, and to avoid cross-contamination between raw and ready-to-eat foods.

Legislation

There are no specific requirements for levels of *Arcobacter* species in foods under EU legislation or in the Food and Drug Administration (FDA) Food Code.

Sources of Further Information

Published

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1.1.3 *BACILLUS*

Hazard Identification

What are Bacillus*?*

The *Bacillus* genus is a group of gram-positive, spore-forming bacteria some of which, notably *Bacillus cereus* and more rarely *Bacillus licheniformis*, *Bacillus subtilis* and *Bacillus pumilus*, have been implicated in food-borne disease. Of the *Bacillus* species, *B. cereus* is recognised as the most frequent cause of food poisoning and therefore much of this section will focus on this pathogen. It is important to note that not all strains of *Bacillus cereus* are capable of causing food-borne illness.

Occurrence in Foods

Bacillus species are found in many raw and unprocessed foods. However, *Bacillus cereus* is commonly associated with dried foods, spices, cereals (particularly rice and pasta), as well as milk and dairy products. The presence of low numbers of *B. cereus* in raw foods is of little concern because large numbers of the bacteria (usually $>10^5$ cfu g^{-1}) are required to cause illness. However, *B. cereus* spores can survive cooking processes and high numbers of *B. cereus* spores in herbs and spices can be a problem if these seasonings are used in processed foods where conditions permit the growth of the vegetative cells.

Hazard Characterisation

Effects on Health

Bacillus cereus food poisoning is caused by toxins produced during the growth of the bacteria, and these toxins cause two distinctly different forms of food poisoning—the emetic or vomiting type, and the diarrhoeal type. Both forms of food poisoning require the bacteria to reach high numbers in the food (usually $>10^5$ cfu g^{-1}) before sufficient toxin to cause illness can be produced.

The more common emetic type is caused by the presence of a pre-formed toxin (a heat- and acid-stable, ring-form peptide called ''cereulide'') in the food. It is important to note that live cells of *B. cereus* do not need to be ingested for this form of *B. cereus* food poisoning to occur and foods containing toxin, but no viable cells, can still cause illness. This form of intoxication is characterised by rapid (0.5–6 hours) onset of symptoms, which include nausea, vomiting and sometimes abdominal cramps and/or diarrhoea. Symptoms usually last fewer than 24 hours.

The less common diarrhoeal type is caused by the formation and release of heat- and acid-labile enterotoxins in the small intestine, although enterotoxin can also be pre-formed in food. This 'intermediate' form of food poisoning has an incubation time of 6–24 hours (typically 10–12 hours). Typical symptoms,

which last for 12–24 hours, are primarily watery diarrhoea, abdominal cramps and pain, with occasional nausea and vomiting.

Some strains of *Bacillus subtilis* and *B. licheniformis* linked to outbreaks of food-borne illness also produce heat-stable toxins similar to cereulide. Rapid onset of vomiting is the main feature of *Bacillus subtilis* food poisoning, usually followed by diarrhoea. However, in outbreaks linked to *B. licheniformis*, diarrhoea is usually the main feature of illness, with vomiting occurring in half of cases.

Recovery from food poisoning caused by *Bacillus* species is usually within 24 hours with no complications. Fatalities have rarely been reported.

Incidence and Outbreaks

Due to the usually mild symptoms and short illness caused by *Bacillus* food poisoning it is highly likely that the true incidence of *Bacillus* food poisoning is under-reported.

Nevertheless, in the EU there were 133 reported outbreaks of *Bacillus* food poisoning during 2008 (an increase of 18.1% from 2007). The highest incidence rates were in France and the Netherlands (83 and 15 outbreaks respectively). In England and Wales there were three reported outbreaks of gastroenteritis associated with *Bacillus* species during the five-year period 2005–2009 affecting a total of 59 individuals. This was a reduction on the 21 outbreaks, with 149 cases, reported in the previous five-year period (2000–2004). Annually in the USA there are an estimated 27 360 cases of food-borne illness caused by *Bacillus* species, with no deaths reported.

Most outbreaks of *Bacillus* food poisoning are associated with the consumption of cooked food which has been cooled too slowly and/or incorrectly stored, providing conditions for the microorganism to increase to significant numbers. Outbreaks caused by the *Bacillus cereus* emetic toxin are most frequently linked to starchy foods such as boiled or fried rice, as well as pasta, potato and noodle dishes. The diarrhoeal form of *B. cereus* food poisoning has been linked to a wide variety of foods but is most commonly associated with meat and vegetable dishes, soups, sauces and puddings.

Food poisoning caused by other *Bacillus* species has also been linked to a wide variety of foods including cooked meat and vegetable dishes, cooked reheated rice, 'ropy' bakery products, custard powder, pastries, infant formula, synthetic fruit drinks, mayonnaise, canned tomato juice, sandwiches and pizza.

Sources

Bacillus species are ubiquitous and are widespread in the environment, being found in dust, soil, water, air and vegetable matter. It is thought that climate can influence *Bacillus cereus* populations in soil with surveys indicating that psychrotrophic strains are more dominant in samples from cold regions. *Bacillus* species are also often present in low numbers in human stools, reflecting dietary intake. During bouts of *Bacillus* food poisoning fairly high numbers of the organism will be excreted for up to 48 hours after onset.

Growth and Survival in Foods

The optimum growth temperature range for *B. cereus* is around 30–35 °C with an upper limit of up to 55 °C. Some strains, particularly from milk and dairy sources, are reported as being able to grow at chill temperatures, having a minimum temperature for growth of 4 °C (these are described as psychrotrophic). These psychrotrophic strains usually have a maximum temperature for growth of 37 °C. Psychrotrophic *B. cereus* strains have been shown to produce enterotoxins and research suggests that this may occur at temperatures of 7 °C. Emetic toxin production at refrigeration temperatures is thought not to occur. Although growth of *B. cereus* can occur at less than 10 °C, both lag time and growth rate are significantly increased at these temperatures.

Data on growth temperature ranges for other *Bacillus* species associated with food poisoning is limited. Although there have been occasional reports of some strains of *B. subtilis* and *B. pumilus* growing at 5 °C, these organisms are not generally considered psychrotrophic.

Bacillus cereus can grow under otherwise ideal conditions at pH values between 4.3 and 9.3. The emetic toxin is stable over the pH range 2–11, but the diarrhoeal enterotoxin is less stable at acid pH values.

Some strains of *B. subtilis* and *B. pumilus* can grow at relatively low pH values, and have been implicated in the spoilage of canned tomato products. Whether these strains are capable of causing food poisoning is not known.

The minimum water activity for the growth of *B. cereus* is generally considered as 0.93 but may be as low as 0.91. *Bacillus* spores can survive for extended periods of time in low-water-activity conditions and are resistant to desiccation.

Bacillus cereus and *B. licheniformis* are facultative anaerobes, being able to grow either aerobically or anaerobically, although studies have shown that both growth and toxin production by *B. cereus* are reduced under anaerobic conditions. *B. subtilis* and *B. pumilus* are obligate aerobes. The growth of *B. cereus* is adversely affected by increasing concentration of carbon dioxide, and the use of appropriate gas mixtures in modified atmosphere packaged products can extend safe shelf-life.

The vegetative cells of *Bacillus* species are not notably resistant to commonly used preservatives and sanitisers, but the spores are much more difficult to destroy. The 'natural preservative' nisin, which prevents spore germination, has been shown to be effective at preventing the growth of *Bacillus* species in various food commodities.

Thermal Resistance

The vegetative cells of *Bacillus cereus* are fairly heat sensitive, being readily destroyed by typical pasteurisation processes, but spores are moderately heat resistant and can survive quite harsh heat treatments. *B. cereus* spores can vary in their resistance to heat with D_{85}-values of 33.8–106 min and D_{95}-values of

between 1.2–36 min being described. Spores are more heat resistant in high-fat or low-water-activity products.

The *B. cereus* emetic toxin is heat stable (withstanding 126 °C for 90 min), whereas the diarrhoeal enterotoxins are heat sensitive, being inactivated at 56 °C for 5 min.

Control Options

Processing

The risk from *Bacillus* species in foods is usually highest where the pH and/or water activity of the product will permit the growth of the pathogen. The risk also applies for products designed to be rehydrated by the consumer prior to consumption, such as infant formula and soup mixes. For these foods, control is achieved by ensuring a low initial level of the microorganism in the product. This can be done by using ingredients with low levels of *Bacillus*, as well as by using well-designed equipment with effective cleaning regimes to prevent biofilm formation.

Further control of *Bacillus* numbers is achieved by the appropriate use of temperature, either to destroy spores (sterilisation temperatures used for many low-acid canned products are effective), or to minimise the germination and outgrowth of spores during the manufacture of chilled foods. Heat processes sufficient to inactivate the very heat stable emetic toxin are not practical, and the preferred approach is to prevent its formation before heat is applied. For many refrigerated products, heating processes should be devised so that foods reach processing temperatures quickly, and are cooled rapidly, particularly over the temperature range 10–55 °C. The cooling of small portions is easier to control than large volumes of product. Published cooling processes devised to control *Cl. perfringens* will usually also control the growth of *B. cereus* and other *Bacillus* species.

Product Use

Manufacturers should ensure that *Bacillus cereus* levels do not reach hazardous levels ($>10^3$ cfu g^{-1}) during the shelf-life of the food. Cooked foods should be held hot (minimum 63 °C) prior to consumption, and refrigerated foods should be held at chill temperatures (ideally 4 °C or below) throughout the shelf-life of the product.

Legislation

There are no specific requirements for *Bacillus cereus* and other species in foods under European Community (EC) legislation. EC legislation does require, however, that foodstuffs should not contain microorganisms or their toxins in quantities that present an unacceptable risk for human health.

The UK Health Protection Agency (HPA) has published guidelines on acceptable levels of microorganisms in various ready-to eat foods (see links

below). These state that a level of $<10^3$ cfu g^{-1} of *Bacillus cereus* and other pathogenic *Bacillus* species in these products is satisfactory.

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1.1.4 *CAMPYLOBACTER*

Hazard Identification

What is Campylobacter*?*

Campylobacter species are gram-negative, non-spore-forming bacteria, some of which (*C. jejuni*, *C. coli*, *C. lari, C. hyointestinalis* and *C. upsaliensis*) are associated with gastroenteritis, although most cases of human campylobacteriosis are caused by *C. jejuni*. *Campylobacter* is now the leading cause of bacterial gastroenteritis in many developed countries.

Campylobacter is unique amongst food-poisoning bacteria in that it is not normally able to grow in foods. This is because it has specific atmospheric requirements (microaerophilic conditions) for growth and can only grow at temperatures above ambient.

Occurrence in Foods

Campylobacter is most often associated with fresh poultry meat and related products. A UK Food Standards Agency (FSA) study in 2007–2008 found that the level of poultry carcass contamination in the UK was 65.2%, and studies elsewhere have also found contamination rates of at least 60%, with up to 10^7 *Campylobacter* cells per carcass being recorded. Fresh poultry is more frequently and more heavily contaminated than frozen.

Campylobacter species have also been isolated from other fresh meats such as beef, lamb, pork and offal, but at lower frequencies than in poultry. *Campylobacter* can also be found in raw milk, shellfish, mushrooms and salads.

Hazard Characterisation

Effects on Health

The infective dose for *Campylobacter* may be fewer than 500 cells. Symptoms associated with *Campylobacter* infections appear between 1 and 11 days (typically 2–5 days) after infection. Symptoms can vary widely and usually start with muscle pain, headache and fever. Most cases involve diarrhoea, and both blood and mucus may be present in stools. Nausea occurs, but vomiting is uncommon. Symptoms can last from 1 to 7 days (typically 5 days). The infection is usually self-limiting. *Campylobacter* enteritis is most commonly associated with children (less than 5 years) and young adults. Death rarely occurs, particularly in healthy individuals. However, mortality rates associated with *C. jejuni* in the USA have been estimated at 1 per 1000 cases.

Although complications of campylobacteriosis are rare, arthritis (*e.g.* Reiter's syndrome) can occur and severe abdominal pain can be confused with appendicitis. Reactive arthritis occurs in 1% of cases and 0.1% can suffer Guillain–Barré syndrome (a severe nerve disorder, which can lead to paralysis). Around 15% of those affected recover from Guillain–Barré syndrome, 3–8%

die and the remainder suffer from some degree of disability. Bacteraemia can also occur, particularly in the elderly.

Incidence and Outbreaks

Campylobacter is recognised as the principal cause of bacterial gastroenteritis in the EU and nearly 200 000 cases were reported in the EU in 2008. The majority of these are thought to be food borne. In the USA, the organism is reported to be responsible for 2.4 million cases of illness and 124 deaths annually. A similar situation exists in Canada and other countries. In 2008, New Zealand was reported to have the highest incidence of *Campylobacter* infection in the developed world.

Most cases of *Campylobacter* enteritis are sporadic, or part of small family-related outbreaks, so definitive sources of infection are difficult to establish. However, most cases are thought to be associated with undercooked, or re-contaminated, poultry. Large documented outbreaks are relatively rare, but have been linked to raw and inadequately pasteurised milk, poultry liver parfait and pâté, salads, raw clams, garlic butter, fruits and contaminated water supplies. In one recorded incident in 2005, at least 80 people working at offices in Copenhagen were made ill by contaminated chicken salad in canteen meals.

Sources

Campylobacters are found in the intestinal tract of many warm-blooded animals, such as cattle, sheep, pigs, goats, dogs and cats, although they are especially common in birds, including poultry. Wild birds are thought to be a reservoir for domestic and food animals. Flies and other insects are also thought to be vectors for *Campylobacter*.

If hygiene is poor, infected humans can transfer *Campylobacter* to food *via* the faecal–oral route and asymptomatic carriers have also been reported. Excreta from infected animals can contaminate water and mud, and *Campylobacter* can survive for some time in these environments, particularly when temperatures are low.

Growth and Survival in Foods

As previously stated, *Campylobacter* is unable to grow at temperatures normally used to store food. The temperature range for growth is 30–45 °C, with an optimum of 42 °C. Although survival at room temperature is poor, *Campylobacter* can survive for a short time at refrigeration temperatures—up to 15 times longer at 2 °C than at 20 °C. The organism dies out slowly at freezing temperatures.

The optimum pH for growth is 6.5–7.5, and the organism does not grow below pH 4.9. Survival at acid pH values is temperature dependent, but inactivation is rapid at pH values less than 4.0, especially above refrigeration temperatures.

The minimum water activity for growth is ≥ 0.987 (2% sodium chloride). The organism is sensitive to salt and, depending on temperature, levels of 1% or more can be bactericidal (less effect being observed with decreasing temperature). Although *Campylobacter* is sensitive to desiccation, there are reports of survival for some time on wooden cutting boards.

Campylobacter is microaerophilic, requiring reduced levels of oxygen (5–6%) to grow. The cells usually die out quickly in air, but survive well in modified or vacuum packaging.

Thermal Resistance

Campylobacter is heat sensitive and the cells are destroyed at temperatures above 48 °C. They do not therefore survive normal pasteurisation processes applied to milk. Heat processes targeted at other poultry pathogens (*e.g. Salmonella*) will easily inactivate *Campylobacter*.

Control Options

Processing

Poultry and poultry products are considered to be the main source of *Campylobacter* food poisoning and controls focus on measures to minimise the level of contamination during primary production and processing of poultry meat.

In many EU countries measures are in place to encourage effective biosecurity and hygiene strategies to prevent the introduction of *Campylobacter* to flocks and reduce the incidence of infection. For example, in Denmark, ''Campylobacter-free'' chicken meat can be marketed at a premium price, providing that it comes from flocks that meet required monitoring standards.

Much attention has also been given to measures designed to reduce high rates of cross-contamination during the processing of poultry, particularly chicken, by improving the hygienic design and operation of equipment such as de-feathering machines and immersion chiller tanks.

Product Use

As previously discussed, *Campylobacter* is unable to grow in foods stored at normal temperatures. However, the potentially low infective dose means that undercooking of raw foods and/or cross-contamination from raw to ready-to-eat foods is a major risk factor for human campylobacteriosis.

Clear and effective cooking instructions can help to ensure that the pathogen is destroyed during the cooking stage. Undercooking and/or cross-contamination at barbeques are thought to be linked to an increase in reported *Campylobacter* infections during summer months.

Consumer education and domestic hygiene training can help prevent the transfer of *Campylobacter* from raw to ready-to-eat foods. Consumers should be advised not to wash meat and poultry carcasses prior to cooking to help

prevent water splashes and aerosols from contaminating kitchen surfaces. Any surfaces that could be potentially contaminated, such as in meat preparation areas, as well as chopping boards, should be thoroughly disinfected after use.

Legislation

No specific requirement is made under EC legislation with regard to levels of *Campylobacter* species in food. Requirements for their control are covered under EU general food safety requirements.

The UK HPA has published guidelines on acceptable levels of micro-organisms in various ready-to eat foods (see link below). These state that ready-to-eat foods on the market should be free from thermotolerant *Campylobacter* species and that their presence in ready to eat foods is ''unsatisfactory: potentially injurious to health'' and/or the product is unfit for human consumption.

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1.1.5 *CLOSTRIDIUM BOTULINUM*

Hazard Identification

What is Clostridium botulinum*?*

Clostridium botulinum is a gram-positive, spore-forming bacterium, which produces neurotoxins. It is these toxins (the most potent natural toxins known) that cause the severe illness known as botulism. Some strains of *Clostridium butyricum* and *Clostridium baratii* have also been found to produce botulinum neurotoxins and there have been outbreaks of food-borne illness associated with these species.

There are at least two types of food-borne botulism:

Classic botulism—an intoxication caused by the ingestion of pre-formed toxins in food.
Infant botulism (also known as floppy baby syndrome)—a condition arising from toxins produced when *Cl. botulinum* grows in the intestines of unweaned infants.

Seven different types of *Cl. botulinum* (A–G) are recognised and are typed by the toxin they produce. These seven types are divided into four groups based on physiological differences. When assessing risk, food safety professionals should consider two of these groups:

Group I: proteolytic, mesophilic (comprising types A, B & F)
Group II: non-proteolytic, psychrotrophic (comprising types B, E & F)

Occurrence in Foods

Clostridium botulinum spores are present at low levels in a wide variety of foods. However, surveys to determine levels in foods have concentrated on fish, meat and honey. The highest incidence is in fish, with *Cl. botulinum* type E commonly associated with farmed trout, Pacific salmon and Baltic herring. Types A and B have been isolated in very low numbers from meats such as pork, bacon and liver sausage as well as fruit and vegetables, including mushrooms. *Clostridium botulinum* has also been isolated, usually at low levels, from some honey samples. However, levels as high as 60 cfu g^{-1} have occasionally been reported, and 80 spores per g of types A and B were found in a sample of honey linked to a case of infant botulism.

It is important to remember that most low-acid (pH >4.6) foods stored in conditions that permit the growth of *Cl. botulinum* have the potential to be associated with botulism unless sufficient thermal processing to inactivate spores has been applied.

Hazard Characterisation

Effects on Health

Botulinum toxins are neurotoxins that affect the neuro–muscular junction, leading to muscle paralysis. Botulism is the most severe form of food poisoning and unless it is recognised and treated promptly, it carries a high risk of mortality (35–40%). Prompt treatment can reduce this mortality rate to below 10%. The presence of live organisms is unnecessary for 'classic' food-borne botulism to occur and very small concentrations of pre-formed toxin (possibly as low as a few nanograms) in food can cause illness. The ingestion of viable *Cl. botulinum* spores, at levels as low as 10 to 100 spores, is required for infant botulism to occur.

All individuals are susceptible to classic food-borne botulism and onset times and the severity of symptoms depend on the amount of toxin ingested. Typically, the onset of symptoms occurs within 12–36 hours, although the recorded range is 4 hours to 8 days. Early symptoms may include abdominal distension, mild diarrhoea and vomiting, before more severe neurological symptoms develop. These include blurred or 'double' vision, dryness of mouth, weakness, and difficulties in talking, swallowing and breathing. Death is usually the result of respiratory paralysis. General paralysis may also develop in some cases.

Infant botulism is associated with babies under a year old and symptoms include constipation, poor feeding, lethargy and an unusual cry, as well as a loss of head control.

Incidence and Outbreaks

The incidence of botulism around the world reflects regional eating patterns and outbreaks are relatively rare. The highest nationally reported incidence of botulism in the world is in the Republic of Georgia, where more than 80% of cases are linked to home-preserved vegetables. However, the highest incidence in the EU is in Poland, where a large number of 'high-risk' home-preserved (bottled/canned) foods are consumed. In the USA, infant botulism is the most common form of botulism. In 2009 there were 84 reported cases (no deaths) of infant botulism compared to 11 cases (9% of all reported botulism cases) of food-borne botulism, including one death.

Notable outbreaks in the UK linked to commercially produced foods have been associated with canned salmon, hazelnut conserve used as a flavouring in yogurt and duck paste. Elsewhere 'unusual' foods causing botulism have been baked potatoes, potato salad made from baked potatoes, un-eviscerated dry salted fish, vegetable-in-oil products (such as garlic and aubergines), Brie and Mascarpone cheeses, cheese containing onion and hot and cold smoked fish. A large outbreak in Thailand linked to dishes containing preserved bamboo shoots occurred during the spring of 2006, when at least 143 individuals were taken ill, although there were no fatalities. More recently, two outbreaks occurred in the USA: one in late 2006 is thought to have been caused by temperature-abused, commercially produced carrot juice; while in 2007, deficiencies in the canning process of canned chilli sauce led to eight cases of botulism.

Honey and possibly glucose syrup, are the only food vehicles known to cause infant botulism. However, infant milk powder may have caused a case in the UK.

Sources

Clostridium botulinum is widely distributed in nature, being found in soil and marine environments throughout the world, as well as in the intestinal tracts of animals (including fish). The frequency of isolation and variation of type varies with geographical region. Type A dominates in the Western USA, South America and China, type B in the Eastern USA and the EU and type E in Northern areas and in temperate aquatic environments.

Growth and Survival in Foods

Cl. botulinum is an obligate anaerobe (only grows in the absence of oxygen), but the risk from the pathogen is not limited to products packaged in obviously anaerobic conditions such as canned, bottled or vacuum/modified atmosphere packaging. Conditions in products packed in air can be anaerobic beneath the surface of the food providing a suitable growth environment for the pathogen.

In other respects Group I (proteolytic) and Group II (psychrotrophic, or non-proteolytic) *Cl. botulinum* differ significantly in their growth and survival characteristics.

Group I

The minimum temperature for growth is 10 °C, with a maximum of 45–50 °C and an optimum of 35–40 °C. Both toxins and spores will survive freezing.

The minimum pH for growth is generally accepted as 4.6. This value is important in defining which foods will receive a botulinum cook (see below). For example, in the UK a low-acid food (*i.e.* low in acid and not low pH) is defined as having a pH value equal to or greater than 4.5. *Clostridium botulinum* toxin is stable at low pH but is quickly inactivated at pH 11.

Although the minimum water activity for growth can be affected by solutes in the product it is accepted that 10% sodium chloride (salt), or a water activity of 0.94 is required to inhibit the growth of Group I *Cl. botulinum*.

Group II

The minimum temperature for growth is 3 °C, with a maximum of 40–45 °C and an optimum of 18–25 °C. The ability of Group II *Cl. botulinum* to grow at refrigerated temperatures has raised concerns over products that receive a mild heat treatment and are given an extended shelf-life at chilled temperature, particularly if the products are modified atmosphere/vacuum packaged.

The minimum pH for growth is 5.0.

The salt concentration and water activity value (A_w) required to inhibit the growth of Group II *Cl. botulinum* are 3.5%, and 0.97 respectively.

Other Toxin-Producing Species

The minimum temperature for growth for *Cl. butyricum* and *Cl. baratii* is 7–8 °C, although for *Cl. butyricum* strains known to produce toxins, it is around 10–11 °C.

A recent study has found that the minimum pH for the growth of other *Clostridium* species that may produce botulinum toxins is 4.1, although minimum pH values are influenced by the type of acid in the product.

Clostridium butyricum and *Clostridium baratii* have minimum water activities for growth of 0.95.

Thermal Resistance

Vegetative cells of *Cl. botulinum* are not particularly heat resistant. Heat processes designed to inactivate *Cl. botulinum* target the much more heat-resistant spores of this pathogen.

Group I

Although heat resistance of spores varies between different strains the most heat resistant spores are found from *Cl. botulinum* types in Group I ($D_{121\,°C} = 0.21$ min). Consequently, foods that will be stored at temperatures at 10 °C or above and where conditions can support the growth of *Cl. botulinum* are usually subject to a heat process (known as a "botulinum cook") designed to inactivate Group I spores. This encompasses many canned or bottled products with a pH >4.6. For commercial food-processing purposes a botulinum cook is a process equivalent to 121 °C for at least 3 min at the slowest heating point in the container (an F_o 3 process).

Group II

Group II (psychrotrophic) *Cl. botulinum* spores are not as heat resistant at Group I spores. For refrigerated foods where psychrotophic *Cl. botulinum* can grow (generally pH >4.9 and A_w >0.96), heat processes to inactivate the pathogen need to be applied to the product when it is in its final packaging and should be the equivalent of a minimum of 90 °C for 10 min. At-risk products—especially, but not exclusively, those that are modified atmosphere or vacuum packed—receiving a lesser heat treatment should have a very limited refrigerated shelf-life to prevent the outgrowth of any viable *Cl. botulinum* spores.

All toxins produced by *Cl. botulinum* are heat labile and can be inactivated by heating at 80 °C for at least 10 min. However, toxins may be more heat stable at lower pH values.

Control Options

The ubiquitous nature of *Cl. botulinum* means it must be assumed that spores could be present in all raw food. It should be remembered that the growth of

Group II, non-proteolytic (psychrotophic) *Cl. botulinum* does not cause obvious spoilage and can easily go undetected in foods. Group I *Cl. botulinum* growth is proteolytic and usually causes detectable spoilage.

Processing

Prevention of spore outgrowth and subsequent toxin production in foods can be achieved both by applying an effective thermal process as described above and by careful product formulation. For at-risk foods, there are a number of published processing guidelines and codes of practice, and these should be strictly followed where applicable.

Any change to a process or product formulation should be carefully evaluated using an HACCP approach and adequate controls implemented to ensure either the destruction or control of the growth of *Cl. botulinum*.

Factors that can be used to control the growth of *Cl. botulinum* are given in Table 1.1.1.

Although Group II *Cl. botulinum* strains will not grow below 3.0 °C, refrigeration alone should not be used to prevent growth for extended periods, except under very controlled and monitored conditions, because of the difficulty in maintaining the very low temperatures required. It is usually recommended that refrigerated processed foods with extended durability (REPFEDS), or sous-vide products, are heated to 90 °C for 10 min, or equivalent, to ensure safety with regard to Group II *Cl. botulinum*.

It is also important to note that *Cl. butyricum* can grow at lower pH values than Group I *Cl. botulinum* strains and this should be considered in acid products with a pH >4.0.

Preservatives can effectively control the growth of *Cl. botulinum* in foods. For example, nitrite is used, in combination with other factors (often referred to as hurdles) in cured meat products. Sorbates, parabens, polyphospates, phenolic antioxidants, ascorbates, EDTA, metabisulfite, *n*-monoalkyl maleates and fumarates, lactate salts and liquid smoke (in fish) can all be used as additional hurdles in the control of *Cl. botulinum* under certain circumstances, although specific use should always be validated. The natural bacteriocin nisin is sometimes used to prevent the germination of *Cl. botulinum* spores in products such as canned vegetables and processed cheese.

Table 1.1.1 Factors that can be used to control the growth of *Cl. botulinum*.

	Group I (proteolytic)	*Group II (non-proteolytic, psychrotrophic)*
pH	<4.6	<5.0
Water activity (A_w)	<0.94	<0.97
Temperature/°C	<10	<3.3
Heat processes (in sealed final container)	121 °C for 3 min, or equivalent	90 °C for 10 min, or equivalent

Product Storage and Use

Foods stored at ambient temperatures should never rely on shelf-life as a control for *Cl. botulinum*. These products should be formulated, and/or heat processed, to ensure the prevention of growth of the pathogen, or the destruction of spores.

For chilled foods where the pH and water activity could potentially permit the growth of psychrotrophic *Cl. botulinum* (*e.g.* many ready meals, chilled low-acid sauces and cooked meat products) and where a 90 °C for 10 min or equivalent process in the final packaging has not been implemented, the UK Advisory Committee on the Microbiological Safety of Food (ACMSF) has given advice on restricting shelf-life to control the growth of *Cl. botulinum*. In 2008 the FSA published guidance on the safety and shelf-life of vacuum packed and modified atmosphere packaged chilled foods with respect to non-proteolytic *Cl. botulinum*, which includes a decision tree for use in determining the shelf-life of these products when stored above 3 °C. A link to the publication is given below.

Well-chosen food packaging can play in role in reducing the risk from botulism. An outbreak of botulism associated with film-wrapped mushrooms in the USA, where product respiration had quickly provided an anaerobic environment permitting growth and toxin production by *Cl. botulinum* naturally present on the produce, led to advice to suppliers to ensure holes at the bottom of containers of pre-packed mushrooms.

Infant botulism is controlled by advice to parents not to give their infants at-risk foods. These foods, notably honey, are recommended to carry warnings on their labels that they are not suitable for infants under 12 months of age. In addition, the ACMSF has recommended that honey should not be added to foods specifically targeted at infants less than 12 months old (unless the foods receive a full botulinum cook or an equivalent process control).

Legislation

No specific requirement is made under EU legislation with regard to levels of *Cl. botulinum* in food. Requirements for its control is covered under EC general food safety requirements in which food should not be sold if it is unsafe.

Sources of Further Information

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1.1.6 *CLOSTRIDIUM DIFFICILE*

Hazard Identification

What is Clostridium difficile*?*

Clostridium difficile is a gram-positive, anaerobic, spore-forming bacterium typically associated with gastrointestinal disease amongst elderly patients in healthcare settings, such as hospitals and long-term care facilities. However, there is some evidence to support the hypothesis that food may play a role in the transmission of the organism, although there has not yet been any direct correlation between illness in humans and a specific foodstuff. It is therefore not unreasonable to regard *Cl. difficile* as a potential food safety hazard.

Pathogenic strains of *Cl. difficile* are toxigenic and can produce two principle toxins, known as *Cl. difficile* toxins A and B, although a small number of strains only produce toxin B. Toxin A is an enterotoxin whereas toxin B is a more potent cytotoxin. A third unrelated toxin, known as *Cl. difficile* binary toxin, is also produced by some strains.

Of growing concern is the emergence, and worldwide spread, of highly pathogenic strains of *Cl. difficile*, in particular ribotype 027.

Occurrence in Foods

Clostridium difficile has been isolated from a variety of foods, including spoiled vacuum-packed meat products, ground beef, veal, chicken, turkey, pork, sausages, braunschweiger, vegetables and ready-to-eat salads. Many of the isolates were found to be toxigenic and were related to strains associated with human illness.

Sources have reported a higher prevalence of *Cl. difficile*-contaminated retail meat products in the winter months. Levels of *Cl. difficile* spores are reported as low in pork and beef samples (on average 30 and 100 spores per g, respectively).

Hazard Characterisation

Effects on Health

Clostridium difficile infections are most commonly associated with patients in hospital who have been given broad-spectrum antibiotic therapy. These antibiotics inactivate other competing bacteria, which normally live in the gut and inhibit the germination of *Cl. difficile* spores. The resulting suppression of the normal gut flora makes these patients more vulnerable to *Cl. difficile* infection. A further risk factor is increasing age; the elderly (>65 years) are much more susceptible to *Cl. difficile* infection and over 80% of reported infections occur in this age group. The organism also causes disease in animals, most notably enteric disease in piglets although it has also been implicated as the agent causing diarrhoea in calves.

Transmission of *Cl. difficile* is normally considered to be by the faecal–oral route, but there is a suggestion that *Cl. difficile* could also be a food-borne pathogen. This hypothesis is supported by a rise in infections amongst individuals in non-healthcare settings (*i.e.* in the wider community), including the young as well as individuals who have not recently been taking antibiotics, and the results of recent studies identifying similarities between isolates from food animals, food, and humans.

The severity of *Clostridium difficile* infections varies. In mild cases the infection causes non-bloody diarrhoea and sometimes abdominal pain, nausea, vomiting, dehydration and low-grade fever. However, infections can be very severe causing ulceration and bleeding from the colon (colitis). This may result in perforation of the intestine leading to peritonitis, which can be fatal. When colitis is present reported death rates vary from 6 to 30%.

The presence of *Cl. difficile* spores in the gut does not necessarily lead to disease. Although not usually considered as part of normal gut flora, asymptomatic carriage of *Cl. difficile* has been reported in around 3% of adults and in up to 70% of newborn babies. Conditions in the gut need to be suitable for the spores of pathogenic strains to germinate and produce the exotoxins that cause illness. Toxin A is an enterotoxin that causes fluid secretion and inflammation whereas toxin B is a more potent cytotoxin. *Clostridium difficile* toxins A and B account for the majority of illness associated with the organism. A third unrelated toxin, known as *Cl. difficile* binary toxin, is also produced by some strains although its role in disease is unknown.

The increasingly frequent and widespread isolation of emerging highly pathogenic strains of *Cl. difficile*, notably ribotype 027, is a particular concern. Along with a high sporulation rate perhaps contributing to greater persistence in the environment, this strain produces all three toxins and has a mutation that allows it to produce those toxins in larger quantities. It is associated with more severe disease, increased transmissibility between individuals, and a three-fold greater mortality rate.

Incidence and Outbreaks

In the USA, reports suggest that more than 3 million *Cl. difficile* infections occur in hospitals each year. In England reported cases have decreased significantly in the last few years. There were 55 498 in 2007–2008, while in the period 2008–2009 only 36 095 cases were reported. Outbreaks of *Cl. difficile* infections have been reported in healthcare settings in a number of European countries and in the USA, Canada and Japan. In the past decade reported outbreaks seem to have increased in occurrence and severity, with high mortality rates and poor response to treatment. However none have been definitely attributed to a food source.

Of growing concern is the increased reported incidence of *Cl. difficile* infections in the community, occurring in individuals with none of the known risk factors. In the USA it is estimated that around 20 000 infections occur in the community, and according to one report, community cases in the UK have

risen from less than 1 case per 100 000 to 22 cases per 100 000 in the ten-year period from 1994 to 2004. It is possible that some of these community cases may be caused by food-borne *Cl. difficile*.

Sources

Clostridium difficile has been found in a wide range of environmental and animal sources. It is a common soil organism and has been isolated from seawater, fresh water and plant material as well as vegetables. It has also been found in the environment in hospitals, in farm animals (including their faeces), the domestic environment, pets and healthy humans. Studies have shown that many of the isolates found in food animals and in humans are indistinguishable, although the original source of these strains is unknown.

There is potential for foodstuffs to become contaminated with *Cl. difficile* at many points in the food production chain. Contamination could arise from soil, irrigation waters and animal manure, the slaughter and/or processing environment, and possibly from infected food handlers.

Growth and Survival in Foods

Although vegetative cells of *Cl. difficile* only survive on surfaces for a few days, spores of the organism can survive in the environment for many months, or even years. Spores are also highly resistant to many disinfectants and it is possible that they survive common cleaning and disinfectant procedures.

Thermal Resistance

Clostridium difficile spores are relatively heat resistant; they have been shown to survive 71 °C for 120 min. Spores of the organism are therefore likely to survive the heat processes recommended for the inactivation of low levels of vegetative pathogens, such as verocytotoxin-producing *E. coli* and *Salmonella*, in meat products. It is probable that, if present in the raw materials, viable spores of *Cl. difficile* strains pathogenic to humans are sometimes ingested in certain food products, including burgers, ready-to-eat cooked meats, chilled ready meals and salads.

Control Options

At present the public health significance of *Cl. difficile* in foods is uncertain. It is highly likely that low numbers of spores of potentially pathogenic strains are occasionally ingested *via* foodstuffs, but whether this is linked to illness in susceptible individuals in the short term, or later if the health status of the individual becomes compromised, is still unknown.

Until the matter is resolved, food producers should continue to apply the food safety and hygiene practices that are used to control known food-borne pathogens.

Legislation

There is no specific legislation in the EC or in the USA regarding the levels of *Cl. difficile* in foods.

Sources of Further Information

Published

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1.1.7 *CLOSTRIDIUM PERFRINGENS*

Hazard Identification

What is Clostridium perfringens*?*

Clostridium perfringens is a gram-positive spore-forming bacterium and is a relatively common cause of food poisoning. It is an anaerobe, although it can also grow in the presence of very low levels of oxygen. *Clostridium perfringens* was previously known as *Clostridium welchii*, and the organism may be referred to by this name in older references. *Cl. perfringens* strains are classified by the types of exotoxin they produce (types A–E). Most cases of *Cl. perfringens* food poisoning are caused by type A strains, although type C strains can also produce the enterotoxins that cause *Cl. perfringens* food poisoning.

Occurrence in Foods

Clostridium perfringens can be found in low numbers in many raw foods, especially meat and poultry, as the result of soil or faecal contamination. Spores of *Cl. perfringens* will survive many heating and drying processes, and the presence of low numbers of the spores in raw, cooked and dehydrated products is not necessarily a cause for concern because high numbers of vegetative cells are required to cause illness. In addition, research has suggested that only strains of *Cl. perfringens* repeatedly exposed to heating are able to cause food poisoning and that strains freshly isolated from the environment do not.

Hazard Characterisation

Effects on Health

Clostridium perfringens food poisoning is a relatively mild form of food poisoning and is caused by strains that produce enterotoxins (it is important to note that not all strains of *Cl. perfringens* are enterotoxin producers). The enterotoxins are produced when vegetative cells of the bacterium start to multiply in the human intestine and then sporulate. During sporulation, the organism also releases the enterotoxin that causes the symptoms associated with food poisoning. Some cases of *Cl. perfringens* food poisoning have a reported very rapid onset of illness, suggesting that toxin was pre-formed in food. However, toxin pre-formed in food is not usually at sufficient levels to cause illness, although low levels may contribute to a rapid onset of symptoms.

High numbers ($>10^5$ per g, usually 10^6–10^8 per g) of viable vegetative cells of enterotoxin-producing *Cl. perfringens* are necessary to cause food poisoning. Symptoms generally appear 8–22 hours (typically 12–18 hours) after ingestion of contaminated food and usually comprise profuse watery diarrhea and severe abdominal pain. Vomiting and nausea occur only rarely. The duration of illness is short, usually lasting for 24 hours and not exceeding 48 hours. In the majority

of cases there is a full recovery, although occasional deaths do occur in elderly and debilitated individuals.

Incidence and Outbreaks

There is little information on the incidence of *Cl. perfringens* food poisoning. However, because of its mild nature, it is probable that it is grossly under-reported, even in countries with well-developed disease-reporting systems. In the EU, the number of reported outbreaks caused by food-borne *Cl. perfringens* has declined in recent years. There were a total of 27 reported outbreaks in England and Wales during the period 2005–2009, with 50–500 people being affected annually. In the USA, the Centers for Disease Control and Prevention (CDC) has estimated that there are around 248 000 annual cases of illness caused by food-borne *Cl. perfringens*, resulting in 41 hospitalisations and seven deaths.

Outbreaks of *Cl. perfringens* are usually associated with meat dishes and are frequently linked to facilities or events catering for large numbers of people, such as institutions, restaurants or receptions. Dishes prepared and cooked in large quantities can be difficult to cool quickly to refrigeration temperatures, or can be held at improper temperatures and served warm instead of piping hot. Slow cooling or holding of food at incorrect temperatures can result in the germination of surviving *Cl. perfringens* spores and the rapid multiplication of vegetative cells. The organism can grow extremely rapidly and relatively short times at abuse temperatures can give rise to sufficient numbers of vegetative cells to cause illness.

Cooked meat and poultry products are often associated with *Cl. perfringens* food poisoning because spores of *Cl. perfringens* are likely to be present and protected from extreme heat at the centre of stuffed poultry, rolled meats and meat pies. Cooling at the centre of these products can be slow, oxygen levels are low and the food is protein-rich, providing ideal conditions for the outgrowth of surviving spores. Anaerobic conditions are also created during the rapid boiling of gravies, casseroles and stews, and if improperly cooled or held at inappropriate temperatures, these products too may be the cause of *Cl. perfringens* food poisoning. Cured meats are rarely involved in *Cl. perfringens* food poisoning; in these products the combined effect of preservatives and heat processing effectively control the growth of the organism.

Non-meat-derived foods such as vegetable curries and soups have also been associated with outbreaks of *Cl. perfringens* food poisoning, although fish and fish products are rarely implicated.

Sources

Cl. perfringens is ubiquitious and spores of *Cl. perfringens* type A are widely distributed in the environment. *Cl. perfringens* spores are found in soil and dust, as well as in the faeces of many animals. Well-manured soil can have high numbers (10^3–10^4 per g) of the spores present. *Cl. perfringens* spores are present as part of the normal faecal microflora of humans (typically 10^3–10^4 per g) and

it has been reported that healthy individuals can act as reservoirs for *Cl. perfringens* type A strains carrying enterotoxin genes. Contaminated food handlers could therefore potentially play a role in the spread of *Cl. perfringens* type A food poisoning.

Growth and Survival in Foods

Cl. perfringens can grow over the temperature range 15–55 °C, and growth does not occur below 10–12 °C. The optimum temperature for growth is 43–47 °C, and at these temperatures *Cl. perfringens* has the fastest recorded growth rate (shortest generation time) of any bacterium. Generation times (time for a defined population to double in size) of around 7 min at 41 °C have been recorded although 10 min is more typical. The optimum temperature for enterotoxin production is 35–40 °C.

Cl. perfringens vegetative cells die out relatively rapidly (93.5% were killed after 30 days at −17.7 °C) at freezing temperatures. However, they die out less quickly during storage at chill temperatures. Spores survive both refrigeration and freezing.

The optimum pH for the growth of *Cl. perfringens* is 6.0–7.0, the pH of most cooked meat and poultry products. The organism is able to grow over the pH range 5.0–8.3 under otherwise ideal conditions. The spores can survive more extreme pH values.

The minimum water activity for spore germination and growth of *Cl. perfringens* is 0.94–0.95, but minimum values will be affected by the nature of the solute. Vegetative cells are not very tolerant of low water activity but spores are very resistant to desiccation.

Cl. perfringens is an anaerobe and grows best when oxygen is absent or present at very low levels, such as at the center of cooked meat and poultry dishes. It is unable to grow on the surface of foods unless they are vacuum or modified atmosphere packaged.

The vegetative cells are not especially resistant to preservatives and sanitisers, but the spores are much more resistant. Curing salts used in meat products can be effective at controlling *Cl. perfringens*, but unacceptably high levels of sodium nitrite are required to inhibit the growth of the pathogen when it is used as the sole preservative. However, in combination with sodium chloride, sodium nitrite can be effective at preventing its growth.

Thermal Resistance

Vegetative cells of *Cl. perfringens* are not very heat resistant and will usually be inactivated at temperatures exceeding 60 °C. The heat resistance of *Cl. perfringens* spores has been shown to vary significantly. D_{90}-Values of between 0.015 and 8.7 min, and at 110 °C of between 0.5 and 1.29 min have been recorded. Some spores have been shown to survive boiling for one hour.

The enterotoxins are heat labile and heating food to >70 °C throughout will inactivate enterotoxin.

Control Options

A HACCP approach to the control of *Cl. perfringens* in food is preferred and control measures focus on effective temperature control.

Processing

The key control for *Cl. perfringens* during processing is the rapid cooling of high-risk product after cooking, especially through the temperature range 55–15 °C, followed by storage at temperatures below 4 °C (although below 10 °C ensures no growth of *Cl. perfringens*, refrigeration below 5 °C is essential to control other pathogens). In some countries there is legislation, and in others published guidelines, for the rapid cooling of various products (see legislation section).

When developing a product, food processors should ensure that the intended use of the product should not pose a risk to the consumer. For example, in a dehydrated soup product, acceptable levels of *Cl. perfringens* in the dried ingredients should take into consideration that the consumer will be re-hydrating the product by adding hot water and that it will later be consumed warm.

Product Use

Food to be served hot should either be freshly cooked and kept hot at temperatures not permitting the growth of *Cl. perfringens* (>63 °C), or if cooked product is reheated, it should reach temperatures that inactivate vegetative cells and enterotoxin (at least 72 °C throughout the product).

Legislation

EU regulations, and the FDA Food Code do not have specific requirements relating to levels of *Cl. perfringens* in foods.

However, the EFSA's Scientific Panel on Biological Hazards has recommended that, "when new or modified products are developed, that might support the growth of *Cl. perfringens* and/or enterotoxin production, processors should ensure that target levels of 10^5 per g are not exceeded under the anticipated conditions of storage and handling." In addition, the HPA has issued guidelines for assessing the microbiological safety of ready-to-eat foods placed on the market. These state that levels of *Cl. perfringens* of 10–10^4 per g in these products is a moderate microbiological risk, and levels >10^4 per g are potentially injurious to health and/or the product is unfit for human consumption.

In the UK and the USA there are requirements for the control of temperature aimed at limiting the growth of *Cl. perfringens* in at-risk foods.

The USA has a mandatory requirement for the times and temperatures used during the cooling of large joints of meat. These are the same as guidelines published in the UK by Campden & Chorleywood Food Research Association (now Campden BRI) for the chilling of a large piece of meat, and they are summarised in Table 1.1.2.

Table 1.1.2 Guidelines published in the UK by Campden BRI for the chilling of a large piece of meat.

	Good practice/hours	*Maximum/hours*
>50 °C	1	2.5
50–12 °C	6	6
12–5 °C	1	1.5
Total cooling time/hours	8	10

For chilled prepared foods (excluding cook–chill foods used within integrated catering systems) a guideline by the UK Chilled Food Association advises that a heated product should be cooled as quickly as possible through the temperature range 63 °C to 5 °C or less to minimise the risk of spore germination and outgrowth. The time taken for cooling will vary from product to product, but as a guideline, should be no more than 4 hours. Rapid cooling for these products can be facilitated by preparing product in relatively small portions/packages and ensuring their separation during the cooling process.

There are also requirements in the USA under the FDA Food Code (2005) for the cooling of potentially hazardous cooked food. The code requires that these should be cooled:

1. Within 2 hours from 57 °C (135 °F) to 21 °C (70 °F); and
2. Within a total of 6 hours from 57 °C (135 °F) to 5 °C (41 °F) or less, or to 7 °C (45 °F) or less (under certain conditions).
3. Product prepared from ingredients at ambient temperatures, such as reconstituted foods and canned tuna, and which are potentially hazardous food, should be cooled within 4 hours to 5 °C (41 °C) or less, or to 7 °C (45 °C) under certain circumstances.

For the hot holding of foods, UK legislation requires food served hot to be held at temperatures >63 °C. In the USA, food that ''is received hot'' should be at a temperature of 57 °C (135 °F) or above.

Sources of Further Information

Published

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1.1.8 *CRONOBACTER*

Hazard Identification

What is Cronobacter*?*

Cronobacter species are gram-negative non-spore-forming bacteria belonging to the family *Enterobacteriaceae* and closely related to *Enterobacter* and *Citrobacter*. *Cronobacter* is a relatively recently described genus (2007) comprising six species, *C. sakazakii*, *C. malonaticus*, *C. turicensis*, *C. muytjensii*, *C. dublinensis*, and a sixth, as yet unnamed species. Prior to 2008, these organisms were referred to as a single species, *Enterobacter sakazakii*, or as yellow-pigmented *Enterobacter cloacae*. The most commonly isolated species is *C. sakazakii*, and it is probable that the majority of older publications refer to this species.

All species of *Cronobacter* have been associated with clinical infection in infants and adults and should therefore be considered as potential pathogens.

Occurrence in Foods

Cronobacter species are of most concern in infant formula, where the organism has been isolated from both powdered and rehydrated product. However, recent studies have isolated *C. sakazakii* from ''follow-on formulas'' (products usually marketed as suitable for infants 6–12 months old) as well as other infant-weaning food and drink products.

Cronobacter species have been found in a wide variety of other food products including dried milk powders, dried infant foods, cereals, rice products, wheat, soy, corn, bread, tea, dried herbs and spices, lettuce, mung bean and alfalfa sprouts, fermented cassava, vegetables, cheese products, confectionery, eggs, crab meat, minced beef and sausages. However, these foods have not been linked to *Cronobacter* infections.

Hazard Characterisation

Effect on Health

Cronobacter infections have most frequently been associated with illness in infants fed with contaminated rehydrated powdered-milk-based formula, particularly those in intensive-care units. Infants at greatest risk of infection appear to be premature babies who are immunosuppressed and those of low birth weight. However cases have also been reported in full-term newborns, as well as infants and children up to three years of age.

The infectious dose is unknown, although reports suggest that 1000 cells may cause illness. Current knowledge indicates that <3 cfu $100\,g^{-1}$ in powdered infant formula followed by multiplication after reconstitution can lead to infection. Symptoms of infection are bloody diarrhoea, and in rare cases sepsis and meningitis, which can result in high death rates. Due to the worldwide

under-reporting of infections, the long-term effects for children who survive infection are unclear, however mental retardation and quadriplegia have been reported.

Cronobacter infections have been reported, albeit rarely, in other age groups. Adults most at risk are the elderly, the immunocompromised and those suffering from conditions where the pathogen can survive passage through the stomach. *Cronobacter* usually cause bacteraemia in adults, although urinary tract and wound infections have also been reported. However, there is little reported evidence of any risk to older children and adults from *Cronobacter* in food. A report published by the New Zealand Food Safety Authority in May 2004 assessing the risk of *Cronobacter* species (then *E. sakazakii*) in dairy products concluded that, ''There is no evidence however that *E. sakazakii* poses any significant risk to general populations consuming food products that comply with recognised international food-processing or public health standards. While there have been eight cases of *E. sakazakii* infections reported in adults suffering from underlying health problems no connections to food could be made in any of these episodes.''

Incidence and Outbreaks

Reported *Cronobacter* infections are rare, though cases in infants have occurred in a large number of countries including England, Canada, the Netherlands, Belgium, Israel, the USA, New Zealand and France. The first documented neonatal death caused by *Cronobacter* was in 1958. However, it was not until 2001 that an outbreak was linked to a food product when the microorganism was isolated from an unopened can of powdered infant formula. On previous occasions it had been difficult to determine whether outbreaks were caused by product contaminated with the pathogen after opening, or the outbreak strain was present as part of the manufacturing process.

According to the World Health Organization (WHO), since 1958 there have been 120 reported cases (to July 2008) of neonatal and infant *Cronobacter* infections worldwide, with at least 27 deaths. However it is highly likely that not all infections are reported. In the USA the reported annual rate of *Cronobacter* infections is around 1 per 100 000 infants, rising to 9.4 per 100 000 in infants of very low birth weight (defined as <1.5 kg). *Cronobacter* infections are ten times more likely in infants fed formula than those fed breast milk, although a case reported in Brazil in 2003 suggested transmission *via* breast milk.

Worldwide there are only 13 documented cases (to 2009) of adults contracting *Cronobacter* infections.

Sources

Cronobacter species are ubiquitous, being widespread in both the environment and in plant material. Studies have isolated the organism from a range of sources including food, water, dust, soil, mud, vacuum cleaner residue, flies, rodents and various sources within clinical environments, including neonatal feeding tubes. Asymptomatic human carriers have also been reported.

Growth and Survival in Foods

Strains of *Cronobacter* have been reported to grow over the temperature range 5.5 to 47 °C. Some outbreaks have been associated with temperature-abused, reconstituted, powdered infant formula, and studies indicate that *Cronobacter* has a doubling time of 40–94 min at 21 °C. Cells survived freezing in reconstituted powdered infant formula for six months without any decrease in numbers.

The organism has a minimum pH for growth of 3.89. It has been found to survive mild acid conditions and induced acid resistance at pH 3 has also been demonstrated.

The maximum reported salt concentration allowing growth is 9.1%. However, *Cronobacter* survives for extended periods in low-water-activity environments such as that found in powdered infant formula (water activity around 0.2), particularly when stored at low temperature. The organism survives desiccation processes (*e.g.* spray drying), and its ability to survive dry conditions for more than two years has been attributed to capsule formation. *Cronobacter* has been reported to be more resistant to osmotic stress than a number of other members of the *Enterobacteriaceae*.

Cronobacter species have been shown to form biofilms on latex, polycarbonate, silicon rubber and glass.

Thermal Resistance

Initially it was thought that *Cronobacter* species were relatively heat resistant. However, there is no evidence to suggest that they survive typical milk-pasteurisation treatments and studies have confirmed that the organism is not as thermotolerant as *Listeria monocytogenes*.

There is some evidence to suggest that when rehydrated, previously desiccated *Cronobacter* cells may have different thermal inactivation characteristics to those not exposed to dry conditions, and there is also a degree of variability in thermal inactivation characteristics between different strains. Therefore reported *D*-values and *z*-values (the temperature change resulting in a ten-fold change in *D*) do vary. Studies in reconstituted powdered infant formula indicate *D*-values of 1.1–4.4 min at 60 °C, with *z*-values of 2–14 °C.

Reheating infant formula by applying microwave heating until the first signs of boiling has been shown to be particularly effective in inactivating *Cronobacter*. However, concerns have been expressed that this may present a scalding hazard to infants.

Control Options

Processing

Powdered infant formula is a product intended for newborn infants through to six months of age. Although it is not a sterile product its intended use results in its manufacture being subject to very strict hygiene controls and

microbiological criteria. In an opinion published in September 2004, the EFSA concluded that, "*E. sakazakii* is inactivated by the pasteurisation processes used in the manufacture of infant formula. However due to the widespread occurrence of the microorganism it appears very difficult to control it in the processing environment, and as a consequence the recontamination of the product does occur during handling and filling processes." EFSA has advised that measures to reduce the risk of *Cronobacter* recontaminating product during manufacture should include:

- Using ingredients of good microbiological quality.
- Closely monitoring and controlling the levels of *Enterobacteriaceae* in the production environment using the results to indicate the likely presence of pathogens such as *Cronobacter*.
- Imposing strict hygiene measures such as the control of movement of personnel, the separation of wet and dry processes, and avoiding condensation and water ingress in dry areas.

Product Use

Infant Formula

Although the dose–response relationship of *Cronobacter* infections in humans is unknown, evidence indicating the widespread occurrence of the organism would suggest that the consumption of low numbers of the pathogen in infant formula is unlikely to cause illness in healthy infants. To protect the most at-risk infants, food safety experts in many countries have advised that where possible commercially sterile ready-to-use infant formula should be used in neonatal intensive-care settings.

The storage of reconstituted product at temperatures in excess of 5 °C can lead to a rapid increase in numbers of pathogens, including *Cronobacter* species and both EFSA (2004) and the WHO (2007) have advised on the safe preparation, handling, storage and use of infant formula in the home and in hospitals. A joint FAO/WHO expert meeting held in January 2006 recommended that product labels should be revised so that safe preparation instructions and other safety information is included on the packaging of dried infant formula products. In 2007 the WHO recommended rehydration of infant formula at no less than 70 °C to minimise the risk from the pathogen, and that the prepared product is used immediately (within three hours). However concerns have been expressed that this could pose a potential scalding hazard to infants and carers, as well as causing nutrient loss and clumping of powder. Nevertheless, the WHO has called for manufacturers to review instructions on product packaging where the use of cooled boiled water is recommended. These instructions can lead to reconstitution at 50 °C, a temperature that generally poses the greatest risk with regard to *Cronobacter* unless the product is consumed immediately. The WHO has called for manufacturers to include

instruction steps that result in sufficiently high temperatures to reduce the risk from *Cronobacter*.

In 2007 the FAO/WHO published an online quantitative risk assessment model for *Cronobacter sakazakii* in powdered infant formula. The model considers the risk of powdered infant formula that is contaminated with *C. sakazakii*, examining the impact of different preparation and handling strategies on the organism (a link to the model is included later in this chapter).

Follow-on Formula

A FAO/WHO report published in 2008 discussed the potential *Cronobacter* risk posed by follow-on formula (products aimed at weaning infants from 6–12 months of age). Sporadic cases of *Cronobacter* infections have occurred in infants >6 months of age and there is also evidence that carers may feed these products to infants less than six months of age (occasionally <1 month old), despite existing regulations and label recommendations. Follow-on formula products can contain a wider variety of dry-mix ingredients aimed at increasing the diversity of foods in an infant's diet. However, the microbiological quality of these additional ingredients may not meet the same standards as those required in the manufacture of powdered infant formula.

In 2009 the Codex Alimentarius Commission (CAC) concluded that based on current epidemiological evidence, there is no requirement to establish microbiological criteria for *Cronobacter* in follow-up formula. Instead the CAC called for clearer labelling of follow-on products so that misuse of the product can be addressed, as well as improving education of healthcare professionals and carers on the appropriate use of these products.

Legislation

The EU regulation for microbiological criteria for foodstuffs (EC Regulation No. 2703/2005), which came into force in January 2006, has specific requirements with regards to limits for *Enterobacteriaceae* and *Cronobacter* species (*Enterobacter sakazakii*) in dried infant formula and dried dietary foods for special medical purposes intended for infants below six months of age. These state that *Cronobacter* species (*Enterobacter sakazakii*) should be absent in 10 g of product.

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1.1.9 ENTEROCOCCI

Hazard Identification

What are the Enterococci?

The enterococci belong to a genus of gram-positive, non-spore-forming bacteria previously known as Lancefield's group D streptococci, or faecal streptococci. At least 20 *Enterococcus* species have been described, but the most common species associated with foods and human disease are *Enterococcus faecium* and *Enterococcus faecalis*. The enterococci are recognised as the causative agents of a number of non-food-borne clinical infections, such as bacteraemia and endocarditis, and in recent years there has been increasing concern over the number of emerging vancomycin resistant enterococci strains (VREs).

However, the enterococci are also important in food microbiology for a number of seemingly opposing reasons. When present in food they can be viewed as potential pathogens very occasionally associated with outbreaks of food-borne disease, as important spoilage microorganisms of dairy and meat products, as starter microorganisms used in the production of various traditional fermented foods, or even as probiotic microorganisms. It is important to remember that the possession of 'virulence' factors (*i.e.* the ability to cause disease) and resistance to antibiotics are strain specific and that many strains are entirely non-pathogenic.

Occurrence in Foods

Enterococci are found in a wide variety of foods. They are common contaminants of milk and meat products and are used as starter cultures in some traditional European cheeses. They are also found on plant materials such as olives and vegetables.

Hazard Characterisation

Effects on Health

Symptoms associated with food-borne outbreaks associated with *Enterococcus* species have been described as "milder than *Staphylococcus* food poisoning." Human volunteer feeding studies have been conflicting and so the description of *Enterococcus* food poisoning is vague and variable. All individuals are thought to be susceptible to food poisoning caused by enterococci.

The infectious dose for food-borne outbreaks is thought to be high ($>10^7$ cells), and the incubation period is reported to vary widely (between 2–60 hours). Symptoms described include abdominal cramps, diarrhoea, nausea, vomiting and dizziness. The disease is thought to be typically of short duration and self-limiting.

Incidence and Outbreaks

There is very little data on the incidence of food-borne enterococcal infections. It is the presence of high numbers of *Enterococcus* species and the absence of other food-borne pathogens that has caused some outbreaks of food-borne disease to be linked with the enterococci. However, it is important to remember that many foods (*e.g.* cheeses) can contain high numbers of these bacteria without causing illness.

Foodborne outbreaks have been associated with sausages, ham, meat croquettes, meat pie, raw milk, pasteurised milk, evaporated milk, cheeses and chocolate pudding.

Sources

Enterococcus species are found in the intestine of most animals, including humans. They are excreted in the faeces of animals leading to contamination of the environment. *E. faecalis* is the species found most frequently in human faeces (10^5–10^7 cells per g of faeces) whereas *E. faecium* is the most common species found in the faeces of cattle. Dairy processing equipment can become contaminated with enterococci and surveys have frequently isolated them from pig, poultry and beef carcasses.

Although associated with faeces, the presence of enterococci in foods is not always related to direct faecal contamination. Due to environmental contamination, the enterococci are also found in soil, insects, water and plant materials such as vegetables.

Growth and Survival Characteristics

The enterococci can grow over a wide temperature range and some strains can grow at temperatures as low as 1 °C. The maximum reported growth temperature is 50 °C, but the optimum for most strains is 37 °C. The enterococci are resistant to freezing and are reported to survive storage at −70 °C for several years.

Growth can occur over the pH range 4.4–10.6. Although the minimum water activity for growth is dependent on solute present, *E. faecalis* is reported to grow at 0.93. The enterococci are generally able to tolerate salt concentrations of 10%. In addition, these organisms are resistant to drying and are extremely persistent in the environment. *E. faecalis* and *E. faecium* are reported to survive for weeks on environmental surfaces, in soil for up to 77 days and in cheese for up to 180 days.

Although the enterococci are generally persistent in the environment they are not particularly resistant to sanitisers (including sodium hypochlorite) or preservatives. There is concern, however, that some enterococci strains isolated from food have demonstrated multiple antibiotic resistance, including resistance to vancomycin.

Thermal Resistance

The enterococci are relatively heat resistant and are able to survive many mild pasteurisation processes. This is often why they are present in, and associated with, the spoilage of some heat processed foods such as pasteurised milk and cooked meats.

E. faecium (D_{70}-value of 1.4–3.4 min) is more heat resistant than *E. faecalis* (D_{70}-values of 0.02–0.6 min).

Control Options

Processing

The enterococci can survive mild pasteurisation treatments and can be present in minimally heat processed, or undercooked foods. Strict adherence to heat-processing regimes and the subsequent control of the chill chain assists in minimising the numbers of any enterococci present in pasteurised foods.

The presence and persistence of enterococci in the environment and on raw materials means that processing equipment and establishments can become sources of the organisms. Strict adherence to cleaning regimes and the use of appropriate sanitisers can control the organisms in food-processing establishments.

The use of enterococci as starter organisms or as probiotics in foods has been a cause for concern even though there is a history of safe use for the organisms in both these roles. Antibiotic resistance and the ability to cause disease appear to be strain dependent however, but these factors should be carefully considered in any risk assessment when selecting an *Enterococcus* strain for use in the food industry.

Legislation

EU legislation has requirements for levels of enterococci in drinking water and for water used in the food industry unless it can be demonstrated that the use of the water does not affect the wholesomeness of the food. These requirements are a level of 0 per 100 ml. For water on sale in bottles or containers there is a more stringent requirement of 0 per 250 ml.

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1.1.10 *ESCHERICHIA COLI*

Hazard Identification

What are Escherichia coli*?*

Escherichia coli are gram-negative, non-spore-forming bacteria belonging to the family *Enterobacteriaceae*. Microbiologists recognise a small number of genera within the *Enterobacteriaceae*, including *Escherichia* species, as the coliform group.

Not all *E. coli* are pathogenic: the organism is found as part of the normal human gut flora as well as in the environment. Therefore the presence of *E. coli* in processed food products can indicate faecal contamination, and it is for this reason that *E. coli* is used as an ''indicator'' organism.

Most strains of *E. coli* do not usually cause illness, but some have been associated with infections resulting in diarrhoea, or occasionally more severe illness. There are at least six different groups of diarrhoea-causing *E. coli* which are grouped by distinct virulence characteristics as follows:

Enteropathogenic (EPEC)	Causing infantile gastroenteritis or summer diarrhoea mostly in the developing world.
Enterotoxigenic (ETEC)	Causing traveller's diarrhoea.
Enteroinvasive (EIEC)	Causing a form of bacillary diarrhoea.
Diffusely adherent (DAEC)	Causing watery diarrhoea most commonly in children aged >1 year.
Verocytotoxin-producing (VTEC) Sometimes referred to as Shiga-like toxin-producing (STEC) This group includes a subset of serotypes often referred to as enterohaemorrhagic *E. coli* (EHEC)	Not all VTEC are associated with human disease but those that are EHEC can cause haemorrhagic colitis (bloody diarrhoea).
Enteroaggregative (EAEC)	Causing acute and persistent diarrhoea.

Both VTEC and EAEC have been associated with food-borne disease and these *E. coli* groups are discussed in the following sections.

1.1.10.1 Verocytotoxin-producing *Escherichia coli* (VTEC)

Hazard Identification

What are VTEC?

The verocytotoxin-producing *Escherichia coli* (VTEC) are a group of strains within the species *E. coli*, some of which are highly pathogenic and capable of causing potentially serious food-borne infections in humans. Of all the *E. coli* groups it is the VTEC—so named because they produce one or more toxins that are toxic to vero cells (a tissue cell culture line derived from the kidneys of an African Green monkey)—that are of most concern in developed countries.

In excess of 200 VTEC have been described and some of these organisms have been associated with outbreaks of severe food-borne disease in many countries. The VTEC most frequently associated with causing food-borne illness is the serotype *Escherichia coli* O157 : H7. Other important VTEC serotypes that have caused food-borne infections are O26, O91, O103, O111 and O145.

Occurrence in Foods

VTEC are usually associated with foods derived from cattle, such as beef products, particularly minced/ground beef, and dairy products derived from raw milk. Although VTEC could be present on any raw beef product, minced meat products are considered more of a risk because the pathogen is transferred from the surface to the center of the product during the mincing process.

Studies in the USA and the UK have found that VTEC can be present, at least occasionally, on most farms. However, surveys of food commodities have found that the prevalence of the organism in beef and raw milk products is generally low. VTEC have also been found on pork and lamb mince, fruits, vegetables and seeds and associated products. Fresh produce can be contaminated at any stage during cultivation or handling, possibly *via* contaminated water supplies, or cattle manure used as a fertilizer.

Hazard Characterisation

Effects on Health

The incubation period for illness caused by VTEC can be between 1 and 14 days, although on average it is 3–4 days. The infective dose is thought to be very low, possibly just 10 cells. This is probably because these bacteria are unusually acid tolerant. Symptoms may be restricted to mild diarrhoea only, and some individuals may be asymptomatic.

However, VTEC infection can cause more serious symptoms in some 50% of those infected, especially in vulnerable groups. These symptoms include bloody diarrhoea, abdominal cramps, vomiting and very occasionally, fever. The

illness typically resolves itself after 5–10 days, but in a small number of cases, particularly in young children under five years of age and the elderly, VTEC infection can lead to haemolytic uraemic syndrome (HUS), potentially resulting in kidney failure. HUS in children can also result in seizures, coma and sometimes death. Thrombotic thrombocytopaenic purpura (TTP) is a form of HUS typically developed by the elderly and includes fever, platelet loss and neurological symptoms. Around one third of individuals showing signs of VTEC infection are hospitalised and the average mortality rate from HUS caused by VTEC infections in the UK and in North America is 3–5%.

Incidence and Outbreaks

Fortunately, in view of its potentially serious symptoms, VTEC infections are comparatively rare. Nevertheless, in the EU between 1995 and 2002, incidence of infection more than doubled to 3.2 cases per 100 000 of the population, before levelling off. During 2008, 3159 cases (53% O157 VTEC) were reported in 25 EU Member States, resulting in an overall incidence of 0.7 cases per 100 000 of population. Ireland, the UK, and Scotland in particular have a higher incidence than many other EU countries, but the reasons for this are not known.

In the USA there are an estimated 110 000 EHEC cases annually, resulting in the hospitalisation of approximately 3200 individuals. During 2009 there were 723 VTEC (63% O157 VTEC) cases reported in 10 USA states, giving an overall incidence of roughly 1.5 cases per 100 000 of the population.

VTEC outbreaks, particularly those caused by *E. coli* O157 : H7, have frequently been associated with undercooked minced (ground) beef products such as hamburgers—it has been dubbed ‘‘hamburger disease’’. However, VTEC outbreaks have also been caused by a wide variety of other foods such as cooked meats, raw and recontaminated pasteurised milk, cheese, yoghurt, mayonnaise, unfermented apple cider, unpasteurised apple juice, melon, salad leaves such as lettuce and spinach, parsley, coleslaw, venison jerky, salami, frozen pepperoni pizza, prepackaged cookie dough, in-shell hazelnuts (filberts), raw shelled walnuts and alfalfa sprouts. Contaminated water sources are also a common source of VTEC outbreaks.

The largest recorded non-O157 VTEC food-borne outbreak occurred during 2011 in Germany, with additional cases amongst individuals who had travelled to Germany but returned to other countries before becoming ill, and a related, though much smaller outbreak in France. Approximately 3900 people developed illness during the outbreak, with at least 800 cases of HUS and 48 deaths being recorded. The outbreak strain was identified as *E. coli* O104 : H4 and unusually, was reported to possess a number of pathogenic features more typical of enteroaggregative *E. coli*, together with the capacity to produce Shiga (vero) toxin. The profile of the outbreak victims was also unusual, with the majority of HUS cases being adult females aged 20 years or more. The outbreak was associated with the consumption of fenugreek sprouts grown from seeds imported from Egypt.

Sources

The main infection reservoir for O157 VTEC is recognised as cattle, which, together with other ruminants such as sheep and camels, are apparently healthy carriers of VTEC. Studies have found that the organism is more likely to be found in cattle faeces during the spring than in the winter. Other animals have also been found to excrete VTEC, including swine, goats, deer, horses, dogs, cats, rats, seagulls, pigeons, and geese. *E. coli* O157 has also been isolated from houseflies. A number of outbreaks have been associated with direct contact with infected animals in petting zoos.

Contamination of water supplies with animal faeces has led to outbreaks linked to drinking water and wells, as well as from recreational waters such as lakes, paddling pools and water parks. Soil manured with animal faeces, or in fields where animals have been grazing, can be contaminated with VTEC and contamination may be transferred to crops. O157 VTEC has been found to survive for 150 days in soil and for 90 days in cattle faeces.

Person-to-person spread *via* the faecal–oral route has also occurred causing outbreaks in institutions and childcare settings such as nurseries. Asymptomatic carriers, a state where individuals show no clinical symptom of the disease but are capable of infecting others, have also been reported.

Growth and Survival in Foods

VTEC can grow over the temperature range 7–46 °C (although some sources suggest possibly up to 50 °C) with an optimum of 37 °C. Some isolates of *E. coli* O157 : H7 have been reported to grow in raw milk at 8 °C. *E. coli* O157 : H7 also grows poorly at 44–45 °C, so that traditional methods to detect *E. coli* in food may not pick up this important pathogen.

VTEC survive well at chill and frozen temperatures. Low temperature is reported to be the primary trigger for VTEC to enter a ''viable non-culturable'' state (VNC) in water. A VNC state means that normal methods of detection are unable to recover the organism, but it is still able to cause illness.

VTEC are unusual amongst *E. coli* because they are relatively acid tolerant. The minimum pH for the growth of *E. coli* O157 under otherwise optimum conditions is reported as 4.0–4.4, although the minimum value is affected by the acidulant, and acetic and lactic acids are more inhibitory than hydrochloric acid. The organism is able to survive acid conditions (down to 3.6) and has been reported to survive for two months at 4 °C at a pH of 4.5.

The minimum reported water activity for the growth of VTEC is 0.95. Salt (NaCl) at 8.5% inhibits the growth of *E. coli* O157 and growth is retarded at 2.5%. VTEC are very resistant to desiccation and are able to survive many drying and fermentation processes. Outbreaks have been associated with salami and jerky type meat products.

VTEC are facultative anaerobes (able to grow with or without the presence of oxygen). Modified atmosphere packaging has little effect on the pathogen although it is reported that it is inhibited on meat packaged under 100% CO_2.

VTEC are not notably resistant to preservatives and sanitisers typically used in the food industry. Organic acids (acetic and lactic acid) are used in the USA to decontaminated beef carcasses.

Thermal Resistance

VTECs are not heat-resistant organisms. However heat resistance can vary in different food matrices. *D*-values are affected by factors such as levels of salt, carbohydrate, protein and fat, as well as the water activity and the pH of the foodstuff. For *E. coli* O157, D_{57}-values of 5 min, and D_{63}-values of 0.5 min have been reported in meat. Research indicates that the thermal resistance of non-O157 VTEC is relatively similar to O157 VTEC.

VTEC present on the surface of the product are likely to be inactivated rapidly during cooking, but cells at the center of ground meat products and rolled meat joints will only be inactivated if the center of the product is sufficiently heated. Advice has been given in the USA and the UK on the cooking of hamburgers (meat patties, beef burgers) to ensure the complete inactivation of the pathogen. In the USA, this advice is that they should reach an internal temperature of 71 °C throughout, and in the UK it is recommended that they be cooked to 70 °C for 2 min, or the equivalent, in all parts of every burger.

Control Options

The control of VTEC starts on the farm with the implementation of good agricultural practices. This can help reduce the shedding of *E. coli* O157 from cattle. Good agricultural practices are extremely important for the production of fresh fruits, salads and vegetables. It is very important to minimise the potential for faecal contamination of all food commodities.

Processing

It is safe to assume that raw products of bovine origin (such as fresh meat and raw milk) are potentially contaminated with VTEC and to treat them accordingly using a HACCP approach. Good hygienic practices should be implemented when handling beef carcasses and the controlled use of chill temperatures will prevent the growth of VTEC in these products. The possible survival of VTEC should also be considered during the development of products such as bovine milk cheeses and fermented meat products. There are published guidelines for producers of such foods, but the use of unpasteurised milk is best avoided.

USA regulations require abattoirs and meat processing establishments to implement a step to eliminate *E. coli* O157 : H7 and this can include decontamination. Non-intact raw beef products (as well as intact raw beef products intended to be processed into non-intact raw beef products) found positive for *E. coli* O157 : H7 are considered ‘adulterated’ and are recalled.

It is important to ensure that heat processes (where appropriate) are designed to inactivate any VTEC. Cross-contamination between raw and processed product must be avoided.

Product Use

Consumers should be advised of the risks associated with raw meat products, in particular those made from minced/ground meat, and that all beef products need to be thoroughly cooked. Advice has been given on the required internal cooking temperature for burgers (see *thermal inactivation*). In the USA consumers are advised that checking the colour of meat patties or burgers (brown as opposed to pink or red) is not a reliable indication that the product has reached a safe temperature and that they should use a thermometer to check that the required temperature has been reached.

Consumers should be advised to avoid unpasteurised dairy products, juice or cider, and to wash fruit and vegetables well (although washing may not remove all contamination). Vulnerable groups (the young, elderly and the immunocompromised) should be advised not to eat raw or lightly cooked sprouts (such as alfalfa and mung beans).

Legislation

EU regulations have some general requirements for *E. coli* as an indicator of faecal contamination in some products. These requirements giving maximum levels for *E. coli* in some products do not pertain specifically to VTEC, but the presence of these organisms in any product that will not receive a heat treatment prior to consumption is unacceptable.

In 2009 the HPA published guidelines for assessing the microbiological safety of ready-to-eat foods placed on the market (see link below). These state that the detection of *E. coli* O157 and other VTEC in these products in unsatisfactory: potentially injurious to health and/or unfit for human consumption.

The FDA Food Code (2005) requires food to be safe and unadulterated and product that will not be heated prior to being consumed would need to be absent from VTEC to conform to this requirement. In addition the FSIS considers *E. coli* O157 : H7 and *E. coli* O26 as adulterants in non-intact raw beef products (ground, minced or chopped), as well as intact raw beef products intended to be processed into non-intact raw beef products.

1.1.10.2 Enteroaggregative *Escherichia coli* (EAEC)

Hazard Identification

What are EAEC?

Enteroaggregative *Escherichia coli* (EAEC) were first described in 1987 as causing persistent diarrhoea in a child in Peru. The organism is called aggregative because of its ability to adhere to epithelial cells in a characteristic 'stacked-brick' pattern. EAEC produce toxins (including a cytotoxin) but do not invade cells. EAEC serotypes include O3, O44, O86, O111 and O127.

EAEC is considered as an emerging enteric pathogen and is the second most common cause of traveller's diarrhoea (the most common is ETEC). In a number of countries EAEC have been identified as the causative organism in large outbreaks of diarrhoea and several of these are thought to have been caused by EAEC-contaminated food.

Occurrence in Foods

EAEC is spread *via* the faecal–oral route; both contaminated water and food can be vehicles for the organism.

Data is limited on the occurrence and frequency of the organism in foods. However, one study found EAEC in 44% of tabletop sauces sampled in Guadalajara, Mexico. A study of street foods in Ghana isolated EAEC from macaroni, rice, shito (hot pepper sauce) and tomato stew and a study in Brazil isolated EAEC from commercially produced ice.

Hazard Characterisation

Effect on Health

EAEC infections are more common in developing countries and illnesses caused by the organism are typically found in young children, although EAEC strains can also can cause infection in adults. Undernourished children and the immunocompromised are particularly vulnerable and EAEC infections have been linked to persistent diarrhoea in HIV-affected individuals.

Recent studies have linked the seemingly symptomless carriage of EAEC with malnutrition and growth retardation and EAEC has also been implicated in the development of irritable bowel syndrome, although this yet has to be confirmed.

The infective dose is high and the incubation time variable. Symptoms are typically prolonged, lasting in many cases for at least 14 days, and include persistent watery diarrhoea (occasionally bloody) without fever, possibly with vomiting, leading to dehydration. The illness is generally mild, but deaths have been reported.

Incidence and Outbreaks

Analysis of published data suggests that EAEC was the cause of acute and persistent diarrhoea in a median of 15% of children in developing countries, and in 4% of children in developed countries.

EAEC infections can occur sporadically or as outbreaks. Since EAEC was first described in 1987 a number of EAEC outbreaks linked to food have been well documented. In the UK EAEC outbreaks were linked with restaurant meals and large catered events, and in Italy two consecutive EAEC outbreaks were associated with the consumption of unpasteurised cheese (although infected food handlers could not be ruled out). In Japan, centrally prepared school lunches were linked to a large outbreak (2697 children at 16 schools) of severe diarrhoea. Although the organism was not isolated from any implicated foods EAEC was isolated from 10% of cases.

Sources

EAEC are found in human and animal faeces and it is thought that transmission usually occurs through faecally-contaminated food or water. Food handlers are also thought to be important reservoirs for EAEC and a study in Kenya in 2003–2004 isolated the organism from 2.1% of participants. Although less common, person-to-person transmission may also occur.

Although the environmental reservoir for EAEC is unknown, the organism is increasingly being isolated from environmental samples.

Growth and Survival in Foods

Little is known about the growth or survival of EAEC in foods. Under otherwise optimal conditions studies indicate that EAEC grow well at both 37 and 41.5 °C.

A study also found that EAEC survived well in bottled spring and mineral water (at least 60 days), at 4, 10 and 23 °C. Higher numbers survived at 10 and 23 °C compared to those in samples stored at 4 °C.

Thermal Resistance

There is no specific information on the heat resistance of EAEC; however the organism is likely to have a heat resistance similar to other types of *E. coli*. EAEC should therefore be inactivated by typical heat processes used for the pasteurisation of food products.

Control Options

Until more is known about the reservoirs for EAEC, measures to prevent food from becoming contaminated with the organism should focus on avoiding the use of faecally-contaminated water to irrigate, wash and prepare foods as well

as the implementation of good hygienic practices for the preparation and storage of foodstuffs. These include minimising the handling of food and insisting on good levels of hygiene to reduce the risk of food becoming contaminated with EAEC from infected food handlers.

One of the risk factors in contracting EAEC infection is travel to developing countries. To avoid contracting enteric diseases, the USA Centers for Disease Control and Prevention (CDC) advises travellers to "boil it, cook it, peel it or forget it".

Legislation

There is no specific legislation regarding EAEC in foods. However, there is general legislation and guidance referring to all groups of *Escherichia coli,* which in some cases is specific for *E. coli* O157 and other verocytotoxin-producing *E. coli*.

To assess the hygiene status of a ready-to-eat food product and for shellfish, legislation and guidance can refer to levels of general *E. coli* (excluding VTEC). As an indicator for faecal contamination, the EU regulation for microbiological criteria for foodstuffs has a requirement for the permitted maximum level of *Escherichia coli* in live bivalve molluscs and live echinoderms, tunicates and gastropods.

In 2009 the HPA published guidelines for assessing the microbiological safety of ready-to-eat foods placed on the market (see link below). These state that a satisfactory level of *E. coli* in these products is <20 cfu g^{-1} (*Note: this level is not applicable for* E. coli *O157 and other verocytotoxin-producing* E. coli).

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1.1.11 *HELICOBACTER PYLORI*

Hazard Identification

What is Helicobacter pylori*?*

Helicobacter pylori is a gram-negative, non-spore-forming, microaerophilic, spiral-shaped bacterium, which is able to survive and grow in the epithelial tissue and mucus lining the human stomach. It is recognised as a major cause of gastric disease in humans and has been classified as a carcinogen.

First isolated in 1982, *Helicobacter pylori* is closely related to the genus *Campylobacter* and was first classified as *Campylobacter pyloridis*, then *Campylobacter pylori*. The genus *Helicobacter* was created in 1989 and now contains approximately 30 species, of which five are reported to have caused infection in humans. Of these, *H. pylori* is by far the most common and the only one so far considered to have potential for food-borne transmission. It is suspected that both contaminated food and water may play a significant role in the transmission of *H. pylori*, although this has yet to be confirmed.

Occurrence in Foods

Food samples are not routinely tested for the presence of *H. pylori* and it is exceptionally difficult to isolate from samples containing large populations of other bacterial species. Therefore its prevalence and distribution in foods is unknown. However, molecular-biology-based studies have shown that DNA sequences specific to *H. pylori* can be isolated from foods, including unpasteurised milk from sheep, goats and cows. It is considered very unlikely to grow and multiply in food, although cells have been reported to remain viable for several days in some foods, such as fresh fruit and vegetables, raw meat and dairy products, allowing the possibility of food-borne transmission. It has been stated that any food product with a pH in the range 4.9–6.0 and a water activity of more than 0.97 could allow the survival of cells of *H. pylori*, particularly at low temperatures.

Hazard Characterisation

Effects on Health

H. pylori is able to colonise gastric mucus and the cells of the gastric epithelium. It is able to survive the harsh acidic conditions in the stomach, principally by production of the enzyme urease, which generates ammonia from urea and raises the pH of the microenvironment around the cells. The infective dose is not known, but infection is thought to occur most often during childhood and will persist unless treated with antibiotics.

Many infections are asymptomatic, but *H. pylori* can cause chronic gastritis, gastric and duodenal ulcers in some people and is also considered to be involved in the development of stomach cancers, including gastric adenocarcinoma.

It has been classified by the International Agency for Research on Cancers (IARC) as "carcinogenic to humans (IARC Group 1)".

Infection can cause an immune reaction leading to a localised inflammation of the stomach and duodenal epithelium. Ammonia and other toxic metabolites produced by the bacteria may also damage epithelial cells. The resulting inflammation may increase stomach acid production and damage the gastric mucus layer. The increased exposure to stomach acids can cause ulceration and lead to stomach cancer in some cases.

The role of *H. pylori* in gastric disease was not recognised until the late 1970s, when two Australian medical researchers, John Warren and Barry Marshall, proposed that a bacterium was responsible for much gastric disease. Although their hypothesis was controversial, Warren and Marshall were able to isolate *H. pylori* and demonstrate a causal link between the bacterium and gastritis.

Incidence and Outbreaks

H. pylori is considered to be one of the commonest bacterial infections found in humans and has been estimated to be present in the upper gastrointestinal tract of more than 50% of the global population. In developing countries, especially in rural areas, the incidence of infection is even higher, with up to 80–90% of people being infected in some regions. In developed countries, incidence is usually less than 50% and is thought to be falling. There appears to be a close association between the incidence of infection and socioeconomic status, with *H. pylori* being more prevalent in poorer communities. This is likely to be linked to standards of hygiene applied to the food and water supply chains, but there may be multiple routes of transmission and the situation is complex.

Sources

The only known reservoir for *H. pylori* at present is the human upper gastrointestinal tract. However, it is possible that other, as yet unidentified, reservoirs exist. It has been isolated from non-human primates such as rhesus monkeys, domestic cats, sheep and cockroaches, but there is no evidence that zoonotic transmission is a significant route of infection.

It seems likely that there are multiple routes of transmission, especially in developing countries. Direct oral–oral transmission is considered likely and some studies have shown that contact with infected individuals is a risk factor for new infection. There is also some evidence for gastro–oral transmission by exposure to the vomitus of infected individuals.

Faecal-oral transmission is the third possible route and *H. pylori* has reportedly been isolated from the faeces of infected children and adults, but these findings have been difficult to reproduce. This may be because of the difficulty of isolation in the presence of competing microflora. It has been suggested that survival of *H. pylori* in faeces is more likely when symptoms of gastrointestinal illness, such as diarrhoea, are present.

The possibility of transmission through contaminated food and water has also been considered and the presence of *H. pylori* DNA in foods and water

sources is indirect evidence for this. There is also some epidemiological evidence for transmission through food. For example, studies in Chile have identified consumption of raw vegetables irrigated with sewage-contaminated water supplies as a risk factor for *H. pylori* infection.

Growth and Survival in Foods

The cells of *H. pylori* have been observed to enter a viable, non-cultivable coccoid form under conditions of metabolic stress and this may aid survival in hostile environments. However, there is little evidence to suggest that *H. pylori* is unusually resistant to environmental factors relevant to food processing. It will only grow within the temperature range 30–37 °C, although survival in food at lower temperatures has been demonstrated. *H. pylori* has been isolated from inoculated milk kept at 4 °C for up to 11 days.

Cells are not acid-resistant outside of the gastric environment and they are also very sensitive to desiccation. They have not been found to survive at water activity levels of <0.97.

Survival of cells within microbial biofilms developing on the surfaces of water supply equipment has been observed.

Thermal Resistance

Formal studies of the heat resistance of *H. pylori* have not been reported, but there is no evidence to suggest that it is any more resistant that the closely related genus *Campylobacter*. It is thought highly unlikely that cells would survive typical milk pasteurisation processes.

Control Options

Although transmission of *H. pylori* through foods remains unproven, the evidence suggests that it is a possibility and controls are therefore necessary. However, since the prevalence of the pathogen in most foods is uncertain, only general measures can be implemented.

Processing

Since the organism is probably unable to multiply in foods, control options should focus on good hygiene and the prevention of contamination. Good hygiene practice, including the use of potable quality water in processing and proper separation of raw and processed food commodities will reduce any possible risk of infection.

Product Use

Consumers can reduce the risk of infection by avoiding consumption of unprocessed foods in developing countries, especially those from street vendors and other uncontrolled sources, and by ensuring that foods are properly cooked before consumption.

Legislation

There are no specific requirements relating to *H. pylori* in foods under EU legislation or the FDA Food Code.

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1.1.12 *LISTERIA*

Hazard Identification

What is Listeria*?*

The members of the genus *Listeria* are gram-positive, non-spore forming, rod-shaped bacteria. The genus contains a number of species including *L. monocytogenes*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, *L. ivanovii* and *L. grayi*. Although the first four of these have all been implicated in human infection nearly all cases of *Listeria* infection are caused by *L. monocytogenes*.

At least 13 different serotypes of *L. monocytogenes* are known. All can cause human listeriosis, but most cases are caused by serotypes 1/2a, 1/2b and 4b. The majority of significant reported food-borne outbreaks have been caused by serotype 4b.

Occurrence in Foods

Listeria monocytogenes has the potential to be present in all raw foods. Cooked foods can also be contaminated, usually as the result of post-process contamination. *L. monocytogenes* has been isolated from a very wide range of processed foods including pâtés, milk, soft cheeses, ice cream, ready-to-eat cooked and fermented meats, smoked and lightly processed fish products and other seafood products. *L. monocytogenes* is usually found only in low numbers ($<$10 cfu g^{-1}) in foods. However, products such as pâtés and soft cheese have occasionally been found to contain populations of $>$10 000 cfu g^{-1}.

Hazard Characterisation

Effects on Health

Listeria monocytogenes causes one of the most severe forms of food-borne infection and it is fortunate that listeriosis is a relatively rare disease. The overall mortality rate associated with the disease is 30%, although it can be as high as 40% in susceptible individuals. Those most at risk of acquiring the disease are pregnant women (20 times greater risk than healthy individuals), the elderly and the immunocompromised, although healthy individuals can develop listeriosis particularly if the food is heavily contaminated. Monitoring in the USA suggests that *Listeria* infections are more likely to result in the hospitalisation of affected individuals in comparison with those affected by other food-borne pathogens, such as *Salmonella* (hospitalisation rate 95% for *Listeria* compared with 21% for *Salmonella*).

The incubation period is 1 to 90 days (mean 30 days). The onset of illness is typically marked by flu-like symptoms (fever and headache), and sometimes by nausea, vomiting and diarrhoea. In some cases these symptoms can lead on to meningitis and septicaemia. Symptoms in pregnant women can lead to infection

of the foetus, which can result in miscarriage, stillbirth, or the birth of an infected infant, although the mother usually survives.

The infective dose is unknown, although it is generally considered to be $>10^3$ cfu g^{-1} for healthy individuals. Due to the length of the incubation period, it can be difficult to determine the numbers of organisms in foodstuffs at the time of consumption and an outbreak associated with frankfurters in the USA in 1998 is thought to have been caused by product containing less than 0.3 cfu g^{-1}, although it is suspected that the causative strain may have carried enhanced virulence.

Incidence and Outbreaks

The first outbreak of *L. monocytogenes* that could be definitely linked to food was caused by commercially prepared coleslaw in Canada in 1981 (at least 41 cases with seven deaths). Manure from *Listeria*-infected sheep had been used as a fertilizer when growing the cabbages used to prepare the salad.

The incidence of reported *Listeria* infections increased dramatically during the 1980s as did the number of food-related outbreaks. An outbreak in Los Angeles County during 1985 was caused by Mexican-style cheese (142 cases with 48 deaths) and during the late 1980s an outbreak in the UK was associated with pâté (>350 cases with >90 deaths).

Notable outbreaks occurring in the 1990s were linked to smoked mussels (1992; New Zealand); 'rillettes' or potted pork (1993, France); pasteurised chocolate milk (1994, USA); raw milk soft cheese (1995, France); frankfurters (1998–1989, USA); butter (1998–1989, Finland) and pork tongue in jelly (1999–2000, France).

During the first decade of the 21st Century there were a number of large *Listeria* outbreaks caused by ready-to-eat (deli) poultry products on the North American continent. In the USA in 2000 a multi-state outbreak (29 cases with seven deaths) was linked to turkey deli meat, and during 2002 another outbreak (at least 46 cases with 11 deaths) was linked to poultry deli products produced by the Pilgrims Pride Corporation. This outbreak resulted in the recall of 27.4 million pounds of product, the largest meat recall in USA history. In Canada in 2008 an outbreak linked to a number of ready-to-eat deli meats resulted in 57 cases of illness and 23 deaths.

Strategies to reduce the incidence of *Listeria* infections were implemented in many countries during the 1990s resulting in a reduction in the incidence of the disease. However, outbreaks have continued to occur and the incidence in the last decade has again risen in some countries. For example, in the UK 278 cases were reported in 1988. Although this figure decreased in the period 1991–2000 to an average annual rate of 110 cases, the number of cases rose again (particularly amongst the over 60s) during 2001–2009 to an average annual rate of 192. A similar pattern has been reported in the EU and Canada. The European Centre for Disease Prevention and Control reported that there were 1645 confirmed cases (including 270 deaths) during 2009 and the Public Health Agency of Canada has reported an increase of cases since 2000 (from 0.23 cases

per 100 000 to 0.42 cases per 100,000 in 2007), with a sharp increase in 2008 resulting in 0.72 cases per 100 000 of population.

However, the USA has reported a downward trend in recent years: the incidence of infection in 2009 was 0.34 cases per 100 000 people, which compared to 1996–1998 rates of *Listeria* infections is a decrease of 26%.

Sources

Listeria is ubiquitous in the environment. It is found in soil, where it can survive for extended periods leading to the contamination of plant material. *Listeria* has been isolated from a wide variety of fresh produce. It is also found in marine environments and the organism is often associated with fish and seafood products. Animals such as sheep, goats and cattle are recognised carriers of the organism, often acquired from the consumption of contaminated (usually poor quality) silage. Healthy humans can also be carriers of the organism.

Kitchen and food-processing environments, particularly those that are cold and wet or moist, can be reservoirs for *Listeria*. The organism can be particularly persistent and difficult to control because of its psychrotrophic nature and resistance to unfavourable environmental conditions. The efficacy of hygiene standards in food production facilities producing ready-to-eat products is usually monitored and this can include environmental swabbing for *L. monocytogenes*. Although other *Listeria* species are not normally associated with human disease, a positive test for *Listeria* species other than *L. monocytogenes* can be a useful indicator that there is the potential for *L. monocytogenes* to be present.

Growth and Survival in Foods

Listeria monocytogenes is psychrotrophic and the ability to grow at chill temperatures is the reason why it is a particular risk in extended-shelf-life chilled foods that can support its growth. Extremely slow growth of *L. monocytogenes* has been recorded at temperatures as low as $-1.5\,^{\circ}C$ and the maximum temperature for growth is generally accepted as $45\,^{\circ}C$. The organism survives well in frozen foods, but survival times can be adversely affected under acid conditions.

The pH range for the growth of *L. monocytogenes* is 4.3–9.4 under otherwise ideal conditions. These values are affected by the specific acid in the product, and the minimum pH is likely to be higher in real foods and at low temperatures. However, *L. monocytogenes* can survive for extended periods in acid conditions, particularly at chilled temperatures.

The minimum water activity for the growth of *L. monocytogenes* is 0.92. The organism is tolerant of high sodium chloride levels and is able to grow in environments of up to 10% salt, and to survive in concentrations of 20–30%. *L. monocytogenes* is also able to survive for some time in low-water-activity environments, and may survive drying processes. Survival times are extended at chilled temperatures.

Listeria monocytogenes grows well in aerobic and anaerobic conditions. Its growth is unaffected by many modified atmospheres even at low temperatures. High concentrations of carbon dioxide are necessary to inhibit growth.

Although *Listeria monocytogenes* is not especially resistant to antimicrobials, it can prove difficult to control on food contact surfaces such as stainless steel because the bacteria can form persistent biofilms. It is important to clean equipment prior to using sanitisers because organic matter can affect their efficacy at inactivating the pathogen.

Thermal Resistance

Although *Listeria monocytogenes* is not particularly heat resistant it is more heat resistant than some other food-borne pathogens, such as *Salmonella* and *E. coli* O157 : H7. It is readily inactivated at temperatures above 70 °C and heat processes such as normal commercial milk pasteurisation will destroy numbers typically found in milk. Typical *D*-values in food substrates are: between 5 and 8 min at 60 °C, and 0.1–0.3 min at 70 °C. Concern about the pathogen in particular food product categories has lead to heating guidelines been issued by various heath authorities. The UK Department of Health advised that ready meals or similar products should receive a heat treatment of at least 2 min at 70 °C, or equivalent, to ensure the destruction of *L. monocytogenes*. For consumers, terms such as heating till 'piping' hot in the UK, and 'steaming' hot in the USA are used to describe heat processes required to ensure the safety of foods identified as being a potential risk of causing *Listeria* food poisoning.

Control Options

The control of *Listeria* in foods relies largely on a HACCP approach and the establishment of effective critical control points in the process.

Processing

The careful design and layout of processing equipment in conjunction with the implementation of regular, thorough cleaning regimes of the processing environment can significantly reduce the level of *Listeria* contamination in many processed foods. However, because of its ubiquitous nature it is virtually impossible to totally eliminate the pathogen from many food products. The organism should be inactivated by heat applied during the cooking process and the presence of *Listeria* in cooked products can indicate poor hygiene either during manufacture, distribution or at retail.

Other critical controls include strict temperature control, the prevention of cross-contamination between raw and processed foods and between the processing environment and processed foods, as well as the use of a restricted shelf-life for potentially contaminated products that could support the growth of the pathogen.

Product Use

Appropriate scientifically-based methods should be used to devise safe shelf-lives for at-risk chilled foods and these restricted shelf-lives should be rigorously implemented and adhered to in order to reduce the risk from *L. monocytogenes*. Clear cooking instructions are needed on the packaging of many chilled foods requiring reheating prior to consumption, to ensure that all parts of the product receive a listericidial process.

Vulnerable individuals, especially pregnant women, the elderly and the immunosuppressed, are advised to avoid eating specific foods to reduce the risk from listeriosis. Health authorities in the UK advise these groups not to eat soft mould-ripened or blue-veined cheeses, all types of pâté (including vegetable) and unpasteurised dairy products. These groups are advised that they may also choose to avoid cold (pre-cooked) meats and smoked salmon, and that they should thoroughly wash pre-packed salads and adequately heat chilled meals before eating. In the USA the FDA also includes hot dogs, luncheon meats, cold cuts and smoked seafood (unless thoroughly reheated) to the list of foods that at-risk consumers should definitely avoid.

Legislation

Countries differ in their regulatory approach to the presence of *L. monocytogenes* in ready-to-eat food.

In the USA a 'zero tolerance policy' is taken on the presence of *L. monocytogenes* in any ready-to-eat food, and the pathogen should be absent in 25 g of product. However in 2008 the FDA published a draft consultation paper proposing to loosen up these strict controls to allow a maximum limit of 100 per g in frozen and refrigerated ready-to-eat foods that do not support the growth of the pathogen. This would be applicable to foods:

1. With a pH $\leq$4.4
2. With an A_w $\leq$0.92, or
3. Frozen foods

EU regulations generally permit a count of up to 100 cfu g^{-1} at the end of shelf-life for ready-to-eat foods, except those intended for infants and for special medical purposes.

Specific regulatory guidance on *Listeria* for food manufacturers is also available in a number of countries.

In April 2011, Canadian authorities published a policy on *Listeria monocytogenes* in ready-to-eat foods, which included the following guidelines: *A ready-to-eat food in which growth of L. monocytogenes will NOT occur includes the following:*

1. Combination of factors, *e.g.* pH <5.0 and A_w <0.94
2. pH <4.4 regardless of A_w

3. A_w <0.92 regardless of pH, or
4. Frozen foods

Various countries have standards/legislation for the pasteurisation of ice cream/frozen desserts; these heat processes are more severe than high-temperature/short-time (HTST) milk pasteurisation (at least 15 s at 72 °C) because ingredients such as sugars, fat, emulsifiers and stabilisers in these products protect *L. monocytogenes* from heat, resulting in an increase in *D*-value. In New Zealand a heat process of at least 15 s at 79.5 °C (or equivalent) is required for ice cream, and in the USA standards require a process of 30 min at 68.3 °C or 25 s for 79.4 °C.

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1.1.13 *MYCOBACTERIUM AVIUM* SUBSPECIES *PARATUBERCULOSIS*

Hazard Identification

What is Mycobacterium avium *subspecies* paratuberculosis*?*

Mycobacterium avium subspecies *paratuberculosis*, often referred to as *Mycobacterium paratuberculosis* or MAP, is a gram-positive, strictly aerobic bacterium belonging to the family *Mycobacteriaceae*. It is a slow growing organism and is difficult to cultivate in laboratory conditions.

It is known to be the causative agent of Johne's disease, a widespread chronic condition in ruminants, particularly cattle. However there is some evidence that it may also have a role in the development of a chronic inflammatory bowel condition in humans called Crohn's disease.

Occurrence in Foods

MAP can be isolated from the raw milk of clinically infected cattle and from the milk of sub-clinically infected, apparently healthy cattle. Surveys in the UK and the Czech Republic also found MAP in about 2% of pasteurised milk, giving rise to concerns that the organism is able to survive standard HTST milk pasteurisation treatments (72 °C for 15 s). In addition MAP has been isolated from commercial milk in the USA and Switzerland.

The bacterium is generally acid resistant and may survive the low-pH conditions in cheese making. It could therefore be present in cheeses made from raw milk, or milk subjected to less severe pasteurisation processes. Surveys of retail cheeses in Greece and the Czech Republic found MAP in 3.6% of samples. Sheep and goats' milk and associated dairy products may also be potential sources of MAP.

The presence of MAP in dried milk infant foods was reported in the EU in 2005, and in 2011 an Egyptian study also detected the organism in imported infant formula.

Infected ruminants excrete MAP into the environment where the organism is known to persist for some time. It is likely that MAP enters the water supply and is present on raw vegetables and fruits, as well as raw meats from ruminants. Although data on its prevalence from these sources is very limited, recent studies suggest that beef can be contaminated with MAP *via* dissemination of the pathogen in the tissues of infected animals. However, a USA study in 2007, which examined 200 samples of retail ground beef, was unable to detect the organism.

Hazard Characterisation

Effects on Health

The evidence to link MAP as the causative agent of Crohn's disease is not conclusive and claims that the two are linked are not widely accepted by

gastroenterologists. There is evidence that hereditary and environmental factors play a role in the development of Crohn's disease, suggesting that if MAP is involved, it is not the sole aetiological agent. However, while research is ongoing to identify any health implications for the presence of MAP in foods, it has been suggested that the food industry should adopt a precautionary approach.

Crohn's disease is a chronic inflammatory disease in humans, which can occur in any part of the gastrointestinal tract, although it usually affects the small intestine. Symptoms, which include loss of weight, abdominal pain and cramps, diarrhoea, fatigue, muscle and joint pains, usually first occur when individuals are 14 to 24 years of age. There is no known cure, and it is a lifelong debilitating illness. The disease is managed by the use of drugs, although surgical intervention is also often necessary. Crohn's disease is rarely fatal, though life expectancy is often reduced.

Incidence and Outbreaks

Cases of Crohn's disease have not yet been conclusively linked to MAP-contaminated food. The incidence of Crohn's disease is higher in developed countries than in the developing world, although some of this difference could be because diagnosis is more likely where there is a higher standard of healthcare. In the EU and North America, Crohn's disease is estimated to occur with an overall incidence of 5.6 cases per 100 000 individuals per year.

If MAP is involved in the development of Crohn's disease, food is likely to be an important vehicle for transmission. An epidemiological study has reported a statistical link between the consumption of beef and Crohn's disease. However, if MAP is a zoonosis, it is thought that the most likely source for humans is cows' milk.

Sources

Infected ruminants are the major source of MAP and transmission of the bacterium is mainly *via* the faecal–oral route. Infected ruminants such as cows, sheep, goats and deer excrete MAP into the environment where the organism is known to persist in pastures for sometime. Non-ruminant wild animals such as rabbits, mice, foxes, badgers and some birds are also known to excrete MAP.

Run-off waters from contaminated pastures can lead to the organism being present in water supplies, where it can persist for some months. MAP may survive processes used to produce potable water and could be present in drinking water.

Breast milk samples from Crohn's disease patients have been found to contain MAP.

Growth and Survival Characteristics

MAP can grow at temperatures from 25–45 °C, with an optimum of 37 °C. It can grow at salt concentrations below 5% and at pH values ≥ 5.5. It is a very

slow growing bacterium, and on laboratory media incubated at 37 °C it can take many weeks for colonies to be visible to the naked eye.

Although MAP is unlikely to increase in numbers in food, the organism can survive for extended periods depending on conditions. It can survive for some time under acid conditions and studies have recorded *D*-values of approximately 10 and 19 days at pH 4.0 and pH 5.0 respectively, when stored at 20 °C. Salt concentrations of between 2 and 6% had little effect on the survival of the organism regardless of pH. It is therefore possible that MAP may survive some cheese-making processes. MAP can survive outdoors in pastures and the environment for up to nine months although exact survival times are dependent on conditions.

Evidence suggests that standard water treatments such as slow sand filtration and chlorination may not be sufficient to remove MAP from drinking water. MAP can survive chlorination at 2 ppm and this resistance is increased in the low-nutrient, low-temperature conditions found in many water systems.

MAP in not usually inactivated by food preservatives.

Thermal Resistance

MAP is more heat resistant that other Mycobacteria of concern in milk, notably *M. bovis* (which can cause tuberculosis in humans). Following extensive studies on the thermal inactivation of MAP in milk, coupled with the fact that MAP can be isolated from commercially pasteurised milk, it has been concluded that the organism may occasionally survive standard commercial milk pasteurisation processes. This has led to recommendations in the UK for extended HTST milk pasteurisation treatments of 72 °C for 25 s, although there is evidence to suggest that even this extended heat process is insufficient to ensure that the organism is absent in pasteurised milk.

Thermal inactivation studies of MAP in meat products suggest that conventional meat cooking processes would inactivate low numbers of the organism.

Control Options

Processing

Strategies to control MAP in milk focus on reducing or even eliminating Johne's disease in dairy cattle on the farm. There are difficulties with this approach, such as the possibility of re-infection of MAP-negative herds from infected wild animal reservoirs. Nevertheless, initiatives such as cattle health schemes, vaccination and veterinary advice to farmers on husbandry, basic hygiene and biosecurity measures are in place in many countries.

Other measures to lessen the risk of MAP-contaminated milk reaching consumers include minimising faecal contamination of raw milk during the milking process to reduce initial MAP numbers in milk—thereby lessening the chance of the organism being present after pasteurisation—and ensuring that

dairies carry out pasteurisation correctly and that cross-contamination between raw and pasteurised milk does not occur.

Product Use

Consumers can reduce the possible risk of MAP by only using correctly pasteurised milk and other dairy products.

Legislation

There is no specific legislation in the EU or the USA on levels of MAP in foods.

There are food hygiene requirements in many countries, which include controls on hygiene standards for the production and distribution of milk and dairy products. In addition there are recommendations on steps to reduce the prevalence of MAP in dairy herds.

The FSA has recommended taking a precautionary approach with respect to MAP and has said that steps should be taken to reduce human exposure to the organism. In the UK it is recommended that the minimum holding time for HTST milk pasteurisation at 72 °C should be increased from 15 to 25 s.

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1.1.14 *PLESIOMONAS SHIGELLOIDES*

Hazard Identification

What is Plesiomonas shigelloides*?*

Plesiomonas shigelloides is a gram-negative, non-spore-forming bacterium that has, on occasions, been thought to have caused food-borne disease. Although the role of the organism in causing enteric disease has yet to be conclusively established, it is strongly implicated as a cause of human diarrhoea by a number of factors.

In many ways the organism is very similar to *Aeromonas*. Indeed the organism was called *Aeromonas shigelloides* for a short time, and for years both genera were included in the family Vibrionaceae. Recently however, *P. shigelloides* has been classified into a different family, the *Enterobacteriaceae*. In addition, unlike *Aeromonas* species, *P. shigelloides* is not regarded as a psychrotroph (it is unable to grow at refrigeration temperatures).

Occurrence in Foods

Plesiomonas shigelloides is primarily an aquatic organism and most infections are thought to be caused by the ingestion of contaminated water. The few studies identifying foods contaminated with the organism have mostly isolated *P. shigelloides* from fish and seafoods, and many food-borne infections are associated with the consumption of raw oysters.

Hazard Characterisation

Effects on Health

All individuals are susceptible to *P. shigelloides* infections, although the organism is likely to cause more severe disease in children and the immunocompromised. Infections peak in the summer months and are more often reported in tropical and subtropical regions.

Plesiomonas shigelloides causes gastroenteritis and in rare cases extraintestinal infections. Although the infective dose is unknown, it is thought to be high ($>10^6$ organisms). The incubation period is not well defined and symptoms may begin between 20 to 50 hours after ingesting the contaminated water or food.

Symptoms of gastroenteritis last from 1–9 days and can include diarrhoea, nausea, vomiting, abdominal pain, chills, fever and headaches. The diarrhoea is usually characterised by watery stools although in severe cases the stools have been described as greenish-yellow, mucoid and blood tinged. This form of the disease is usually self-limiting.

Occasionally, extraintestinal infections, such as meningitis and septicaemia, can occur, particularly in immunocompromised individuals. These infections can be very severe and are associated with a high mortality rate.

Incidence and Outbreaks

There is very little reported information on the incidence of food-borne *P. shigelloides* infections and food-borne outbreaks are not often reported. However, food-borne disease caused by the organism has been associated mostly with raw oysters. Other foods thought to have caused outbreaks of *P. shigelloides* gastroenteritis are chicken, fish, shrimp, cooked crab and temperature-abused buffet food comprising cold fish and egg with mayonnaise.

Sources

Plesiomonas shigelloides is regarded as an aquatic microorganism, and is found in fresh and marine waters, especially during warm weather. The organism is unable to grow below 8 °C, so is more often found in tropical and subtropical waters and in river water from temperate climates during the summer months.

The organism is naturally found in finfish and shellfish, again more often in those originating from warmer waters. During the warmer months samples can be heavily contaminated. *P. shigelloides* has also been isolated from snakes, toads, dogs, cats, cattle, pigs, goats and birds.

P. shigelloides has been found in healthy humans at very low rates in Japan (<1%) but at higher rates in developing countries such as Thailand (23–24%).

Growth and Survival Characteristics

P. shigelloides is not regarded as being psychrotrophic and most strains will not grow below 8 °C. However, at least one strain has been reported to grow at 0 °C and the organism can be isolated from waters in cold climates, such as those in the Northern EU. The maximum temperature for growth is around 45 °C.

P. shigelloides can survive freezing temperatures and the organism has been isolated from foods stored at −20 °C for some years.

P. shigelloides has been shown to grow in salt concentrations up to 5% and the pH range for growth is generally 4.5–8.5. However, a few isolates have been shown to grow at low pH values of 3.5, and some at high pH values of 9.0.

P. shigelloides is a facultative anaerobe (it is able to grow in the presence or absence of oxygen). Studies using vacuum/modified atmosphere (80% CO_2) packaged cooked crayfish tails have shown some inhibition in the growth of *P. shigelloides* compared to product stored in air.

Thermal Resistance

The organism is not particularly heat resistant and a pasteurisation process of 60 °C for 30 min or equivalent heat processes will ensure its inactivation.

Control Options

P. shigelloides is primarily a risk when contaminated water and raw seafoods are ingested. It is easily inactivated by heat and normal cooking processes should ensure its destruction.

Processing

Using water from a potable source in food-processing establishments, and ensuring that cross-contamination between raw and cooked foods does not occur reduces the risk of infection. High numbers of cells are thought to be necessary to cause illness, so ensuring adequate refrigeration of raw and cooked foods will limit the growth of any *P. shigelloides* present.

Product Use

Consumers can reduce the risk from *P. shigelloides* infections by avoiding the consumption of raw shellfish and contaminated water.

Legislation

There is no specific legislation in the EU or the USA on levels of *Plesiomonas shigelloides* in foods.

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1.1.15 *PSEUDOMONAS AERUGINOSA*

Hazard Identification

What is Pseudomonas aeruginosa*?*

Pseudomonas aeruginosa is a gram-negative, non-spore-forming, strictly aerobic bacterium. The organism has on rare occasions been implicated in cases of food poisoning, but is more often associated with disease in the immunocompromised, in hospital patients and in infants. It is very rarely a problem for healthy individuals and is generally regarded as an opportunistic pathogen.

Occurrence in Foods

Pseudomonads are ubiquitous and are normal contaminants of vegetables, meats, milk and water. In many foods, these organisms, including *Ps. aeruginosa*, are regarded as potential spoilage microorganisms. *Ps. aeruginosa* contamination is of particular concern in potable water supplies and bottled water.

Hazard Characterisation

Effects on Health

Pseudomonas aeruginosa can cause a range of infections, such as soft tissue, respiratory tract, urinary tract and systemic infections in at-risk individuals. However, it can also invade the intestinal tract sometimes leading to acute gastroenteritis.

Pseudomonas aeruginosa infection in healthy individuals can, on occasion, lead to mild gastroenteritis whereas in susceptible individuals (in particular, infants) it can lead to serious diarrhoea sometimes resulting in death.

Incidence and Outbreaks

There is little information on the incidence of food-borne *Ps. aeruginosa* infections, but outbreaks of infections in hospital caused by the bacterium being introduced from water or food sources have been documented. Recently there have been reports of *Ps. aeruginosa* outbreaks in neonatal units caused by contaminated feeding bottles or from the mineral water used to prepare milk.

Outbreaks caused by the organism are not necessarily associated with gastroenteritis. For example, an outbreak of pneumonia in an intensive care unit was traced back to patients drinking *Ps. aeruginosa*-contaminated bottled mineral water.

Sources

Pseudomonads are ubiquitous and are commonly present in environmental sources such as soil and water. They are frequently found on plant surfaces, and

occasionally on the skin of animals. *Ps. aeruginosa* can be found on the skin or in the throat of some healthy human individuals.

It is thought that *Ps. aeruginosa* may enter hospital environments on foods such as fruits and vegetables and surveys have found that *Ps. aeruginosa* is present on vegetables and meats as well as in frozen foods.

Growth and Survival Characteristics

Pseudomonads are sensitive to heat and are readily inactivated by normal cooking processes. They are sensitive to desiccation and are not tolerant of acid pH.

However pseudomonads, including *Ps. aeruginosa*, are notable for their relative resistance to many disinfectants, and they can form biofilms on surfaces, making them very difficult to remove.

Control Options

Processing

Low levels of *Ps. aeruginosa* are not usually a concern in foods destined for consumption by healthy individuals. However, the organism should be considered when designing and preparing foods intended for consumption by the immunocompromised such as those found in intensive-care units. Mild heat processes readily inactivate the microorganism, but high hygiene standards need to be implemented to prevent post-process contamination.

Product Use

Healthy consumers need not be unduly concerned about the presence of low levels of *Ps. aeruginosa* in foods.

Legislation

There are requirements within EC legislation for *Ps. aeruginosa* in water offered for sale in bottles or containers. These require that no *Ps. aeruginosa* cells can be detected in 250 ml of water.

Sources of Further Information

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1.1.16 *SALMONELLA*

Hazard Identification

What is Salmonella*?*

The salmonellae are gram-negative, non-spore-forming rod-shaped bacteria belonging to the family *Enterobacteriaceae*. However, *Salmonella* is not included in the group of organisms referred to as coliforms. *Salmonella* is one of the principal causes of food-borne gastroenteritis worldwide and is also an important pathogen of livestock. Salmonellosis is a zoonotic infection (can be transmitted to humans from animals).

Salmonella nomenclature has been revised over the years and is based on biochemical and serological characteristics. Many microbiologists now use a classification that recognises only two species of *Salmonella*. These are *S. enterica* (which includes six subspecies) and *S. bongori*. The subspecies most important in food-borne disease is *S. enterica* subspecies *enterica*.

The genus *Salmonella* can be further divided into serotypes, of which there are a great many (>2500). Most serotypes (sometimes referred to as serovars) belong to the species *S. enterica* and only 20 belong to *S. bongori*. *Salmonella enterica* subspecies *enterica* contains nearly 1500 serotypes, including many of the serotypes that are known to cause food-borne disease. Under the currently accepted classification, an example of the correct way to denote a serotype would be *Salmonella enterica* subspecies *enterica* serotype Enteritidis, although fortunately convention allows this to be abbreviated to *Salmonella* Enteritidis (*S.* Enteritidis). In addition, each *Salmonella* serotype can be divided further by phage typing. A particular phage type can be denoted using the term PT. For example, *Salmonella* Enteritidis PT4 is an organism commonly associated with eggs and human illness. Other common serotypes involved in human illness are *S.* Typhimurium and *S.* Virchow.

Occurrence in Foods

Food animals can become infected with *Salmonella* from feed and from the environment, and many foods of animal origin such as meat, poultry, eggs and raw milk can be contaminated with the pathogen. Many studies to determine *Salmonella* contamination rates in food commodities have been conducted. For example, in 2005 an EU-wide study found that about one in five large-scale commercial egg producing facilities had hens infected with *Salmonella*, with the lowest levels of infection being found in Sweden and Luxembourg, and the highest levels in Portugal, Poland and the Czech Republic. A UK study reported contamination levels in poultry of 5.7% in 2001, and a 2003 study of UK produced shell eggs found contamination levels of 0.34%. In the USA, testing during 2003 found that 3.6% of raw meat and poultry samples were contaminated with *Salmonella*.

Fresh produce may also become contaminated with *Salmonella* from animals and environmental sources. The pathogen has been isolated from tomatoes, lettuce and salad greens, peppers, sprouting seeds, fruit juice, cantaloupe melons and nuts.

Cooked ready-to-eat foods can become contaminated as the result of cross-contamination from raw foods. Although contamination can occur as the result of direct contact, it can also occur *via* food preparation surfaces or equipment used for both raw and cooked foods. A wide variety of processed foods have been found to be contaminated with *Salmonella*, including chocolate, breakfast cereal, flavoured potato crisps and similar snack products, peanut butter, fermented meats, cheeses, milk powder and ice cream.

Hazard Characterisation

Effects on Health

Some *Salmonella* serotypes have a limited host spectrum (*i.e.* they cause specific and often serious clinical disease in one or a few animal species), such as *S.* Typhi and *S.* Paratyphi in humans (causing typhoid fever), *S.* Dublin in cattle, and *S.* Choleraesuis in pigs. These are not considered further here.

The more usual food-borne form of the illness is caused by non-typhoid salmonellae, which invade the cells lining the small intestine. These organisms cause gastroenteritis lasting between 1–7 days, with symptoms that include diarrhoea, abdominal pains, nausea, vomiting, and chills, leading to dehydration and headaches. Susceptible individuals, such as the young, the elderly and those who are immunocompromised can sometimes develop more severe symptoms from non-typhoid salmonellae, such as septicaemia, or chronic conditions, such as reactive arthritis. The death rate for infection by non-typhoid salmonellosis is $<1\%$ although this figure is higher amongst some groups, particularly the elderly.

The incubation time is between 6 and 48 hours (usually 12–36). The infective dose is thought to vary widely and can depend on the individual consuming the infected food, the type of food involved and possibly the serotype involved. Small numbers (between 10–100) of cells can cause illness if consumed by the young or the elderly, or if the food consumed has a high fat content (*e.g.* chocolate, cheese or peanut butter) because the fat is thought to protect the cells from gastric acids. In general however, it is thought that high numbers (between 10^5–10^6 cells) of salmonellae need to be consumed to cause illness.

Individuals recovering from salmonellosis can continue to shed *Salmonella* in their stools for some time. Food handlers reporting *Salmonella* gastroenteritis should be excluded from work until shedding has stopped.

Incidence and Outbreaks

The incidence of human salmonellosis in the EU has been declining steadily since 1995. In 2008, just over 131 000 cases were reported in 27 countries, but there is likely to be considerable under-reporting. The decline is thought to be

mainly due to the success of measures taken to reduce *Salmonella* Enteritidis contamination in hens' eggs. Similar trends have been observed in other developed countries, including the USA, where the incidence of salmonellosis fell sharply between 1996 and 2001, but has since remained at approximately 15 cases per 100 000 of the population. Annually in the USA there are around 40 000 confirmed cases (with 400 deaths) of non-typhoidal *Salmonella* illness.

Food-borne *Salmonella* outbreaks are commonly associated with inadequately cooked eggs and poultry, or products containing these ingredients, such as egg mayonnaise. However, many other food types have been linked with outbreaks. These include dairy products (such as milk, cheese and ice cream), fruit juice, tomatoes, melons, lettuce and other salad leaves, sprouted seeds, jalapeño and serrano peppers, cereals, potato crisps and similar snack products, coconut, black pepper, chocolate, almonds, products containing sesame seed paste (tahini), peanut butter and peanut paste, herbal infusions, cooked meats, fermented meats such as salami, bottled water and reconstituted dried infant formula.

Outbreaks involving processed foods can be very large. For example, an outbreak of *S.* Enteritidis associated with ice cream that occurred in the USA in 1994 may have affected as many as 224 000 people.

Sources

Salmonella can be shed in the faeces of infected humans. Shedding can occur for some time after symptoms have subsided and some individuals become chronic carriers. However, food-borne illness caused by an infected food handler is rare and is the result of poor personal hygiene.

Many *Salmonella* infections in animals are asymptomatic, and many animals such as birds, rodents, reptiles, frogs, fish and snails can be infected with *Salmonella*. This can result in contamination of the soil and surface waters, leading to the infection of food animals and contamination of fruits and vegetables, herbs, spices, seeds, nuts and shellfish. In addition food animals can also become infected *via* their feed or from other infected food animals. Although some *Salmonella* serotypes are species specific, many are able to cross between species and cause disease in man (zoonoses). Both poultry and pigs are considered to be significant reservoirs of *Salmonella* but many foods of animal origin, such as raw meats and unpasteurised milk, are also important sources of the pathogen.

Growth and Survival in Foods

Most *Salmonella* serotypes can grow over the temperature range 7–48 °C, although growth is reduced at temperatures below 10 °C. Reports in the literature suggest that some serotypes can grow at temperatures as low as 4 °C, but this is not universally accepted.

Although most *Salmonella* serotypes are unable to grow at refrigeration temperatures, the organism is able to survive for extended periods at chill temperatures, particularly under freezing conditions.

A few *Salmonella* serotypes can grow over a range of pH values from 3.7–9.5 under otherwise ideal conditions, but the optimum is 6.5–7.5. Other factors such as temperature, the type of acid present and the presence of antimicrobials can affect the minimum pH for growth. Although *Salmonella* cannot grow under very acid conditions, the organism is able to survive for some time in acid environments. Survival times are dependent on type of acid present and temperature (chill temperatures favour survival).

Salmonellae are able to grow at water activities down to 0.94 (and possibly 0.93), lower values are dependent on serotype, food sources, temperature and pH. *Salmonella* will die out at water activities below that permitting growth, but inactivation can be extremely slow in some products (measured in years), particularly those with very low moisture and high fat content, such as chocolate. *Salmonella* is relatively resistant to drying and can survive on food production surfaces for some time.

Salmonellae are facultative anaerobes (can grow with or without oxygen) and growth is only slightly reduced under nitrogen. The organism is able to grow in atmospheres containing high levels of carbon dioxide (possibly up to 80% in some conditions).

Salmonella is not especially resistant to sanitisers used in the food industry, but is able to form biofilms that may reduce the efficacy of a sanitiser if cleaning is inadequate.

Thermal Resistance

The majority of *Salmonella* serotypes are not particularly heat resistant and are usually inactivated by pasteurisation or equivalent heat processes. *D*-Values are typically 1–10 min at 60 °C and <1 min at 70 °C, with typical *z*-values of 4–5 °C. However, there are some important exceptions. Some rare serotypes such as *S.* Senftenberg are much more heat resistant (approximately 10–20 times) than other *Salmonella* serotypes at high water activities, and some foods such as those with high fat content or with low water activities reduce the effectiveness of heat treatments normally expected to inactivate the organism.

Control Options

A HACCP approach is essential for the effective control of *Salmonella* in food production.

Processing

The control of *Salmonella* in food should start on the farm with the careful production of animal-derived raw materials, such as eggs, poultry, pork and fresh produce. Many countries have policies that encourage measures to reduce the levels of *Salmonella* in egg production units, in poultry houses, during the growing of fresh produce and also during transport of raw commodities. Such measures are especially important for products that will not receive a heat treatment prior to consumption. Food manufacturers should carefully source

their ingredients and supplies from producers implementing such measures, or purchase pasteurised products (such as milk or egg) to reduce the risk of *Salmonella* entering their facilities or reaching the consumer.

Salmonella can be effectively controlled by relatively mild heat processing (*e.g.* milk pasteurisation), but it is essential that adequate measures are in place to avoid cross-contamination between raw and cooked product. HACCP should be used to identify and implement adequate controls for *Salmonella* (ensuring the organism is absent) in all foods that will be supplied to the consumer as ready-to-eat (or drink). The HACCP plan should be rigorously reviewed when product is reformulated as such exercises can affect the efficacy of heat treatments, or the use of acid or solute as a control for *Salmonella*. General good hygiene procedures and effective temperature controls are also very important.

Product Use

To ensure that ready-to-eat foods remain free from *Salmonella*, careful handling and storage of product should be encouraged at the retail stage and in the consumer's home. Avoidance of cross-contamination is particularly important in this respect.

In the UK, consumers and caterers are encouraged to refrigerate eggs once purchased and to adhere to the "use by date" stamped on the egg, which should mean it is consumed within three weeks of date of laying.

Careful labelling and cooking instructions for raw product is very important, especially when it may appear cooked. Raw chicken entrée products have caused illnesses in the USA because they were not clearly labeled and appeared ready to eat. Consumers should also be advised to wash fresh produce, such as bagged lettuce, even when it appears ready prepared.

Consumers should be advised of high-risk foods. These include raw or partly cooked egg products, such as home made mayonnaise and ice cream, undercooked meat and meat products, unpasteurised dairy products, unpasteurised fruit juices and raw or lightly cooked seed sprouts.

Legislation

There are codes of practice in many countries around the world for the production of various food commodities that include measures to control *Salmonella*. Although it is unacceptable for any ready-to-eat product to contain viable salmonellae, there are regulations in many countries enforcing requirements in specified products.

EU regulations have specific requirements pertaining to *Salmonella* in a wide range of products, including meat and meat products, cheese, butter and cream that have not undergone standard pasteurisation processes, milk powder, whey powder, some ice cream and egg products, various shell fish products, ready-to-eat sprouted seeds, ready-to-eat fruit and vegetables, unpasteurised fruit and vegetable juices and infant formula and dried dietary foods. Sampling plans

and absence requirements vary depending on product. There are also EU requirements for *Salmonella* testing of cattle, sheep, goats, horses, poultry and pig carcasses.

USA food law also requires *Salmonella* to be absent from ready-to-eat food products that are not intended to be heated before being consumed. There are also specific requirements for the labelling of eggs not treated to inactivate the pathogen and for control of *Salmonella* in foods prepared for vulnerable populations.

Some countries have specific storage, labelling requirements and heat treatments for foods that are aimed at controlling food-borne salmonellosis. In the USA these include mandatory refrigerated storage of eggs (from farm to the consumer) and labeling requirements for egg boxes advising of safe egg handling practices. In the EU, legislation requires many eggs to be stamped with a distinguishing mark and country of origin to help trace the farm of origin in case of an outbreak.

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1.1.17 *SHIGELLA*

Hazard Identification

What is Shigella*?*

Shigella species are gram-negative, non-spore-forming bacteria belonging to the group *Enterobacteriaceae*. They have many similarities with *E. coli*, but are not included in the group microbiologists refer to as coliforms. There are four *Shigella* species, *S. sonnei*, *S. dysenteriae*, *S. flexneri* and *S. boydii*, which cause the disease known as shigellosis (also called bacillary dysentery). Although the most common route of transmission is from person to person *via* the faecal–oral route, all have been linked to food-borne outbreaks. *Shigella* infections can also occur as the result of drinking, or swimming in, contaminated water.

S. sonnei is the leading cause of shigellosis from food as well as being the leading cause of shigellosis in industrialised countries. The other three species are largely associated with contaminated water. *S. dysenteriae* is the cause of epidemic dysentery and *S. flexneri* is largely sexually transmitted.

Occurrence in Foods

Humans are the main reservoir for *Shigella* and almost all food can become infected if it is contaminated with faecal material from infected individuals, or with sewage contaminated water. Foods that require a lot of handling during preparation and are not subsequently cooked, such as salads and sandwiches, are at particular risk of contamination from infected food handlers.

Hazard Characterisation

Effects on Health

Shigellae usually only infect humans and some other primates. In humans all individuals are susceptible to *Shigella* infections but infants, the immunocompromised, and the elderly are at risk of developing the severest form of the disease.

The infective dose can be very low—as few as 10 cells can cause illness. The incubation time for illness ranges from 12 hours to 7 days (usually 1–3 days). *Shigella* species can cause an asymptomatic infection, mild diarrhoea, or can cause acute dysentery. Typical symptoms are abdominal pain and cramps, fatigue, fever and diarrhoea with mucus and sometimes blood occurring in the faeces. Frequent bowel movements can lead to dehydration. Typically, symptoms last for 3–14 days although longer term complications such as Reiter's disease, reactive arthritis and haemolytic uraemic syndrome can occur as a result of infection.

The estimated fatality rate is 0.16% although this can increase to 10–15% with some particularly virulent strains.

Incidence and Outbreaks

In countries where hygiene standards are good, the incidence of shigellosis is low. In the USA there are about 18 000 cases reported each year, although the actual figure is thought to be considerably greater (estimated to be around 450 000) because of incorrect diagnosis and under-reporting. However the incidence of cases attributable to food is unknown.

In the EU in 2008, just over 7100 confirmed cases of shigellosis were reported across 27 countries, of which 250 cases were attributed to a food vehicle (239 to *S. sonnei*). In England and Wales there were approximately 1000 cases reported each year in the period 2000–2006. However the number of cases in these two countries has increased in recent years and provisional data for 2010 indicates that the annual figure was the highest for 13 years, at around 1750 cases.

In developing countries, where hygiene standards are low, shigellosis is much more common, and each year an estimated 1.1 million people die from *Shigella* infections.

A wide variety of foods have been implicated in food-borne shigellosis. These include various salads, lettuce, green onions, spinach, uncooked baby maize, milk, soft cheese, cooked rice, spaghetti, deli meats, prawn cocktail, raw oysters, orange juice, strawberries, mashed potato, chocolate pudding and stewed apples.

Notable recent food-borne outbreaks include an outbreak of *S. sonnei* infections in 1994 affecting several Northern European countries, which was associated with imported Spanish Iceberg lettuce. In 1998, chopped parsley used as garnish was implicated in a number of outbreaks, involving 493 confirmed and probable cases of *S. sonnei* infection in the USA and Canada. *S. flexneri* caused an outbreak in the UK during 1998 with 46 cases linked to fruit salad purchased from a supermarket, and in the USA during 2001 the organism caused a large multi-restaurant outbreak linked to tomatoes.

Sources

Humans and higher primates are the main reservoir for *Shigella* species. Individuals recovering from infection can continue to shed the pathogen for weeks after the symptoms have ceased and the organism can survive for sometime in faeces.

The organism is not normally found free living in the environment and is only present in food as the result of faecal contamination.

Sewage-contaminated water can be a source of *Shigella* contamination. Although it is commonly thought that water, rather than food, is the more important vehicle for *Shigella*, public health data suggests that the reverse may be the case. Food can become contaminated from soiled hands, from contaminated water, from the use of night soil as manure and from flies that have been feeding on human faeces.

Growth and Survival Characteristics

Shigella species have a minimum temperature for growth of 6.1 °C, and a maximum of 47 °C. Although little is known about the growth of the organism in foods, it has been shown to grow on parsley, as well as on sliced fruit at ambient temperatures. However, *Shigella* does not need to grow in food to cause illness, as the very low infective dose means that the presence of the organism in food is sufficient to cause infection. *Shigella* species survive at frozen and chill temperatures, although the time of survival depends on the type of food environment as well as the temperature.

The reported pH range allowing growth of *Shigella* species is 4.8–9.3, although actual values will depend on acid type. *Shigella* species are gradually inactivated at pH values <4.0, but the organism can survive for some time in acid conditions. Fresh orange juice has been linked to a *S. flexneri* outbreak in South Africa, and *Shigella* species survived for up to 14 days in tomato and apple juice stored at 7 °C.

Shigella species can grow at water activities down to 0.96 (maximum salt concentration 5.2% NaCl). The organism dies out slowly at low water activities. Even at high NaCl concentrations (10%) some strains can survive for four days.

Shigella species are facultative anaerobes (can grow with or without oxygen). At room temperature *S. sonnei* rapidly increased in numbers in shredded cabbage stored in vacuum/modified atmosphere (30% N_2, 70% CO_2) packaging, and *Shigella* numbers remained static when stored under similar conditions at chilled temperatures.

Shigella species are not particularly resistant to commonly used preservatives and sanitisers and 200 ppm free chlorine has been shown to give a >6 $\log_{10}$ reduction of *Shigella sonnei* on parsley held at 21 °C for 5 min.

Thermal Resistance

Shigella species are easily inactivated by heat and death is rapid at temperatures above 65 °C.

Control Options

Measures to prevent food becoming contaminated with *Shigella* species should focus on preventing faecal contamination of raw and processed foods and using safe or treated water supplies for irrigation of crops and for food processing.

Processing

Washing of fresh produce, even in water containing a disinfectant, does not ensure inactivation/removal of any *Shigella* present. Good hygiene standards in countries supplying salad crops and fruit are very important to prevent the import of contaminated produce. Minimising handling, and insisting on good

levels of personal hygiene, both reduce the risk of food becoming infected from food handlers.

Food handlers suffering or suspected of suffering from *Shigella* infections or individuals who have been in contact with people suffering from shigellosis should be excluded from food handling areas until it is ensured they are free from the pathogen (typically three consecutive negative stool samples are required).

Product Use

The importance of good hygiene should be emphasised to consumers. When traveling to developing countries where shigellosis is endemic, consumers should be advised to only drink treated or boiled water, and only eat cooked foods and fruits that they have peeled themselves.

Legislation

No specific requirement is made under EC legislation, or in the FDA Food Code (2005) with regard to levels of *Shigella* in food.

Control of the pathogen is required under EC general food safety requirements in which food should not be sold if it is unsafe. The presence of *Shigella* species in food indicates poor hygiene, is unacceptable and the food is unfit for human consumption.

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1.1.18 *STAPHYLOCOCCUS AUREUS*

Hazard Identification

What is Staphylococcus aureus*?*

Staphylococcus aureus is a gram-positive, non-spore-forming bacterium that is able to grow both aerobically or anaerobically (a facultative anaerobe). Some strains of the organism have the ability to produce toxins (enterotoxins) in food, and it is the ingestion of these pre-formed enterotoxins that causes the symptoms associated with staphylococcal food poisoning.

Although *Staph. aureus* is the principle *Staphylococcus* species to cause food poisoning, other staphylococci have also been shown to produce enterotoxins. These include *Staph. intermedius, Staph. hyicus, Staph. xylosus, Staph. cohnii, Staph. epidermis* and *Staph. haemolyticus*, although *Staph. intermedius* is the only non-*Staph. aureus* species to be clearly implicated in food-borne outbreaks.

To date, 21 different staphylococcal enterotoxins have been described (known by letters of the alphabet, A–V, although a few letters are missing from the sequence). All are heat stable, water-soluble proteins that resist most proteolytic enzymes, such as pepsin or trypsin, therefore retaining their activity in the digestive tract after ingestion. Studies of outbreaks in many countries have found that staphylococcal enterotoxin A (SEA) either alone or in combination with other staphylococcal enterotoxins is the enterotoxin most frequently found in foods, as well as causing staphylococcal food poisoning outbreaks. In addition, *Staph. aureus* isolates from outbreaks are frequently found to carry the SEA gene.

It is important to note that not all enterotoxin producing staphylococci strains are coagulase or thermonuclease positive (tests for these enzymes are commonly used to indicate potential food poisoning strains). In addition, commercial kits used to test for staphylococcal enterotoxins in foods usually test for the enterotoxins classically causing staphylococcal food poisoning (A–E) and do not test for all staphylococcal enterotoxins that have been described.

In recent years there have been concerns that some strains of meticillin-resistant (or methicillin-resistant) *Staph. aureus* (MRSA) may occasionally be food-borne.

Occurrence in Foods

Foods that have caused outbreaks of staphylococcal food poisoning have usually been temperature abused, either during processing, or refrigerated storage. Foods particularly at risk of causing staphylococcal food poisoning are those that are handled and where the competing microflora has either been destroyed, or inhibited, by cooking or salting.

Foods involved in outbreaks have included milk and milk-based products, such as chocolate milk, cream, custard or cream-filled pastries, butter, ham and

other cured meats such as corned beef and bacon. Cooked meats and poultry products are also commonly implicated, as are cheeses—especially where there has been a slow start in the fermentation process leading to a delay in acid production. Other foods linked to outbreaks have included sausages, canned meat, salads, cooked meals (particularly pasta-based products), rice balls, ice cream, crepes and sandwich fillings.

Low numbers of MRSA have been found in raw meats, including pork, lamb, beef, rabbit, turkey and chicken, and in dairy products.

Hazard Characterisation

Effects on Health

Staphylococcal food poisoning is considered a mild form of food-borne disease, although all individuals are thought to be susceptible. The toxin is pre-formed in the food, so the onset of symptoms is rapid, 30 min to 7 hours (average 2–4 hours). The severity of symptoms is related to the amount of enterotoxin ingested and the susceptibility of the individual to the particular enterotoxin.

No live *Staph. aureus* cells need to be ingested for staphylococcal food poisoning to occur. However, for sufficient quantities of enterotoxin to be produced to cause illness, the organism needs to reach levels of 10^5–10^6 cfu g^{-1} in food. It is thought that the amount of enterotoxin needed to cause illness is between 0.1–1 μg. In instances where lower levels appear to have been involved, it is possible that more than one toxin type may have been present, with one or more types going undetected (see below).

Symptoms are usually nausea and vomiting with abdominal cramps, sometimes followed by diarrhoea. In more severe cases, headache, muscle cramping, dehydration and low blood pressure occur, but patients usually recover within 2 days. Although deaths have occurred amongst children and the elderly, these are rare.

Many MRSA strains have the potential to produce enterotoxins and cause 'classic' staphylococcal food poisoning. However, of more concern is the possibility of the pathogen being spread as a contaminant in food, especially among patients in a healthcare setting. Wound and systemic infections in susceptible individuals are persistent and difficult to treat.

Incidence and Outbreaks

The European Food Safety Authority reported that in Member States during 2008, staphylococcal enterotoxins were involved in 5.5% of all notified food poisoning outbreaks. In England and Wales *Staph. aureus* was linked to 1.5% of all outbreaks from 1992 to 2009 and during this period was ranked as the sixth most common bacterial cause of food poisoning. In the USA during the five-year period between 1993 and 1997, bacterial pathogens were linked to 655 outbreaks involving 43 821 cases. Of those outbreaks, 42 outbreaks involving 1413 cases (including one death) were caused by staphylococcal enterotoxins. However the usually mild nature of staphylococcal food poisoning means that

it is probably a very under-reported illness and its true incidence is uncertain. Nevertheless a number of significant outbreaks have been recorded.

In 1986 a large outbreak linked to chocolate milk in the USA affected schoolchildren and was estimated to have been caused by quantities of enterotoxin as low as 144 ng (±50). The toxin was apparently produced during a period of temperature abuse prior to pasteurisation.

A mass outbreak (>10 000 cases) of staphylococcal food poisoning occurred in Japan during 2000 and was linked to milk from a single dairy. This outbreak was thought to have involved SEA at a very low level (80 ng), but later research suggested that samples of implicated product may have contained other enterotoxins (SEH), which had been overlooked in the original testing (only 'classical' staphylococcal enterotoxins (A–E) are detected by most commercial kits).

In 2004 a very large staphylococcal food poisoning outbreak affected around 4000 people (with 16 deaths) at a celebration in Brazil. Food handlers who tested positive for enterotoxigenic S*taph. aureus* from nasopharyngeal and fingernail swabs were found to have contaminated food prepared for the occasion.

Two food-mediated MRSA outbreaks have been described in the literature. In the first incident, food contaminated by a food handler with no direct patient contact caused an MRSA infection in a severely immunocompromised individual on a haematology unit in the Netherlands. The infection was subsequently spread to other patients *via* healthcare workers, resulting in 27 cases and five deaths. The second report concerned an outbreak of acute gastroenteritis in the USA linked to enterotoxin C-producing MRSA in coleslaw purchased from a delicatessen. The outbreak MRSA strain was isolated from a food handler, food sample and three affected adults.

Sources

Humans are a primary reservoir for staphylococci. *Staph. aureus* is carried in the throats and nasal cavities of around 40% of healthy humans and also in infected cuts and sores. Almost any foodstuff can potentially become contaminated with *Staph. aureus* during physical handling and food handlers play a major role in contaminating foods with the pathogen. It can be transmitted to foods *via* manual handling as well as by coughing and sneezing.

Animals are also a key source of *Staph. aureus*. Mastitis in cows can be caused by *Staph. aureus* resulting in the contamination of raw milk and raw milk products, such as cheeses. Raw meat, particularly pork, can be contaminated with the organism, as can raw poultry and seafood.

The organism is also able to persist in the food-processing environment. It is quite resistant to desiccation and can survive on dry surfaces such as glass, metal and porcelain. It is often found in dust in ventilation systems.

Humans and animals, including pets and livestock, may also carry MRSA asymptomatically. A relatively new MRSA strain of unknown origin (MRSA CC398) has been found in livestock in the EU and North America and is

thought to be widespread in intensively reared pigs, cattle and possibly poultry. However, to date there is no evidence of food-borne transmission of MRSA CC398.

Growth and Survival in Foods

Staph. aureus can grow over the growth range 7–48 °C and the optimum temperature for growth is 37 °C. Enterotoxin can be produced over the temperature range 10–45 °C, with an optimum temperature for production of around 40 °C. The cells survive frozen storage well.

The pH range for the growth of *Staph. aureus* is 4.2–9.3, and the optimum is around 7.0. Enterotoxin can be produced between pH 4.8–9.0, although production is usually inhibited below pH 5.0. The optimum pH for enterotoxin production depends on strain and type of toxin and is between pH 6.5 and 7.3.

Staph. aureus is noted amongst food-poisoning bacteria as being unusually tolerant of low water activities. It is also more tolerant of salt (NaCl) than many other organisms and is generally able to grow in 7–10% NaCl, although some strains can grow at levels as high as 20%. Enterotoxin production has also been shown at around 10% NaCl. The minimum water activity for growth is generally considered to be 0.86. The ability to grow at such low-water-activity values confers a competitive advantage to *Staph. aureus* in low-water-activity products. Enterotoxin can be produced at A_w values as low as 0.87, but the optimum is $\geq$0.90. *Staph. aureus* is very resistant to drying and can survive for extended periods in dried foods.

Staph. aureus is best able to grow and produce enterotoxin in the presence of oxygen, but it is also able to grow and produce small quantities of enterotoxin under anaerobic conditions. High concentrations of carbon dioxide (80%) effectively inhibit *Staph. aureus* growth.

Thermal Resistance

Under normal circumstances *Staph. aureus* is not particularly heat resistant and cells are inactivated by normal pasteurisation temperatures. D_{60}-Values of around 2 min are typical in high-water-activity substrates. However, at reduced water activities, such as in salty foods (cheese, ham and bacon), pasta, or high fat foods, heat resistance is enhanced and D_{60}-values of up to 50 min have been documented.

Staphylococcal enterotoxins are very heat resistant. Inactivation of enterotoxin is affected by the water activity and pH of the substrate. Although heating at 100 °C for a minimum of 30 min will generally inactivate enterotoxin, the time for inactivation will be extended at lower water activities. If enterotoxins are present in sufficient quantities, it is possible for them to survive heat processes used in the sterilisation of low-acid products. Correctly processed canned mushrooms were implicated in an outbreak of staphylococcal food poisoning in the USA.

It is important to remember that heating of product is likely to inactivate *Staph. aureus* cells, but may not inactivate enterotoxin. Temperature abuse of

product prior to heat processing could result in staphylococcal food poisoning even though no viable *Staph. aureus* is detectable in the product.

Control Options

Processing

The presence of low levels of *Staph. aureus* in raw products is not necessarily a cause for concern—it is the prevention of staphylococcal enterotoxin production that should be considered in risk assessments. However, measures to reduce the risk of *Staph. aureus* food poisoning during processing should focus on keeping levels low. This can be achieved by minimising physical handling of product, keeping work preparation areas clean and by the implementation of good temperature control.

Using utensils and disposable gloves can help reduce direct human contact with food products. Individuals suffering from infected cuts and sores and from colds should be temporarily excluded from dealing with ready-to-eat products.

Systems where rework is fed back into the process (*e.g.* pasta/batter production), and where temperatures may permit the growth of *Staph. aureus*, can lead to fresh product being inoculated with increasing levels of the pathogen. Cooking processes applied to these products will not usually be sufficient to inactivate enterotoxin. In these circumstances, short run-times, discarding any remaining unused product and good cleaning regimes are important factors for minimising the risk from *Staph. aureus*.

Product Use

After processing, the physical handling of at-risk processed foods or cured/salted products should be kept to a minimum to reduce the risk of contamination with *Staph. aureus*.

At-risk products should either be kept well refrigerated (<5 °C) or kept hot (>63 °C): under these conditions any contaminating *Staph. aureus* cells will be unable to grow.

Legislation

EU legislation has requirements governing sampling plans and limits for coagulase-positive staphylococci in various cheeses, milk powder and whey powder. For these foods levels of coagulase-positive staphylococci below $10–10^4$ cfu g^{-1} (depending on product) at the time of removal from the premises are generally satisfactory. However, tests for staphylococcal enterotoxin are required where levels of coagulase-positive staphylococci are detected at $>10^5$ cfu g^{-1}, and these toxins should be absent in 25 g. If coagulase-positive staphylococci are found at levels $>10^3$ cfu g^{-1} in shelled and shucked products of cooked crustaceans and molluscan shellfish, EU regulations require improvements in production hygiene.

The US FDA food compliance program suggests that any cheese, fish or seafood product could be removed from the market place if it is found positive for staphylococcal enterotoxin or if levels of *Staph. aureus* are $\geq 10^4$ cfu g^{-1}.

In 2009 the HPA published guidelines for assessing the microbiological safety of ready-to-eat foods placed on the market (see link below). These state that levels of *Staph. aureus*, and other coagulase-positive staphylococci, of 20 per g to $<10^4$ per g in these products is borderline unsatisfactory, and levels $>10^4$ per g are unsatisfactory: potentially injurious to health and/or unfit for human consumption (not applicable to foods fermented with *Bacillus* species).

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1.1.19 STREPTOCOCCI

Hazard Identification

What are Streptococci?

Streptococcus is a genus of gram-positive, non-spore-forming bacteria. Most species are facultative anaerobes, but some are strict anaerobes and will not grow in the presence of oxygen. Although some streptococci have been implicated in human disease, the majority of species are non-pathogenic.

Some of the streptococci implicated in human illness, notably but not exclusively *Streptococcus pyogenes* and *Streptococcus equi* subspecies *zooepidemicus*, may be transmitted by food and have been linked to food-borne outbreaks associated with salads, milk and dairy products.

Streptococcus pyogenes is a member of the Lancefield Group A streptococci (often abbreviated to GAS). There are around 80 distinctly different serological types of *Str. pyogenes*. It is a facultative anaerobe and it displays β-haemolysis on blood agar.

Str. zooepidemicus belongs to the Lancefield Group C streptococci, and it too is β-haemolytic on blood agar. The organism is a cause of zoonotic disease (transmitted from animals to humans).

Occurrence in Foods

Str. pyogenes and *Str. zooepidemicus* can both be present in unpasteurised milk taken from cows suffering from mastitis. Either organism could therefore be present in dairy products made from raw or inadequately pasteurised milk. *Str. pyogenes* can also be present in foods as the result of poor hygienic practices by food handlers suffering from *Str. pyogenes* infections.

Hazard Characterisation

Effects on Health

The main mode of transmission for *Str. pyogenes* infections is person-to-person contact, or *via* airborne droplets, but the organism can also be food borne. Typically, *Str. pyogenes* causes pharyngitis, but it can also cause tonsillitis, scarlet fever, septic sore throat and skin infections (such as impetigo). The organism is occasionally associated with very severe skin/wound infections sometimes leading to necrotising fasciitis—in these cases the organism is often described in the media as "flesh eating".

All individuals are susceptible to infection. Although unknown, the infectious dose is thought to be relatively low (<1000 organisms) and onset of symptoms is 12–72 hours after infection. Typically, these include a sore throat, fever, headache, runny nose, nausea and vomiting. Occasionally a rash occurs. Complications very occasionally occur and the fatality rate is low. If untreated

the condition can remain infective for around 10–21 days, although proper treatment can reduce the infectious period to 24–48 hours.

Many *Str. zooepidemicus* infections in humans are linked to handling animals, but food-borne outbreaks have also been reported. Typically, food-borne infections of *Str. zooepidemicus* cause pharyngitis, but it has also been associated with acute post-streptococcal glomerulonephritis (an inflammation of the kidney tubules) and sometimes meningitis. In the USA in 1983, a food-borne outbreak associated with *Str. zooepidemicus* reportedly caused a range of symptoms, from fever and chills to systemic infections, such as pneumonia, endocarditis and pericarditis.

Incidence and Outbreaks

There is little information on the incidence of food-borne streptococcal infections.

Foods associated with outbreaks of Group A streptococci infections include milk, yoghurt, ice cream, custard, rice pudding, meats, seafood, corn, devilled eggs, salads and sandwiches made from eggs or mayonnaise. In many cases the foods had been prepared by infected food handlers and then stored at room temperature for a few hours prior to consumption.

Foodborne outbreaks of *Str. zooepidemicus* infections have been associated with unpasteurised milk and dairy products. For example, an outbreak occurred in the USA during 1983 caused by contaminated "queso blanco", a homemade white cheese made from raw milk. Unpasteurised milk contaminated with *Str. zooepidemicus* caused an outbreak involving seven deaths in the UK in 1984. More recently in 2006, an outbreak of *Str. zooepidemicus* infections in Spain was associated with inadequately pasteurised cheese and involved 15 cases resulting in five deaths.

Sources

The natural reservoir for *Str. pyogenes* is humans. However, humans can transmit the organism to cows on occasion, causing mastitis. The organism is found on human skin, mucous membranes (particularly in the respiratory tract) and can sometimes colonise the rectum.

Although *Str. zooepidemicus* has been isolated mainly from horses, it has also been found in a wide range of animals including sheep, cattle and pigs.

Growth and Survival Characteristics

Streptococci cannot grow at chill temperatures, and although some species can grow at elevated temperatures (*Str. thermophilus* can grow at 52 °C), this is not typical of the genus. The minimum temperature for the growth of *Str. pyogenes* is around 20 °C, with a maximum of 40 °C.

Str. pyogenes has been shown to survive in various environments outside the host. It can survive in cheese for up to 126 days, on the rim of a drinking glass for two days, on blankets for up to 120 days, and in dust for up to 195 days.

Outbreaks of *Str. pyogenes* infections have been associated with food vehicles with relatively low pH, such as yoghurt and products containing mayonnaise.

Thermal Resistance

Streptococcus species are not heat resistant bacteria and are inactivated by normal milk pasteurisation processes.

Control Options

Processing

The control of food-borne *Streptococcus* infections relies upon the implementation of strict hygiene, ensuring the rapid cooling of foods to refrigerated temperatures, and avoiding the use of unpasteurised milk. Food handlers with skin lesions or symptoms of respiratory illness should be excluded from food handling duties.

Product Use

Consumers should be advised to avoid the consumption of raw milk and associated dairy products.

Legislation

There are no specific requirements for levels of *Streptococcus* species in foods in EU or USA legislation.

Sources of Further Information

Published

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1.1.20 *VIBRIO CHOLERAE*

Hazard Identification

What is Vibrio cholerae*?*

Vibrio cholerae is a gram-negative, non-spore-forming bacterium. It is the causative organism of cholera, a serious human disease responsible for many fatal outbreaks throughout history. Although cholera is usually associated with poor hygiene and faecal contamination, the disease can also be food borne.

Not all strains of *V. cholerae* cause cholera. Strains (or serotypes) causing classic epidemic cholera are O1 and O139, but there have been rare reports of non-O1/O139 serotypes causing cholera-like disease.

Occurrence in Foods

Vibrio cholerae can be present on food if it is contaminated by polluted water, or by food handlers carrying the pathogen. Contaminated water used to make ice can lead to the contamination of beverages.

In the developed world *V. cholerae* infections are usually associated with the consumption of seafood (including fish, shellfish, crabs, oysters and clams). Shellfish can become contaminated from environmental sources and most non-O1/O139 cholera infections are associated with the consumption of raw oysters. Other foods implicated in *V. cholerae* infections are fruits and vegetables, grains, poultry, meat and legumes.

Hazard Characterisation

Effects on Health

For O1/O139 cholera, symptoms can occur between 5 hours and 6 days after infection. The infective dose is thought to be 10^6–10^8 cells. Those most at risk of developing severe cholera are individuals with impaired or undeveloped immunity, such as the immunocompromised and young children, and those suffering from malnutrition. Typically, symptoms start with mild diarrhoea, leading to more severe diarrhoea typified by the production of grey 'rice water' stools. Nausea, abdominal pains and low blood pressure can also occur. If untreated, the infection can lead to dehydration, and in severe cases this can result in death. Healthy individuals usually recover in 1–6 days.

For non-O1/O139 *V. cholerae* infections, symptoms usually occur within 48 hours of infection and last for around 6–7 days. A much milder form of diarrhoea occurs than with O1/O139 cholera, but it can be bloody and is accompanied by abdominal cramps and fever. Sometimes nausea and vomiting also occur. In rare cases the infection can result in septicaemia, and deaths have been reported.

Incidence and Outbreaks

The incidence of infections caused by *V. cholerae* in the developed world is low and is usually caused by serotypes of the organism that cause less severe forms of disease (non-O1/O139 serotypes). In the USA since 2000, on average around 40 cases of non-O1 and non-O139 *V. cholerae* are reported to the CDC each year.

However, *V. cholerae* is a major health problem in parts of India, Asia, Latin America and Africa, and in these regions O1/O139 cholera is endemic. In these parts of the world the disease is linked to poverty and poor sanitation, and large water-borne epidemics and food-borne outbreaks occur. Although in 2007 there were, worldwide, 178 000 reported cases of cholera (with 4031 deaths), the WHO estimates that the number of cases was under-reported and that as many as 120 000 people died from the disease, while millions more individuals were infected. A severe outbreak of cholera in Zimbabwe during 2008–2009, which was linked to a breakdown of clean water supplies as well as to poor health services, affected over 92 000 people, with more than 4000 deaths.

Although most cholera outbreaks are caused by contaminated water, food-borne outbreaks have been reported, but are rare in developed regions. Although primarily associated with shellfish, other fish, as well as vegetables, fruit, meat, frozen coconut milk and cooked rice have been implicated as vehicles for the pathogen. A cholera outbreak in Zambia during 2004, in which raw vegetables were implicated as the vehicle, involved an estimated 4343 cases, with 154 deaths.

Sources

Humans are the main reservoir for *V. cholera* O1 and O139 strains. Individuals suffering from cholera excrete large numbers of cells into the environment. In addition, asymptomatic carriers of the organism are known to occur. Contamination of raw or processed food is usually the result of faecal contamination (either directly or indirectly from faecally contaminated water).

V. cholerae O1 survives for short periods in fresh water, but it can survive in seawater for longer periods. Fish and shellfish from contaminated estuarine environments may become colonised by the pathogen and are a particular risk. *V. cholerae* O1 can persist in contaminated shellfish for many weeks without requiring continuous contamination from human faeces.

Non-O1/O139 *V. cholerae* strains are part of the natural marine environment although the existence of a natural aquatic reservoir for O1/O139 strains is uncertain.

Growth and Survival in Foods

V. cholerae can grow over the temperature range 10–43 °C, with an optimum of 37 °C. The organism can increase rapidly in temperature abused processed foods where there is little competing microflora. It can also survive for extended periods under refrigeration and is reported to survive in moist, low-acid chilled

foods for two or more weeks. It can also survive for long periods at freezing temperatures.

The pH range for the growth of *V. cholerae* is 5.0–9.6, with an optimum value of 7.6. It is tolerant of high pH conditions, but not acid and is rapidly inactivated at pH values of <4.5 at room temperature.

V. cholerae, unlike other *Vibrio* species, does not have an absolute requirement for salt to grow, although its growth is enhanced in the presence of low concentrations of salt. The organism is sensitive to desiccation and survives for fewer than 48 hours in dry foods.

V. cholerae is a facultative anaerobe (grows with or without oxygen). It grows best, however, under aerobic conditions.

The organism is not resistant to sanitisers normally used in food-processing environments.

Thermal Resistance

V. cholerae is not heat resistant and is killed by pasteurisation temperatures with D_{60} of 2.65 min and D_{71} of 0.30 min being reported. Cooking to 70 °C is normally adequate to ensure inactivation of *V. cholerae*.

Control Options

Measures to prevent food becoming contaminated with *V. cholerae* should focus on preventing faecal contamination of raw and processed foods and using safe or treated water supplies for irrigation of crops and for food processing. Raw sewage should not be used as a fertilizer for crops.

The WHO advises that there need not be an embargo on importing foods from cholera-affected areas. It is suggested that importers agree with food exporters on the good hygienic practices that need to be implemented during food handling and processing to prevent, minimise, or reduce the risk of any potential contamination.

Legislation

EU regulations, and the FDA Food Code do not have specific requirements relating to levels of *V. cholerae* in foods.

The presence of *V. cholerae* (toxigenic O1 or O139 or non-O1 and non-O139) in a 25 g sample of ready-to-eat fishery products (minimal cooking by consumer) is an action level in the FDA's Fish and Fishery Products Hazards and Controls Guidance (4th edn, April 2011).

The HPA guidelines for assessing the microbiological safety of ready-to-eat foods placed on the market state that if *V. cholerae* (O1 and O139) is detected in 25 g, these foods are considered unsatisfactory: potentially injurious to health and/or unfit for human consumption.

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1.1.21 *VIBRIO PARAHAEMOLYTICUS*

Hazard Identification

What is Vibrio parahaemolyticus*?*

Vibrio parahaemolyticus is a gram-negative, non-spore-forming bacterium normally found in marine environments. It is the most likely *Vibrio* species to be implicated in food-borne disease, although both *V. vulnificus* and *V. cholerae* may also cause food-borne infections and are covered elsewhere in this book. Other *Vibrio* species associated with food-borne disease to a much lesser extent are *V. alginolyticus*, *V. mimicus*, *V. damsela*, *V. hollisae* and *V. fluvialis*.

Not all strains of *V. parahaemolyticus* cause illness and two distinct groups have been defined: pathogenic 'Kanagawa-positive' strains, which cause *V. parahaemolyticus* food poisoning, and 'Kanagawa-negative' strains, which do not.

Occurrence in Foods

Vibrio parahaemolyticus is found mainly in foods of marine origin, and studies carried out in the USA found that 60–100% of seafood samples were contaminated with the organism. When present, it is usually at levels of around $10\,\text{cfu}\,\text{g}^{-1}$, although levels can be around $10^3\,\text{cfu}\,\text{g}^{-1}$, or even higher in the warmer summer months. Seafood from warm waters presents a greater risk of *V. parahaemolyticus* food poisoning, with 89% of oysters causing the illness reported as originating from waters where the temperature was above 22 °C.

Cases of illness caused by *V. parahaemolyticus* have also occurred when seafoods have been cross-contaminated by raw fish after cooking and subsequently temperature abused. Implicated seafoods in outbreaks include clams, oysters, scallops, shrimp and crab.

Hazard Characterisation

Effects on Health

Kanagawa-positive strains of *V. parahaemolyticus* produce a heat-stable haemolysin, which can be pre-formed in food. This haemolysin is thought to be responsible for the illness, although other toxins could also be involved.

Although the minimum infective dose for *V. parahaemolyticus* is unknown, volunteer studies with healthy individuals have shown that high numbers (10^5–10^7) of Kanagawa positive *V. parahaemolyticus* cells are required to cause illness. The infective dose may be lower when the organism is consumed at the same time as antacids or foods. All individuals are susceptible to infection by *V. parahaemolyticus*.

The incubation time for the infection is 4–96 hours (average 15 hours). The organism usually causes a mild-to-moderate form of gastroenteritis with abdominal cramps and watery diarrhoea. Nausea, vomiting, headache and

fever can also occur. Some affected individuals can require hospitalisation. Symptoms can last for 1–7 days, although the average is 2.5 days and the illness is usually self-limiting. Deaths rarely occur.

Incidence and Outbreaks

The consumption of raw seafood products (such as oysters and sashimi/sushi) from high-risk waters significantly increases the risk from *V. parahaemolyticus* food poisoning. The pathogen is a major cause of food poisoning in Asian countries, but in the UK illnesses caused by *V. parahaemolyticus* are usually associated with the consumption of imported seafoods, or with foreign travel.

In Japan, *V. parahaemolyticus* reportedly accounts for approximately half of cases bacterial food-borne infection. In the USA, *V. parahaemolyticus* illnesses prior to 1997 were infrequently reported; however during 1997 and 1998 there were four multi-state outbreaks associated with the consumption of raw or undercooked oysters, affecting over 700 individuals. This dramatic increase in illnesses caused by *V. parahaemolyticus* in the USA has been attributed to the emergence of a new pandemic strain (O3 : K6); previously this strain had only been associated with illness in Asia. The USA Center for Disease Prevention and Control (CDC) estimates that annually there are 4500 cases of *V. parahaemolyticus* in the USA, although on average only 215 (with 1–2 deaths) of these are confirmed.

In the EU *V. parahaemolyticus* infections are rarely reported. However, a review of clinical data in Spain published in 2005 has concluded that they are more common than previously thought and a *V. parahaemolyticus* outbreak in Spain in 2004 caused by seafood harvested from European waters has been linked to the pandemic strain O3 : K6.

Sources

Vibrio parahaemolyticus is a normal inhabitant of the marine environment and is an obligate halophile (having a minimum requirement for salt to grow). Favourable conditions for its growth are found in tropical and temperate seawaters. For this reason the organism is usually associated with seafood from estuarine or coastal marine environments where water temperatures are highest, such as the Southern coastal USA states and Japan, particularly during the summer months. However, an outbreak of *V. parahaemolyticus* in 2004 was linked to Alaskan oysters and rising seawater temperature is thought to have lead to the organism proliferating in shellfish from this Northerly latitude.

Seasonal temperature variations influence the presence of the organism and although levels are highest in shellfish during the warmer months, the organism can over-winter in sediment and can be difficult to detect in water or fish samples during the winter period. However, more than 99% of environmental isolates are not pathogenic (*i.e.* they are Kanagawa-negative).

Human asymptomatic carriers of *V. parahaemolyticus* are known to occur and they can act as a source of environmental contamination.

Growth and Survival in Foods

The temperature range for growth of *V. parahaemolyticus* is 5–43 °C, with an optimum temperature of 37 °C. Under optimal conditions growth can be very rapid. The organism declines (but is not eliminated) in numbers during chilled (0–5 °C) storage.

The organism survives freezing although numbers will initially be reduced.

The pH range for growth is 4.8–11, optimum 7.8–8.6. The organism is not particularly tolerant of low-pH environments and the minimum pH for growth decreases as the storage temperature increases towards optimum.

V. parahaemolyticus is unable to grow unless salt (NaCl) is present. The optimum salt concentration for growth is 3% (equating to 0.980 water activity). The organism can grow in salt concentrations from 0.5–10%, representing a water activity range of 0.996–0.940.

The organism is inactivated by desiccation and by exposure to fresh water.

V. parahaemolyticus is a facultative anaerobe (can grow in the presence or absence of oxygen) and can grow in foods that are either vacuum or aerobically packaged. It grows best however under aerobic conditions.

Thermal Resistance

V. parahaemolyticus is not heat resistant and is inactivated at temperatures >65 °C. *D*-values of <1 min at 65 °C, and 2.5 min at 55 °C have been reported.

Control Options

Seafood should be considered potentially contaminated with *V. parahaemolyticus*, particularly if it has been harvested from tropical and subtropical waters. However, it should be noted that seafood from what are considered 'colder' seawaters may be contaminated, particularly shellfish harvested during the summer months. The risk of *V. parahaemolyticus* food poisoning is increasing with the worldwide growth in the consumption of raw fish.

Processing

Decontamination processes such as depuration or relay technologies are not effective at removing *V. parahaemolyticus* from shellfish, and effective control of the organism should focus on keeping numbers low. Measures to ensure this include maintenance of the cold chain (<5 °C) from harvest to consumer, minimising delays between harvesting and landing, and avoiding further exposure to untreated seawater and soiled containers. Shellfish-growing areas can also be monitored for the presence of pathogenic strains of *V. parahaemolyticus*, with the closure of waters for harvesting if levels of the pathogens are deemed to be too high.

Seafood should be handled carefully to avoid cross-contamination between raw and cooked product and avoiding temperature abuse is also very important.

Product Use

Consumers should be encouraged to cook seafood thoroughly and not to eat product raw. In the USA, raw oysters and restaurants offering raw oysters on their menus are required to carry health warnings about eating raw shellfish.

Legislation

EU regulations and the FDA Food Code do not have specific requirements relating to levels of *V. parahaemolyticus* in foods.

The FDA's Fish and Fishery Products Hazards and Controls Guidance (4th edn, April 2011) has an action level of $\geq 10^4$ cfu g^{-1} (Kanagawa-positive or negative) for ready-to-eat products (minimal processing by the consumer). For post-harvest processed clams, mussels, oysters and whole and roe-on scallops, fresh or frozen, that make a label claim of "processed to reduce *Vibrio parahaemolyicus* to non-detectable levels" this guidance states that levels of the organism must be <30 per g (by the most probable number (MPN) method).

The HPA guidelines for assessing the microbiological safety of ready-to-eat foods placed on the market states that levels of *V. parahaemolyticus* in these products of <20 cfu g^{-1} are satisfactory, 20–1000 cfu g^{-1} is likely evidence for poor processing or cross-contamination and levels of >1000 cfu g^{-1} are unsatisfactory/potentially injurious to health.

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1.1.22 *VIBRIO VULNIFICUS*

Hazard Identification

What is Vibrio vulnificus*?*

Vibrio vulnificus is a gram-negative, non-spore-forming bacterium normally found in marine environments. It is an occasional cause of serious infections, which may sometimes be food borne. *Vibrio vulnificus* is an obligate halophile (having a minimum requirement for salt to grow) and favourable conditions for growth are found in tropical and temperate seawater.

Occurrence in Foods

This pathogen is usually associated with seafoods from estuarine or coastal marine environments where water temperatures are highest, such as the Southern coastal USA. Although *V. vulnificus* is most often associated with filter-feeding shellfish, such as oysters, which concentrate the bacteria within the tissues, potentially the organism could contaminate any fish from the marine environment. It is mostly associated with shellfish and crustaceans, but can also be found in the guts of fish feeding on plankton or other fish.

Oysters collected monthly from 14 states in the USA contained *V. vulnificus* levels of 0 to 1 100 000 cfu g^{-1}, with water temperature and salinity having a dramatic influence on numbers present. Warm summer temperatures see concentrations of the organism at their highest in oysters. During the summer months it has been estimated that nearly 100% of oysters from the Gulf of Mexico are contaminated with *V. vulnificus*, with levels usually around 10^3–10^4 per g and most infections caused by the organism occur during the summer months when seawater temperatures are between 20 and 30 °C.

Hazard Characterisation

Effects on Health

V. vulnificus can cause three types of illness. Gastroenteritis (5–10% of cases), primary septicaemia (45% of cases), or wound infections (45% of cases). In healthy individuals the consumption of *V. vulnificus* contaminated seafood can cause gastroenteritis, but in susceptible individuals (those suffering from some form of chronic disease such as liver disease, or AIDS) it causes primary septicaemia and these infections are very severe (associated with a mortality rate >50%). Around 90% of *V. vulnificus* infections require hospitalisation.

The infective dose for healthy individuals is unknown and the gastroenteritis (diarrhoea, vomiting and abdominal pain) suffered by these individuals usually occurs about 16 hours after infection. This form of the disease is considered self-limiting.

The infective dose for at-risk groups could be fewer than 100 cells and onset of primary septicaemia can occur from 7 hours to 2 days after exposure. Initial

symptoms include chills, fever and malaise, and septicaemia can occur 36 hours after symptoms first occur. Secondary, bulbous lesions may occur, especially in the extremities, which can lead to amputation.

V. vulnificus wound infections occur when an open lesion is infected by contaminated seawater. Seafood handlers are at risk if they cut themselves while cleaning and harvesting oysters and if the lesion is exposed to contaminated seawater.

Incidence and Outbreaks

The consumption of raw seafood products by susceptible individuals, in particular oysters, from high-risk waters significantly increases the risk from *V. vulnificus* food poisoning. In the USA most cases are reported in the states bordering the Gulf of Mexico, where oysters are harvested from the warm waters. However, recent trends in Florida indicate that vibriosis associated with raw oyster consumption has decreased, whilst the incidence associated with wound infections has increased.

Although there are not many reported cases annually (around 90 cases are reported in the USA each year and not all of these are associated with the consumption of contaminated seafood), the high mortality rate associated with *V. vulnificus* infections has made this organism an important public health issue, particularly in the USA. Infections due to *V. vulnificus* have also been reported in Korea, Taiwan, Japan, Israel and Turkey, and cases have also occasionally occurred in the EU.

No major food-borne outbreaks have been caused by this pathogen and cases tend to be sporadic, the frequency increasing during the summer months. *V. vulnificus* infections are rarely reported during the winter months even though most oysters are eaten during this period.

Sources

V. vulnificus is naturally present in coastal seawater in tropical and temperate regions throughout the world. Numbers of the organism relate to water temperature with higher numbers found during summer months. *V. vulnificus* is thought to enter a viable but non-culturable state (VNC) in cold winter waters and although still present can be difficult to detect. The low numbers of reported illnesses suggests that either many *V. vulnificus* strains are not pathogenic to humans, or that the infective dose is high for healthy individuals.

Growth and Survival in Foods

V. vulnificus can grow over the temperature range 8–43 °C, with an optimum temperature of 37 °C. In live oysters the organism does not grow below 13 °C indicating the importance of chilling shellfish as soon as possible after harvesting. *V. vulnificus* survives in oysters at chill temperatures (0–4 °C) but can be difficult to culture from chilled environments. This can make the detection and enumeration of the organism from chilled foods unreliable.

Although freezing initially reduces levels of the pathogen in oyster tissue, the surviving *V. vulnificus* population remains stable throughout frozen storage.

The pH range for growth of the pathogen is 5–10, and the optimum is 7.8. The organism is inactivated at pH values <5.0.

V. vulnificus is a halophile and is able to grow at salt levels between 0.5–5%, although the optimum concentration for growth is 2.5%. This equates to a water activity range of 0.996–0.960. The pathogen is sensitive to dehydration.

V. vulnificus is a facultative anaerobe (able to grow in the presence or absence of oxygen). Vacuum packing combined with frozen storage was found to reduce levels of *V. vulnificus* in oysters more effectively than frozen storage alone but cannot be relied upon to completely eliminate the pathogen.

Thermal Resistance

V. vulnificus is not a heat-resistant organism and is easily destroyed during cooking processes. A low temperature pasteurisation of 10 min at 50 °C for shellstock oysters has been found to ensure inactivation.

Control Options

Processing

Decontamination processes such as depuration or relay technologies are not effective at removing *V. vulnificus* from shellfish, so strategies should focus on keeping levels low and encouraging consumers not to eat raw shellfish. Shellfish should be harvested from approved waters. In California there are restrictions on the sale of oysters from the Gulf of Mexico from April to October unless the oysters are treated with a scientifically validated method to eliminate *V. vulnificus*.

Levels of the pathogen increase in temperature-abused shellfish and the time taken from harvesting to refrigeration is known to be critical. In the USA the time permitted from harvest to refrigeration can depend on whether an area has been associated with *V. vulnificus* infections, as well as the temperature of the seawater, the season and the air temperature. Oysters harvested during the warmer months can be diverted for cooking, pasteurisation or irradiation to avoid the possibility of them being consumed raw.

Product Use

Consumers, particularly those with medical conditions that make them more at risk of contracting *V. vulnificus* infections, should be advised of the risks of consuming raw or undercooked shellfish.

Legislation

EU regulations, and the FDA Food Code do not have specific requirements relating to levels of *V. vulnificus* in foods.

The presence of *V. vulnificus* in ready-to-eat fishery products (minimal cooking by consumer) is an action level in the US Food and Drug Administration's: Fish and Fishery Products Hazards and Controls Guidance (4th edn, April 2011). For post-harvest processed clams, mussels, oysters and whole and roe-on scallops, fresh or frozen that make a label claim of "processed to reduce *Vibrio vulnificus* to non-detectable levels" this guidance states that levels of the organism must be <30 per g (by the most probable number (MPN) method).

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1.1.23 *YERSINIA ENTEROCOLITICA*

Hazard Identification

What is Yersinia enterocolitica*?*

Yersinia species are gram-negative, non-spore-forming, facultatively anaerobic bacteria belonging to the group *Enterobacteriaceae*. Two species of *Yersinia* have been associated with food-borne disease in man, *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* (see elsewhere in this book).

Not all strains of *Y. enterocolitica* are pathogenic. In fact, only a proportion of isolates can cause disease and these potentially pathogenic isolates carry a piece of genetic material known as a 'virulence' plasmid. There are a large number of different serotypes, but the most common cause of disease worldwide is serotype O : 3. In the EU and the USA 90% of cases of yersiniosis are caused by this serotype. Other important pathogenic serotypes are O:9, O:8 and O:5,27, although at least another eight serotypes are recognised as potential causes of yersiniosis.

Occurrence in Foods

Yersinia enterocolitica is most often associated with pork products and milk, because food-borne outbreaks are often linked to these foods. However, the organism has been isolated from other foods such as fruits, vegetables, dairy products, various meats and poultry, oysters, fish, salads, sandwiches, pastries and tofu, although isolates from these sources frequently include non-pathogenic types.

Hazard Characterisation

Effects on Health

The infective dose for *Y. enterocolitica* infection is unknown, but the severity of the symptoms is thought to be related to the number of organisms ingested. Those most at risk of developing the disease and its associated long-term effects are infants, the elderly and the immunocompromised. Yersiniosis is more common in males than females.

The incubation time for *Y. enterocolitica* infections is from 1–11 days (usually 1–2 days). The disease is usually self-limiting and of short duration, and symptoms typically cease after 2–3 days. Occasionally, symptoms can last for 1–3 weeks, or even a few months.

Symptoms vary and in adults can include abdominal pain, fever, vomiting, nausea and diarrhoea. The infection is often confused with appendicitis and unnecessary appendectomies can be carried out as a result of the abdominal pain. *Y. enterocolitica* infections in children usually cause gastroenteritis and inflammation of the lymph glands.

Longer term effects include reactive arthritis and skin disorders, such as painful red skin lesions. In rare cases, bacteraemia can occur (when the organism enters the blood stream), which may occasionally be fatal. But this tends to affect individuals who have other underlying disease.

Incidence and Outbreaks

Yersiniosis is a relatively common food-borne infection in the Northern EU, Japan and Scandinavia and it is the third most common cause of gastroenteritis in Finland and Norway. In 2008 the overall notification rate for yersinosis in 22 EU countries plus Liechtenstein and Norway, was 2.66 per 100 000 of population. *Yersinia enterocolitica* was the causative organism in 92% of these cases (*Y. pseudotuberculosis* also causes the disease yersiniosis). The highest reported rates were in Lithuania and Finland, with 15.9 and 11.5 cases per 100 000 of population respectively. Infection is often acquired through the consumption of raw or undercooked pork products, or from contaminated milk and fresh produce. Most cases of yersiniosis appear to be sporadic but outbreaks do occur. In Norway in 2011 an outbreak was linked to bagged salad mix containing radicchio rosso, and a previous outbreak during 2005–2006 was associated with a ready-to-eat pork product (sylte).

In the USA and Canada, where food-borne outbreaks of yersiniosis are relatively unusual, cases have mainly been linked to the consumption of raw, or recontaminated pasteurised milk. In the USA there is approximately one culture-confirmed case of yersiniosis per 100 000 of population every year, with infections occurring most often in children and in the winter months. In 1976 an outbreak involving 217 individuals in the USA was linked to the consumption of a chocolate milk drink. Chitterlings, a speciality prepared from raw pig intestines, have been associated with outbreaks amongst the African–American community in the USA.

Sources

Yersinia enterocolitica is ubiquitous; it can be found in a wide range of animals and in the environment. However, many strains found in soil and water are non-pathogenic. The organism has been isolated from water supplies (drinking and surface) and infections have been caused by contaminated water.

The most common reservoir for the organism amongst food-producing animals is the throat and tonsil area of pigs. However, the organism can be carried at a lower rate by sheep, poultry and cattle. Data from the USA suggests that *Y. enterocolitica* in cattle faeces is a potential source of contamination for raw milk.

Low numbers of *Y. enterocolitica*, many of which are non-pathogenic, can be part of the transient intestinal flora of healthy humans. Food handlers have been implicated in cases of food-borne disease, and person-to-person transmission, *via* the faecal–oral route, has been reported as the cause of yersiniosis infections.

Growth and Survival Characteristics

Yersinia enterocolitica is psychrotophic and is able to grow at chill temperatures. The organism can grow over the temperature range 0–44 °C, although there have been reports of extremely slow growth at −1.3 °C. The optimum temperature for growth is 28–29 °C. *Yersinia enterocolitica* survives freezing and there have been reports that it can survive in frozen foods for some time.

The pH range for growth is 4.2–10, although minimum pH values depend on the type of acid present and the storage temperature—the minimum of 4.2 is more likely to occur with inorganic acids. With organic acids, such as acetic or citric acids, the minimum pH for growth is around 5.0. *Y. enterocolitica* is inactivated at lower pH values, but can survive in acid conditions for some days at refrigerated temperatures.

The minimum water activity for growth is 0.945. Levels of salt between 5–7% inhibit growth.

The organism is a facultative anaerobe, and is able to grow with or without oxygen. Vacuum packaging and some modified atmospheres (100% N_2 or CO_2/N_2 mixtures) can slow down or inhibit growth, particularly at chill temperatures.

Thermal Resistance

Yersinia enterocolitica is sensitive to heat and is easily inactivated at temperatures above 60 °C. *D*-Values of around 0.5 min and 2 s at 60 °C and 65 °C, respectively, have been recorded. Typical pasteurisation treatments should easily ensure that the organism is destroyed.

Control Options

Processing

The level of *Y. enterocolitica* in raw pork can be reduced by using measures to limit the level of faecal contamination on pig carcasses after slaughter. Careful removal of the tongue from the head of pigs soon after slaughter can also help to minimise carcass contamination. Raw pork should always be regarded as a potential source of *Y. enterocolitica* and should be handled as such.

Control of the pathogen on fresh produce should focus on avoiding contamination. Measures include implementing good practices in growing and harvesting that are designed to minimise the risk of faecal contamination. The use of irrigation water from clean, uncontaminated sources is also important.

Cooking and milk pasteurisation processes are adequate means of destroying the pathogen, and care should be taken to ensure that recontamination of heat processed foods does not occur after the cooking process. For example, a multistate outbreak in the USA was blamed on the use of dirty, contaminated crates to transport pasteurised milk. The presence of *Y. enterocolitica* in any heat-processed food indicates inadequate heating or post-process contamination,

and is unacceptable. The organism may increase during chilled storage and therefore refrigeration is not an effective means of control.

Product Use

The risk of contracting yersiniosis increases with the consumption of raw pork, or pork cooked rare. Consumers should be advised on measures to ensure that pork products are cooked thoroughly and that cross-contamination from raw pork to ready-to-eat products should be avoided.

Consumers should also be advised of the potential health risks from drinking raw milk, and water from untreated sources, particularly in areas where pigs are kept.

Legislation

There are no specific requirements for levels of *Y. enterocolitica* in foods under EU legislation or in the FDA Food Code.

Sources of Further Information

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1.1.24 *YERSINIA PSEUDOTUBERCULOSIS*

Hazard Identification

What is Yersinia pseudotuberculosis*?*

Yersinia pseudotuberculosis is a gram-negative, non-spore-forming, bacterium belonging to the family *Enterobacteriaceae*. Although another species, *Y. enterocolitica*, is the primary cause of the disease known as yersiniosis in humans, *Y. pseudotuberculosis* has also been associated with the infection. There is increasing evidence that disease caused by *Y. pseudotuberculosis* can be food borne, and in the past decade food-borne outbreaks have been reported in the literature.

Since the beginning of the twentieth century the classification of this species has changed repeatedly and it has been known by a number of names. Initially it was called *Pasteurella pseudotuberculosis*, and then *Shigella pseudotuberculosis* until the current name *Yersinia pseudotuberculosis* was established in the 1960s. Very old references may still refer to the organism using either of these previous names. Not all strains of *Y. pseudotuberculosis* are pathogenic, but the potential pathogenicity of isolates can only be determined by laboratory testing.

Occurrence in Foods

There is little data on the occurrence of *Y. pseudotuberculosis* in food. A study in Italy, which examined 10 842 food samples for the pathogen failed to recover it from a food source. However, the organism is reported to be difficult to isolate from food and from the environment. Cases of yersiniosis caused by *Y. pseudotuberculosis* have been associated with the ingestion of contaminated drinking water, vegetable juice, pasteurised milk, salad leaves and raw vegetables.

Hazard Characterisation

Effects on Health

The infective dose for *Y. pseudotuberculosis* infections is unknown, but is probably between 10^6–10^9 viable cells. The incubation time for the pathogen to cause illness is uncertain, but the literature suggests that it varies 3–10 days. The illness manifests itself as fever, a rash, and severe abdominal pain and it is often confused with acute appendicitis. Diarrhoea is uncommon but can also occur. Long-term complications can include reactive arthritis, and in immunocompromised patients with liver disease it can occasionally cause sepsis.

Infections are normally self-limiting, although in patients developing sepsis because of acute liver disease, the mortality rate can be high (>75%). *Y. pseudotuberculosis* infections occur most frequently in children 5–15 years of age (>75% of cases). Individuals recovering from *Y. pseudotuberculosis* infections can excrete the pathogen for a number of weeks after the illness.

Incidence and Outbreaks

There is very little published information on the incidence of food-borne *Yersinia pseudotuberculosis* infection, but the organism is mostly a health concern in countries with a temperate climate, such as Japan, the Northern EU and the former Soviet Union, and cases seem to occur more frequently during the winter months.

Yersinia enterocolitica is the main cause of yersiniosis, however in EU Member States in the year 2008, *Y. pseudotuberculosis* was the reported cause of 8% of all recorded cases. In Finland, where a number of outbreaks have occurred in recent years, 252 confirmed cases were reported in 2006 (mainly from two identified outbreaks), the highest number ever recorded. In 2007 the number of confirmed cases was 56, reflecting an average year without outbreaks.

Outbreaks associated with foods have occurred in Canada, Finland, Japan and the former Soviet Union. In 1998, an outbreak in Canada was associated with the consumption of contaminated homogenised milk. Again in 1998, an outbreak in Finland was linked to the consumption of Iceberg lettuce, and in the same country outbreaks of *Y. pseudotuberculosis* infections in 2003, 2004, 2006 and 2008 were all traced to grated carrots.

Sources

Yersinia pseudotuberculosis is found in the faeces of a wide number of wild and domestic animals in Eurasia and North America, and it is thought that wild mammals and birds are the main reservoir for infection-causing *Y. pseudotuberculosis*. The organism can cause disease in a number of animal species, but is also carried by apparently healthy animals. *Y. pseudotuberculosis* infection is a zoonosis, but not all strains of the organism are pathogenic.

Animals, such as rodents, deer, hares and birds (*e.g.* ducks and geese), can excrete the pathogen leading to the contamination of soil and water sources. However, the organism is isolated from environmental sources far less frequently than *Y. enterocolitica*. In an outbreak of *Y. pseudotuberculosis* infections associated with the consumption of raw carrots it is thought that the vegetables were contaminated *via* the faeces of rodents, and possibly other wild animals, which had access to the barn where the produce was stored in open containers during the winter.

Studies indicate that sources of *Y. pseudotuberculosis* are seasonal, with the organism only being recovered from rivers and small mammals during the winter and spring months.

Growth and Survival Characteristics

The physicochemical parameters affecting the growth and survival of *Y. pseudotuberculosis* are probably similar to those relating to *Yersinia enterocolitica*. The organism is psychrotrophic, and growth may not be prevented by storage of product at chill temperatures. It is thought that cold temperatures

during the winter in temperate climates provide an advantage to the organism when present in water and on fresh produce, and may explain why more cases of *Y. pseudotuberculosis* cases occur during these months.

The organism is able to persist in environmental sources for extended periods. It has been reported that *Y. pseudotuberculosis* isolates indistinguishable from an outbreak strain were still present in soil samples two months after the outbreak concerned was investigated.

Y. pseudotuberculosis is a facultative anaerobe: it is able to grow with or without oxygen.

Thermal Resistance

Yersinia pseudotuberculosis is not a heat-resistant microorganism and normal pasteurisation processes used in the food industry should inactivate the cells. In buffer at pH 7.0, *D*-values of around 23 min and 2.6 min, at 53.9 °C and 57.8 °C respectively have been recorded, with a *z*-value of 3.75 °C. These *D*-values are reduced significantly when the organism is heated in fruit (apple or orange) juices.

Control Options

Fresh produce can become contaminated with pathogens at any time during growing, harvesting, packing, shipping and processing. However, the refrigeration temperatures often used during transportation actually favours the survival and growth of *Y. pseudotuberculosis*. Therefore, strategies to reduce the risk of food-borne *Y. pseudotuberculosis* infections need to focus on ensuring that contamination is prevented in the first place, and need to be implemented at all stages of production, including at the farm. These should include preventing the access of wild animals to growing areas and water supplies by erecting fences, as well as preventing animals accessing fresh produce storage facilities. Treated water should be used to wash and process fresh produce.

In Finland, where the consumption of domestically grown raw carrots has caused a number of *Y. pseudotuberculosis* outbreaks in recent years, the Finnish Food Safety Authority has issued advice on the handling and processing of carrots. The advice includes the removal of poor quality carrots during storage and prior to processing, and the voluntary microbiological testing of carrots that have been stored over winter. In addition, kitchens in institutional settings have been advised to wash carrots prior to use, even those that are delivered pre-peeled and washed.

Processing

Equipment used to process produce can spread contamination and processing equipment should be cleaned regularly and thoroughly. It has been recommended that any inadequate cleaning regimes should be identified and corrected by routine inspections of production facilities.

Product Use

To reduce the risk of food-borne disease, including *Y. pseudotuberculosis* infections, consumers should be advised to thoroughly wash fresh produce prior to consumption.

Legislation

There are no specific requirements for levels of *Y. pseudotuberculosis* in foods under EU legislation or in the FDA Food Code.

Sources of Further Information

Published

Fredriksson-Ahomma, M., Lindström, M. and Korkeala, H. *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*, in ''Pathogens and Toxins in Foods, Challenges and Interventions'', ed. Juneja, V.K. and Sofos, J.N., ASM Press, Washington DC, 2010, 164–80.

Tauxe, R.V. Salad and pseudoappendicitis: *Yersinia pseudotuberculosis* as a foodborne pathogen. *The Journal of Infectious Diseases*, 2004, 189, 761–63.

1.1.25 OTHER ENTEROBACTERIACEAE

Hazard Identification

What are Enterobacteriaceae*?*

The *Enterobacteriaceae* are a family of gram-negative, facultatively anaerobic (able to grow in the presence, or absence of oxygen) non-spore-forming bacteria that includes a number of genera and species (*Salmonella*, *Escherichia coli*, *Cronobacter* species, *Shigella* species and *Yersinia* species) that are well known causes of food-borne disease and are covered in detail elsewhere in this book. However, there are a number of other, less well known species that have also been implicated in food-borne disease. Although more often associated with food spoilage, it is thought that some strains of *Citrobacter* species (notably *Citrobacter freundii*), *Klebsiella* species, *Providencia* species *Enterobacter* species and *Proteus* species, may occasionally cause what is often described as opportunistic gastroenteritis.

Occurrence in Foods

Enterobacteriaceae are found as contaminants in a wide variety of raw and processed foods. They are often involved in spoilage of dairy products, meat, poultry, fresh fruits and vegetables, usually as a consequence of temperature abuse. However, the prevalence of potentially pathogenic strains of *Citrobacter freundii*, *Klebsiella* species, *Providencia* species, *Enterobacter* species and *Proteus* species in foods is unknown.

High numbers of these bacteria in foods may be a cause for concern. For example, fresh sprout products (such as alfalfa) have been recalled in Canada because they have been found to be contaminated with *Klebsiella pneumoniae*.

Hazard Characterisation

Effects on Health

These organisms are considered to be opportunistic pathogens, and healthy adults are not considered to be at high risk of developing infections and illness. Young children, the elderly and the immunocompromised are most at risk in the developed world. People in developing countries with poor sanitation and inadequate nutrition are at higher risk.

The infectious dose of these potentially pathogenic strains is unknown. Typically, onset of illness occurs 12–24 hours after the ingestion of the contaminated foodstuff. Symptoms include flu-like symptoms, fever, nausea, stomach cramps, vomiting and watery diarrhoea. The illness can, on occasions, be chronic and last for some months. In infants and under-nourished children the disease caused by these organisms can result in death.

Incidence and Outbreaks

The incidence of food-borne infection by these bacteria is uncertain, but outbreaks of disease have been reported.

Outbreaks associated with *Citrobacter freundii* in the USA have been associated with the consumption of imported semi-soft cheeses (Brie or Camembert). In Germany an outbreak associated with *Citrobacter freundii* caused gastroenteritis amongst nursery children, followed by haemolytic uraemic syndrome with acute renal failure. It was linked to the consumption of green butter sandwiches (butter containing parsley leaves from an organic garden). Contaminated infant formula has also been implicated as the vehicle of infection in an outbreak of *Citrobacter freundii* infection.

Klebsiella pneumoniae infections have been linked to the consumption of a contaminated hamburgers and cooked turkey. In 2008 a hospital outbreak of extended-spectrum β-lactamase-(ESBL) producing *K. pneumoniae* was found to have been transmitted by contaminated food.

In 1996 a large outbreak of gastroenteritis caused by *Providencia alcalifaciens* at three schools in Japan was linked to a lunch cooked at a single catering facility. At least 610 individuals were affected.

A study of bacterial food-borne disease in China found that most *Proteus* species food poisoning events were caused by *Proteus mirabilis* (49.19%). Outbreaks of *Proteus* species infection have been linked to meat and seafood products, including sliced baked ham, meatballs and cockles.

Sources

These organisms are found in the environment, in the soil and in freshwater. They have been isolated from fresh vegetables and herbs, such as parsley and alfalfa sprouts. They occur in shellfish harvesting waters and have been found in raw shellfish. They have also been found in raw milk and dairy products.

These bacteria can be isolated from the stools of healthy individuals and are part of the normal intestinal flora of animals.

Growth and Survival Characteristics

Opportunistically pathogenic *Enterobacteriaceae* are not particularly heat resistant. Normal pasteurisation and cooking processes used by the food industry will inactivate these bacteria.

These organisms survive relatively well in the environment for non-spore-forming bacteria. Some species, including *Citrobacter freundii*, *Citrobacter koseri* and *Klebsiella pneumoniae* have been isolated from dried infant formula indicating that they can survive desiccation for some time.

Control Options

Effective control of these bacteria focuses on good hygiene practice and temperature control.

Processing

Fresh produce should be sourced from suppliers implementing good agricultural practices.

The rapid chilling of cooked foods after cooking is extremely important to prevent an increase in numbers of potentially pathogenic microorganisms.

The implementation of good hygienic practices by food handlers is extremely important to prevent the contamination of foods that will not be further heated prior to consumption.

Product Use

Consumers should be advised to wash fruit and vegetables well prior to consumption. They should also be reminded of the importance of good hygienic practices when preparing and storing foods to reduce the risks associated with food-borne disease.

Legislation

Although there are no specific requirements for each individual microorganism covered in this section, there may be requirements/standards/guidelines for levels of *Enterobacteriaceae* or coliforms (a group containing some, but not all, genera from the *Enterobacteriaceae*) in some foods and beverages, as an indication of hygienic status.

Sources of Further Information

Published

Stiles, M.E. Less recognized and suspected foodborne bacterial pathogens, in "The microbiological safety and quality of food." ed. Lund, B.M., Baird-Parker, T.C. and Gould, G.W., Aspen Publishers, Gaithersburg, 2000, vol. 2, pp. 1394–1419.

CHAPTER 1.2

Viruses

1.2.1 ADENOVIRUSES

Hazard Identification

What are Adenoviruses?

Adenoviruses are icosahedral-shaped, double-stranded DNA viruses belonging to the family *Adenoviridae*. This group of viruses has been found to infect many different animals, but human adenoviruses are classified in the genus *Mastadenovirus*.

More than 50 different human adenovirus serotypes have been identified, most of which cause respiratory infections. However, two serotypes, 40 and 41, are specifically associated with gastroenteritis, especially in infants and young children. It is thought likely that transmission of these viruses may occasionally be food borne, but this has not been confirmed.

Occurrence in Foods

Human adenovirus serotypes 40 and 41 are difficult to grow in culture and their presence in specific foods has rarely been reported. However, polymerase chain reaction (PCR)-based detection methods indicate that they can be present in shellfish, including oysters and mussels, growing in waters contaminated with human sewage. Human adenovirus DNA has been investigated as a potential indicator of faecal contamination in shellfish.

Adenoviruses could potentially be present in any ready-to-eat food where faecal contamination has occurred, either through contaminated water or an infected food handler.

The Food Safety Hazard Guidebook, 2nd Edition
Richard Lawley, Laurie Curtis and Judy Davis

Published by the Royal Society of Chemistry, www.rsc.org

Hazard Characterisation

Effects on Health

Human adenovirus serotypes 40 and 41 cause acute gastroenteritis, most commonly in children. The incubation period is typically about 10 days (range 2–15 days) and the principle symptom is watery diarrhoea, but fever, vomiting and abdominal pains may also be observed in some cases. Respiratory illness can sometimes accompany gastrointestinal infections. Symptoms are usually mild and self-limiting, lasting up to 10 days, but may be more serious in individuals who are immunocompromised.

Incidence and Outbreaks

Adenoviruses are very common causes of acute gastrointestinal illness in children, especially in developing countries. They are reported to be the second most prevalent cause of viral child diarrhoea worldwide after rotaviruses.

Confirmed food-borne infections and outbreaks have not been documented, but are considered credible.

Sources

The human gastrointestinal tract is the main reservoir for human adenovirus serotypes 40 and 41. Infected individuals may continue to shed live viruses for some time after symptoms have ceased and transmission of infection occurs mainly by the faecal–oral route, either through direct contact, or *via* contaminated water and potentially, food.

Growth and Survival Characteristics

Like all other viruses, adenoviruses are unable to multiply outside the host and cannot grow in foods or in water. However, in common with some other enteric viruses, they are unusually resistant to physical and chemical factors, including detergents, low pH and desiccation, and so may survive for long periods outside the human body.

Thermal Resistance

Mastadenoviruses have been reported to be inactivated by being held at 56 °C for 30 min in aqueous suspension, although some avian adenoviruses have been shown to be more heat resistant. There are no documented reports of human adenoviruses surviving typical thermal processes applied to shellfish and other foods.

Control Options

The control of adenoviruses should focus on good hygiene practice by food handlers, particularly when handling ready-to-eat foods that receive no further processing.

Processing

Food handlers should be properly trained in effective hand-washing techniques and encouraged to apply strict standards of personal hygiene. Those who act as carers for children with gastrointestinal illness should be especially vigilant. Individuals suffering from viral gastroenteritis should be excluded from work for at least 48–72 hours after symptoms have ceased.

Product Use

Consumers should be advised to eat only properly cooked shellfish, particularly oysters and mussels, harvested from approved waters. They should also apply caution when consuming ready-to-eat foods from uncontrolled sources in developing countries.

Legislation

There is no specific legislation in the EU or in the USA relating to levels of enteric viruses, such as adenoviruses, in foods.

Sources of Further Information

Published

Richards, G. Foodborne and waterborne enteric viruses, in "Foodborne Pathogens: Microbiology and Molecular Biology", ed. Fratamico, P.M., Bhunia, A.K. and Smith, J.L., Caister Academic Press, Wymondham, Norfolk, 2010, pp. 121–44.

1.2.2 ASTROVIRUSES

Hazard Identification

What are Astroviruses?

Astroviruses are spherical, positive-sense, single-stranded RNA viruses belonging to the family *Astroviridae*. These viruses are host-specific and a number of different astroviruses have been described (*e.g.* bovine astrovirus, feline astrovirus, human astrovirus), many of which cause gastroenteritis in the host. Human astroviruses are classified in the genus *Mamastrovirus* and at least eight human serotypes (human astrovirus 1 through to human astrovirus 8) have been recognised.

Astrovirus infections are mainly spread by person-to-person transmission *via* the faecal–oral route, however a very small percentage of infections are estimated to be food borne (<1%).

Occurrence in Foods

Evidence of astroviruses in naturally grown oysters has been reported in Japan, particularly in product sampled during the winter season.

A food handler infected with astrovirus could potentially contaminate almost any foodstuff. This could present a risk of infection if it is consumed without a further heating step.

Hazard Characterisation

Effects on Health

Astrovirus infections are mostly associated with young children (between 6 months and 2 years old), but they can also cause a mild infection in adults. The infective dose is thought to be <100 virus particles and symptoms occur 3–4 days after infection. Astrovirus infections are associated with watery diarrhoea, nausea, fever, abdominal pain and vomiting. The diarrhoea usually lasts for 2–3 days and is self-limiting, but it can sometimes last as long as 14 days. During infection the virus is excreted in high numbers in the faeces of the affected individual.

Incidence and Outbreaks

Although occurring all year round, outbreaks of astrovirus infections peak in temperate climates during the winter and spring, and in tropical climates they occur more frequently in the rainy season.

Outbreaks occur mostly in childcare situations, paediatric wards and amongst the institutionalised elderly. In many instances, astroviruses are second only to rotaviruses as a cause of childhood diarrhoea. Based on this fact, immunity to astrovirus infections is thought to be acquired during childhood, be maintained during adult life, and to diminish in old age.

Although astrovirus infections usually occur *via* person-to-person transmission through the faecal–oral route, food-borne infections and outbreaks associated with these viruses are described in the literature. Infections associated with shellfish and water have occasionally been reported. Probably the largest reported outbreak, involving thousands of children and adults from 14 different schools in Japan in June 1991, was caused by school lunches from a common supplier.

Sources

Humans are the reservoir for human astroviruses and infected individuals can excrete very high numbers of viruses. Infections are usually spread *via* the faecal–oral route. Faecally contaminated water sources (both drinking and recreational), shellfish from contaminated water and foods contaminated by infected food handlers can also be sources of human astroviruses.

Growth and Survival Characteristics

Viruses, including astroviruses, are unable to multiply outside of the host. Although they cannot grow in food or water, astroviruses can survive for some time in the environment, particularly when protected by organic matter at low temperatures. Astroviruses can survive in un-chlorinated water and when dried onto porous and non-porous materials, again particularly at low temperatures. Astroviruses are acid stable, and are resistant to freezing at –20 °C.

Thermal Inactivation

Astroviruses can survive heat treatments of 50 °C for 1 hour. A heat process at 60 °C for 15 min is reported to give a 6 $\log_{10}$ reduction in astrovirus titre.

Control Options

The control of astroviruses should focus on the implementation of strict personal hygiene by food handlers. Ready-to-eat foods that are handled, but will receive no further cooking pose the greatest risk.

Processing

Food handlers should be trained in effective hand-washing techniques and should wash hands after visiting the toilet as well as before handling foods. Those suffering from viral gastroenteritis should be excluded from work for at least 48–72 hours after symptoms have ceased.

Product Use

Consumers should be educated on the importance of adhering to good personal hygiene during food preparation and should be advised to consume only

adequately cooked shellfish, especially oysters, harvested from approved waters.

Legislation

There is no specific legislation in the EU or in the USA regarding levels of enteric viruses, such as astroviruses, in foods.

Sources of Further Information

Published

Greening, G.E. Human and animal viruses in food (including taxonomy of enteric viruses), in ''Viruses in Foods'', ed. Goyal, S. Springer, New York, 2006, pp. 2–42.

1.2.3 HEPATITIS A VIRUS

Hazard Identification

What is the Hepatitis A Virus?

The hepatitis A virus (HAV) is an enteric virus, which causes a liver disease in humans now known as hepatitis A (previously known by other names including infectious jaundice, viral hepatitis and infectious hepatitis). There are a number of different hepatitis viruses but only the HAV, and possibly the hepatitis E virus, can cause food-borne disease. HAV is a single-stranded RNA virus belonging to the *Picornaviridae* family and the genus *Hepatovirus*.

Although HAV is most commonly spread by direct person-to-person contact *via* the faecal–oral route, there are many documented food-borne outbreaks in the literature. Food-borne outbreaks can often be traced back to an infected food handler or foods that have come into contact with faecally contaminated water.

Occurrence in Foods

The HAV can only be present in foodstuffs as the result of faecal contamination. Although this means that any food that is handled under poor hygienic practices could potentially be contaminated with the pathogen, it is bivalve molluscan shellfish, such as oysters, cockles and mussels, which are most often considered to be a source of food-borne viruses. These shellfish concentrate any virus particles present in their tissues during filter feeding in faecally contaminated water. Depuration techniques used to decontaminate shellfish are more successful in reducing bacterial loading than in mitigating viral contamination.

In recent years, fresh produce, such as salads, fresh fruits and vegetables, has increasingly been implicated in food-borne outbreaks of hepatitis A. These products are likely to be consumed raw or lightly cooked, and can become contaminated with faecal matter at almost any point during growing, harvesting, transport and packing.

Hazard Characterisation

Effects on Health

The infective dose for the HAV is unknown. However it is thought that as few as 10–100 virus particles could cause disease. The incubation time for symptoms to appear is on average about 4 weeks, but it can vary from 2–6 weeks. This long incubation time before the illness becomes evident can mean that it may be difficult to trace the exact source of the infection, and it can also mean that large numbers of individuals are affected before it is evident that there is viral contamination in the food chain.

Many cases of HAV infection are asymptomatic, particularly in children. When disease is evident, hepatitis A infection is usually a mild illness. Initial symptoms include headache, fatigue, fever, poor appetite, abdominal discomfort, nausea and vomiting. After a week or so, viraemia (where virus can be detected in the blood stream) and liver disease in the form of jaundice, or liver enzyme elevation, occurs. Hepatitis A is usually a self-limiting disease lasting up to two months, but in a small group of affected people, the HAV can cause long or recurring illness lasting up to six months. Infection can be fatal, particularly in people over 50 years old. In the USA, this age group has a mortality rate reported as 1.8%.

During infection, individuals can excrete high numbers of virus particles ($>10^6$ particles per g of faeces). The shedding of particles can start in the last two weeks of the incubation period and in some individuals can continue for up to five months after infection.

Incidence and Outbreaks

In many developing countries the disease is endemic and exposure during early childhood because of poor hygiene is common. Early childhood infections are usually asymptomatic and confer lifelong immunity.

Outbreaks of hepatitis A are more likely to occur in developed nations, or amongst travellers from developed countries to the developing world, because exposure to the virus during early childhood in individuals from developed regions is low. Countries where the adult population has no immunity are at risk of large hepatitis A outbreaks when food or water supplies are contaminated with the virus.

In 2007 there were an estimated 25 000 new hepatitis A infections in the USA, although only a small percentage ($<5\%$) of these are likely to have been food or water borne. In the EU in 2008 there were just over 17 000 reported cases of hepatitis A reported by 29 countries. The highest rate was in Latvia with 123 cases per 100 000 of population. In England and Wales the incidence of hepatitis A has decreased from just over 1800 in 1997 to around 400 in recent years.

Contaminated water and bivalve shellfish such as oysters, cockles and mussels, are often associated with hepatitis A infections. The largest recorded food-borne outbreak of hepatitis A , involving 290 000 cases, was in Shanghai, China in 1988 and was caused by clams harvested from waters polluted by raw sewage.

Fresh fruits, such as strawberries, blueberries and raspberries harvested by infected pickers, and associated products such as orange juice, have caused outbreaks in the UK, New Zealand and the USA, respectively. Imported lettuce, and more recently in 2003 imported raw/undercooked green onions (601 cases with 3 deaths), have also caused large outbreaks in the USA.

Other foods linked to outbreaks include bakery products, sandwiches, iced beverages, milk and milk products, semi-dried tomatoes, raw beef, beer and soft drinks.

Sources

The human intestine is the main reservoir for the HAV and asymptomatic infected individuals, especially children, are an important source of the virus.

Transmission can occur by the faecal–oral route by direct person-to-person contact, or from the ingestion of faecally contaminated food or water. It has been reported that transmission of the virus can occur as the result of using contaminated drinking glasses. Infected food handlers with poor hygiene are a potential source of the virus in food. The virus could potentially be present in any water source or soil that is faecally contaminated.

Growth and Survival Characteristics

Viruses, including the HAV, are unable to multiply outside of the host. Although the HAV cannot grow in food or water, it can survive in many environments for some time. When excreted in human faeces the HAV can survive in the environment in water or soil for at least 12 weeks at 25 °C. The HAV has a high resistance to many chemicals and solvents and it is more resistant to heat and drying than other enteroviruses. It can survive refrigeration and freezing for up to two years and it is resistant to acid (pH 1 for 2 hours at room temperature).

The HAV is resistant to low levels of free chlorine (0.5–1 mg free chlorine per litre for 30 min). It is also resistant to perchloroacetic acid (300 mg l^{-1}) and chloramines (1 g l^{-1}) for 15 min at 20 °C. The virus can be inactivated on surfaces with a 1 : 100 solution of sodium hypochlorite, or household bleach in tap water.

Thermal Inactivation

The HAV is relatively heat resistant, although thorough cooking at higher temperatures will usually inactivate the virus. It is resistant at 70 °C for up to 10 min but is inactivated at temperatures of 85 °C for 1 min. In the UK it has been recommended that cockles are heated to an internal temperature of 85–90 °C for 1.5 min to inactivate HAV and data from the World Health Organization (WHO) suggests that shellfish from HAV-contaminated areas should be heated to 90 °C for 4 min or steamed for 90 s.

Control Options

Strategies to reduce the risk of food-borne outbreaks of hepatitis A should focus on preventing foods from becoming contaminated. In developing countries young children should be kept away from areas where fresh produce is grown and harvested, and clean water should be used for the irrigation, washing and processing of foods. Shellfish harvesting areas should be monitored for sewage contamination.

Processing

Food handlers should implement frequent hand washing and the wearing of gloves particularly at points in the food chain where foodstuffs that will receive no further cooking are handled. In addition those suffering from symptoms of hepatitis A should be removed from the food production area until they have a medical release. In some parts of the USA food handlers are immunised against hepatitis A, but the effectiveness of such a policy is uncertain.

A 2011 European Food Safety Authority (EFSA) report, *Foodborne Viruses: occurrence and control*, recommends using HAV as a model to validate the virucidal effectiveness of post-harvest treatments and to ensure that they can be applied consistently before they are implemented.

Product Use

If food could be contaminated with the HAV, consumers should be advised only to eat thoroughly cooked foods from known sources and not to eat uncooked fruits or vegetables that they have not peeled or prepared themselves.

Legislation

There is no specific legislation in the EU or in the USA regarding levels of enteric viruses, such as HAV, in foods. However, the EFSA has recommended the development of microbiological criteria for viruses in bivalve molluscs unless they are labelled, ''to be cooked before consumption''.

Sources of Further Information

Published

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Koopmans, M. and Duizer, E. Foodborne viruses: an emerging problem. *International Journal of Food Microbiology*, 2004, 90, 23–41.

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On the Web

Opinion of the Scientific Panel on Biological Hazards on the present knowledge on the occurrence and control of foodborne viruses – European Food Safety Authority (July 2011). http://www.efsa.europa.eu/en/efsajournal/doc/2190.pdf

Hepatitis A – World Health Organization (2000). http://www.who.int/csr/disease/hepatitis/HepatitisA_whocdscsredc2000_7.pdf

1.2.4 HEPATITIS E VIRUS

Hazard Identification

What is the Hepatitis E Virus?

The hepatitis E virus (HEV) is an enteric virus, which causes a liver disease in humans now known as hepatitis E (other names for the disease include, enterically-transmitted non-A non-B hepatitis and faecal–oral non-A non-B hepatitis). The HEV is distinctly different from the hepatitis A virus and is a single-stranded RNA virus, which has recently been classified in the family *Hepeviridae* and the genus *Hepevirus*.

Studies have found that there are distinct similarities between HEV strains affecting humans and HEV strains found in pigs in developed countries. This has led to the conclusion that HEV is a zoonosis and, potentially, a food-borne pathogen.

Occurrence in Foods

The virus is most often associated with pigs, and surveys to determine the frequency of the HEV in pig populations and in pork livers have been conducted in a number of countries. Pigs carrying the HEV do not show any signs of disease and the virus is now known to be present in most pig populations throughout the world. It is reported to have been present in pigs in the UK since at least 1986 and it is estimated that it is present in 75% of pigs in the country.

Studies in Japan and the Netherlands to determine the incidence of HEV in raw retail pig livers found detectable levels of the virus in three of 197 (1.9%), and four of 62 (6.5%) of samples, respectively. However, in the UK a recent study of samples of retail pig livers from 80 outlets in Cornwall found none positive for the virus.

Hazard Characterisation

Effects on Health

Although all individuals are susceptible to contracting hepatitis E, the disease is most frequently seen in the 15–40 year-old age group. The infective dose for the HEV is unknown and the incubation time for the disease can vary from 2 to 9 weeks. Many HEV infections are asymptomatic (cause no sign of disease), and where hepatitis E does occur it is usually a mild illness lasting 3–4 weeks.

The symptoms for this mild form of the disease include general fatigue, jaundice, production of darker urine and pale stools, abdominal pain, vomiting and nausea. However, the virus can occasionally cause a severe disease with complete liver failure and even death, especially amongst individuals who are pregnant or immunocompromised, suffering from chronic liver disease, or from older age groups. In pregnant women the disease may also cause a miscarriage.

In the general population the mortality rate associated with hepatitis E is 0.5–2.0%, but amongst groups susceptible to the more severe form of the disease the fatality rate can be as high as 30%.

Incidence and Outbreaks

In developing countries with poor sanitation hepatitis E is common. In these regions most cases of the infection are sporadic, although large outbreaks associated with contaminated water are not infrequent.

In industrialised countries, cases of hepatitis E have traditionally been associated with foreign travel to the developing world and large outbreaks of the disease have not occurred. However, there is an increasing body of evidence to suggest that a significant number of hepatitis E infections in developed countries are acquired 'at home' (in the UK up to 50% of cases may be domestically acquired). In England and Wales there are around 200 cases of hepatitis E cases reported each year, of which more than 20% are in older individuals and thought to be acquired without foreign travel.

There have been reports in the literature of cases of food-borne transmission of hepatitis E. These have been associated with the consumption of unpasteurised milk, and raw or undercooked meat (pork liver, deer and wild boar). In France, since 2007 a number of hepatitis E cases have been associated with the consumption of raw figatellu (pork liver sausage) and manufacturers of these products are now required to recommend that they are thoroughly cooked prior to consumption.

There is also some evidence suggesting that the infection may also be acquired from the consumption of raw, or poorly cooked, shellfish. In the UK in 2008, a number of passengers returning to the UK from a cruise ship had contracted hepatitis E. An investigation concluded that it was most probably a common-source food-borne outbreak and there was a strong association with consuming shellfish whilst on board.

Sources

In developing regions the main source of the virus is drinking water contaminated with human faecal material.

In developed countries the main source of the virus is from direct or indirect contact with animals. In these regions the main reservoir for the HEV is pigs and pig faeces. Pork and associated products may also be contaminated. Transmission of the virus between pigs is thought to be *via* the faecal–oral route.

Other animals have also been reported to have antibodies to the HEV, and these include deer, wild boar, cattle, goats, chickens and sheep, domestic animals such as dogs and cats, and rodents such as rats and mice.

Growth and Survival Characteristics

Viruses, including the HEV, are unable to multiply outside of the host. Although the HEV cannot grow in food or water, it can survive and still remain

infective. There is, however, very limited data on factors affecting the survival of the HEV in the environment and in food.

The virus is known to survive frozen storage for extended periods and is also able to survive in the gastrointestinal tract indicating that it is relatively resistant to acid conditions. It does appear, however, to be very sensitive to high salt concentrations and is inactivated in chlorinated water.

Thermal Inactivation

Only a few studies to determine the thermal inactivation of the HEV have been conducted. However, in 2011 the EFSA concluded that heating at 70 °C for 10 min or at 95 °C for 1 min seems to be sufficient for the inactivation of HEV. Elsewhere it has been concluded that, although the HEV is less heat resistant than the hepatitis A virus, some HEV is likely to survive the internal temperatures reached in rare-cooked meat.

Control Options

Processing

The risk of acquiring hepatitis E through the ingestion of contaminated food is considered low. However, the risk can be reduced further by ensuring that all pork and pork products (including liver) are cooked thoroughly during processing.

Product Use

The risk of acquiring travel-associated hepatitis E can be reduced by avoiding drinking water or drinks containing ice made from water of an unknown purity in areas where the disease is endemic. In addition, travellers should be advised not to eat uncooked shellfish, or uncooked fruits or vegetables that they have not peeled or prepared themselves.

In industrialised countries where sanitary conditions are good it has been recommended that consumers should be advised that pork products should not be consumed rare. In the UK, the Advisory Committee on the Microbiological Safety of Food (ACMSF) concluded that the risk of acquiring hepatitis E through the food chain in the UK is likely to be low. However the expert committee concluded that searing the outside of meat joints would be insufficient to destroy viruses, such as hepatitis E, that may be present in meat muscle, and recommended that pork and pig products (including liver) should be cooked all the way through prior to consumption. The EFSA has recommended that high-risk groups (people with underlying liver disease, immunocompromised persons and pregnant women) should be discouraged from eating meat and liver derived from pigs and wild boars without proper cooking for the prevention of hepatitis E.

Legislation

There is no specific legislation in the EU or in the USA regarding levels of enteric viruses such as the HEV in foods.

Sources of Further Information

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1.2.5 HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUSES

Hazard Identification

What are Highly Pathogenic Avian Influenza Viruses?

Highly pathogenic avian influenza (HPAI) viruses belong to the family *Orthomyxoviridae*, and within this family these viruses are in the group known as influenza type-A viruses. Influenza type-A viruses are classified into sub-types, and are named according to two main surface proteins, haemagglutinin (HA) and neuraminidase (NA). For example the subtype H5N1 has an HA 5 protein and a NA 1 protein. To date 16 HA subtypes, and nine NA subtypes have been described and many different combinations of HA and NA proteins are known to exist (*e.g.* H5N1, H1N1, H7N3 and H7N7).

Although influenza A viruses can infect many animals including birds, humans, pigs, dogs, cats and horses, wild birds are the natural hosts for these viruses. Avian influenza A virus strains are grouped, based on genetic and pathogenic criteria, as either low-pathogenic avian influenza (LPAI), causing mild disease in birds, or HPAI, having enhanced virulence and causing the rapid onset of severe disease with high mortality rates in birds.

Some avian influenza viruses can be transmitted to humans and cause illness. LPAI viruses cause mild symptoms in humans, whereas HPAI can cause severe disease with high mortality rates. The type of HPAI virus that causes the most severe form of avian influenza (AI) in humans is the H5N1 virus. In recent years this virus has crossed the species barrier between birds and humans on a number of occasions, and an outbreak that began in South-East Asia during 2003 became widespread, even reaching a number of EU countries. The presence of HPAI H5N1 virus in birds is of concern for a number of reasons: it can cause severe disease in domestic poultry flocks resulting in up to 100% mortality; it can be spread to humans from infected birds; and it could potentially develop the ability to spread easily from human to human resulting in a severe influenza pandemic.

There have been concerns that humans may become infected with the H5N1 virus by the handling and consumption of contaminated poultry and poultry products, and this has led to research into the virus and its potential as a food-borne pathogen. However, it is important to note that, although there is a theoretical potential for food-borne transmission of the virus, this has not yet been conclusively demonstrated. Most public health authorities, including the WHO, do not currently consider HPAI H5N1 to be a food safety hazard. However, in 2009 the WHO stated that 25% of human cases had an unknown source of exposure.

Occurrence in Foods

Poultry, such as chickens and turkeys are particularly susceptible to HPAI viruses such as H5N1. All parts of the infected bird, including blood, meat and

bones, are potentially contaminated with virus. The virus is also present in the saliva, nasal excretions and faeces of infected birds resulting in the contamination of feathers. Evidence suggests that the risk of exposure to the H5N1 virus is high during the slaughtering and handling of affected birds, or in meat prior to cooking. There have also been reports of two cases of H5N1 infections in humans possibly linked to the consumption of uncooked poultry products (raw blood-based dishes), and cats are thought to have contracted the H5N1 virus through eating uncooked infected chicken carcasses, or possibly infected wild birds.

The HPAI H5N1 virus is also present on the inside and on the surface of eggs laid by infected birds. To date, there is no evidence to suggest that humans have contracted the H5N1 virus through the consumption of eggs or egg products.

Hazard Characterisation

Effects on Health

There are many strains of avian influenza A viruses, however only four subtypes (H5N1, N7N3, H7N7 and H9N2) are known to cause illness in humans. Usually these viruses cause mild influenza-like symptoms such as fever, muscle aches, cough and a sore throat or sometimes conjunctivitis.

However, in many individuals infected with the H5N1 virus the course of the disease is different. Most reported cases of H5N1 infections have occurred in previously healthy children and young adults and the infectious dose is unknown. It is thought that the incubation period for the H5N1 virus in humans is 2–8 days but may be as long as 17 days (the WHO advises that an incubation time of seven days be used to monitor patient contacts for the disease).

Initial effects may include influenza-like symptoms, a temperature of greater than 38 °C, or acute encephalitis. Sometimes watery diarrhoea without blood, vomiting, chest pain, abdominal pain, and bleeding from the nose and gums have been described. Typically initial symptoms are followed around five days later by lower respiratory tract illness such as breathing difficulties, respiratory distress, a hoarse voice, a crackling sound when inhaling and sometimes the production of sputum, which may contain blood. Deterioration is rapid with the development of acute respiratory distress and possibly multi-organ failure. The disease has an associated mortality rate of 55%.

The majority of reported cases occur as the result of close contact with H5N1-infected poultry or H5N1-contaminated surfaces. There have been a few reports of person-to-person transmission occurring between family members suggesting that very close contact for prolonged periods is needed to contract H5N1 AI from this source.

Incidence and Outbreaks

The outbreak of H5N1 in poultry that began in Asia during 2003 is the largest and most severe on record. It is known to have spread to birds in more than

50 countries in Africa, the EU, Asia and the Near East, and has resulted in at least 561 reported human cases with 328 deaths across 15 countries. Two cases may possibly have been caused by the consumption of infected raw duck blood products, however contact with infected live birds or carcasses cannot be ruled out, so the infected product may not have been the only source of infection.

Sources

Wild water birds are thought to be the main reservoir for the H5N1 virus, and some species, particularly ducks, are thought to act as asymptomatic carriers. Pigeons may also play a role in the spread of the virus. Mammals such as cats, tigers and ferrets have also been infected with H5N1 virus and have died from the disease. Other mammals, such as dogs, have also tested serologically positive for the virus in outbreak areas, indicating that they too can become infected.

Contaminated bird faeces can lead to the contamination of the environment, where the virus can survive for some time, particularly at low temperatures. The virus can also cause infection by air-borne transmission if birds are close together. However, there is no evidence to confirm that water-borne transmission of the virus occurs between birds, and it is thought that the risk of water-borne transmission of the virus to humans is small.

There is evidence to suggest that the HPAI H5N1 virus is excreted in the faeces of infected humans. However, data is limited on the extent of H5N1 virus excretion in urine and faeces in all infected mammals, including humans. It is not yet known whether this is another possible source of the virus.

Growth and Survival Characteristics

Viruses, including influenza viruses, are unable to multiply outside the host. However, the H5N1 virus is able to survive, sometimes for extended periods, in the environment.

The survival of AI viruses in water is dependant on the temperature, pH and salinity. Specific data on the survival of the H5N1 virus in water is limited, but in general for AI viruses, survival in natural water (fresh, brackish and seawater) decreases with increasing salinity and increasing pH values above neutral. Different strains of avian influenza have been shown to survive in water at 17 °C, and at 28 °C, for up to 207 days and 102 days, respectively.

The WHO suggests that the avian influenza virus cannot generally be detected in birds four weeks after infection. However, the survival of the highly pathogenic H5N1 virus in bird faeces is dependant on initial concentration, temperature and pH. Studies using H5N1 viruses circulating during 2004 found that in faeces held at 4 °C and 37 °C, live viruses survived for 35 and 6 days respectively. On surfaces such as that found in poultry house environments, avian influenza viruses are reported to survive for a few weeks.

If the H5N1 virus is present in poultry meat, it can survive in this environment under chilling and freezing conditions with little affect on levels or the viability of the virus. In general, low temperatures actually prolong the survival of the virus in poultry tissue. In 2007, an outbreak of H5N1 on poultry holdings

in Germany led to testing for the virus on retained frozen duck carcass samples. Detection of the virus on carcasses from one farm indicated that silent infection had occurred for some time before the outbreak was detected.

Avian influenza viruses are reported to be more sensitive to low pH than very high pH values. The H7N2 virus was completely inactivated when held at a pH 2 for 5 min whereas exposure to pH 10 or pH 12 for 15 min had no effect on infectivity. HPAI are also sensitive to desiccation. All avian influenza viruses are reported to be relatively susceptible to most disinfectants, including chlorine.

Thermal Inactivation

HPAI viruses are inactivated when held at 121 °C for 15 min, 60 °C for 30 min, or at 56 °C for 3 hours. In foods, the H5N1 virus is inactivated when all parts of the item reach 70 °C or above. Therefore, properly cooked poultry products are safe to eat. The WHO advises that the virus is inactivated during conventional cooking practices used to cook poultry products where temperatures reach 70 °C or above at the centre of the product.

It has been reported that most standard pasteurisation temperatures for eggs used by industry will inactivate HPAI viruses (*e.g.* whole egg, 60 °C, 210 s; liquid egg white, 55.6 °C, 372 s; 10% salted yolk, 63.3 °C, 210 s). However, the industry standard of treating dried egg white of 54.4 °C for 7–10 days would not be sufficient to inactivate HPAI viruses.

Following a risk assessment and advice from the UK Advisory Committee on Microbiological Safety of Food (ACMSF) in 2007, the Food Standards Agency has suggested that even if the HPAI virus was present after cooking, factors in humans such as saliva, gastric acid, and the lack of appropriate receptors in the gut needed for the virus to enter the body would prevent or limit infection following ingestion.

Control Options

Control of HPAI viruses currently focuses on containing outbreaks in poultry by culling infected birds, implementing strict biosecurity measures and limiting movement of poultry within designated areas. However, there are also sensible preventative measures that may be relevant to the food industry.

Processing

Although there is no evidence to suggest that there is a risk of acquiring infection of the HPAI H5N1 virus through the consumption of properly cooked poultry and egg products, there are risks associated with the slaughtering, de-feathering and eviscerating of infected birds, or the handling of raw or partially cooked contaminated eggs. In outbreak areas, diseased birds or those found dead should never be used for human consumption. In addition, good hygiene practices are essential during slaughter and the post-slaughter handling of poultry carcasses to prevent any possible exposure *via* raw poultry meat, or cross contamination from poultry to other foods, food preparation

surfaces, or equipment. Good hygiene is also essential when handling prepared poultry meat and eggs from outbreak areas and thorough cooking of all egg and poultry meat products should be ensured.

It should be noted that the likelihood of the HPAI virus being present in poultry in non-outbreak areas is negligible, and the possibility of infected meat being sold and handled by a consumer in most regions is extremely low.

Product Use

Poultry meat should be thoroughly cooked (heated to 70 °C in all parts) to ensure the inactivation of food-borne pathogens in general. Similar comments also apply to eggs and egg products.

Legislation

There is no specific legislation in the EU or in the USA regarding avian influenza viruses in foods. However, it is highly likely that there will be import and animal movement restrictions applying to areas affected by avian influenza outbreaks.

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1.2.6 NOROVIRUSES

Hazard Identification

What are Noroviruses?

Noroviruses is the name given to a group of related non-enveloped, single-stranded RNA viruses that have recently been classified in the family *Caliciviridae*, genus *Norovirus*. These highly infectious enteric viruses are a major cause of acute gastroenteritis in humans (the infection is often called viral gastroenteritis). Although many cases are caused by person-to-person spread, the ingestion of contaminated food or water also plays a significant part in their transmission.

Noroviruses were first described following an outbreak of gastroenteritis in a school in Norwalk, Ohio in 1968. For many years they were known as the Norwalk group, as Norwalk-like viruses (NLV), or as ''small round structured viruses'' (SRSVs), because of their morphological characteristics. However, the name Norovirus (NoV) has recently been recognised as the official genus for this group of human caliciviruses. NoV strains are named after the location from which they were first associated, *e.g.* Norwalk virus, Southampton virus, Snow Mountain virus and Mexico virus.

Occurrence in Foods

Noroviruses are non-culturable in the diagnostic laboratory and there is no known animal model. Until relatively recently they could only be detected when present in high numbers using electron microscopy. Recent technological advances have enabled noroviruses to be detected and characterised by molecular methods, but the detection of these viruses in foods is extremely difficult and has only been successful in shellfish.

Food vehicles for noroviruses are thought to include sewage-contaminated bivalve shellfish, foodstuffs that are contaminated by an infected handler, fruits and vegetables contaminated during irrigation or washing, and water (including drinking water and ice).

Infected food handlers can contaminate any foodstuff, and outbreaks of NoV infections can be associated with any food that is handled and will be eaten without a further cooking step. Contamination can occur during the preparation of foods as well as during the harvesting of fresh produce such as soft fruits.

Hazard Characterisation

Effects on Health

Noroviruses can cause illness in any age group, although the elderly and the immunocompromised are particularly susceptible. Recent evidence suggests that susceptibility to NoV infection could be genetically determined, and people

with blood group O seem more likely to develop a severe infection. Illness can occur at any time of year but in temperate climates is more common during the winter months. Noroviruses are very contagious, however the illness is usually mild and self-limiting.

The infective dose is low, and as few as 10 virus particles may be sufficient to infect an individual. Signs of infection first appear from between 10–50 hours, typically 24–28 hours, after ingestion of the virus. The onset of illness is abrupt and typical symptoms are vomiting (often projectile), diarrhoea, abdominal pains, nausea, headache, stomach cramps and occasionally low-grade fever. The illness is typically relatively short, lasting from 12–60 hours, although there are reports that symptoms in some individuals last for more than two weeks. Recovery is usually complete with no long-lasting effects.

During the illness high numbers of the virus are generated in the vomit of affected individuals as well as being shed in their faeces. Virus shedding appears to occur before symptoms start and continue for up to two weeks after symptoms have ceased. Outbreaks associated with an infected food handler have been associated with foods prepared before the onset of symptoms.

Incidence and Outbreaks

Norovirus outbreaks are very common, but there is little published information on the incidence of food-borne infection.

In the USA it is estimated that there are more than 21 million cases of acute gastroenteritis each year due to NoV infection, and that these viruses cause more than 50% of all food-borne disease outbreaks. There were 382 confirmed outbreaks (not necessarily food borne) recorded in the USA during the period October to December 2006 and rising incidence is thought to be linked to the appearance of new strains of the virus.

In the UK, the incidence of norovirus infections has also been rising steadily since the 1980 s. In 2010 more than 11 500 confirmed cases were recorded, although there is no indication of the proportion that were food borne. However it has been estimated that noroviruses cause 200 000 cases of food-borne illness annually in England and Wales, with many going unreported.

In New Zealand there are an estimated 403 000 norovirus infections annually. These infections have an estimated annual cost to the health care system of $7.6 million, with food-borne infections costing $3.0 million. It is thought that shellfish causes 40% of food-borne infections in New Zealand, with the remaining 60% being transmitted through foods contaminated by infected food handlers.

Contaminated water is the most common source of NoV outbreaks and has caused very large outbreaks of viral gastroenteritis. Outbreaks have been linked to water from wells, municipal water supplies, swimming pools, lakes and water stored on cruise ships. In the USA, commercially prepared ice from a production facility that was contaminated during flooding was associated with a widespread outbreak.

Food-borne outbreaks of NoV infections are frequently caused by infected food handlers. Foods associated with this source of contamination are cold, ready-to-eat foods such as prepared salads, fresh cut fruits, sandwiches and bakery products. Large outbreaks have been caused when liquid foods such as icings or salad dressings have become infected during preparation and then mixed leading to widespread distribution of the virus.

Shellfish, in particular oysters, from sewage-contaminated water, when eaten raw, or lightly cooked, have also caused large outbreaks of NoV illness.

Contaminated fresh produce, in particular salads and raspberries, has been associated with large food-borne outbreaks of NoV infections. These foods may be contaminated either from irrigation water, during washing or spraying, or during harvesting by infected handlers. In recent years frozen raspberries have caused extensive food-borne outbreaks in Canada and in the EU. The viruses are able to survive the freezing process and frozen fruits are often exported to other countries resulting in the wide distribution of the virus.

Sources

Humans are the only known reservoir for noroviruses. It has been hypothesised that there may also be an animal reservoir, but, although related caliciviruses have been found in many animal species, there have not been any documented cases of cross-species transmission.

Faeces or vomit from infected individuals can lead to the environmental contamination of soil, water and surfaces. Airborne droplets produced during vomiting are a particularly effective method of distribution for viruses.

Noroviruses can accumulate and concentrate in the guts of bivalve molluscs, such as oysters and mussels, growing in sewage-contaminated waters. Depuration processes designed to reduce the bacterial contamination of these shellfish are ineffective for removing viruses. Faecal contamination of water supplies can be a potential source of noroviruses. Live viruses have even been detected in commercially available bottled mineral water, although cases of illness have not yet been traced to this possible source of infection.

Growth and Survival Characteristics

Viruses, including noroviruses, are unable to multiply outside of the host. Although noroviruses cannot grow in food or water, they can survive in many environments for significant periods. The virus can remain infective when held at ambient, chilled and freezing temperatures. In chilled and frozen environments survival can be measured in months or even years. Noroviruses are resistant to acid and can survive gastric acid at pH 3–4. They have also been shown to still be infective when exposed to a pH of 2.7 for 3 hours at ambient temperature. The virus can survive in water environments and in shellfish for extended periods (possibly months). It is resistant to drying, and is reported to persist on environmental surfaces, such as carpets, for up to 12 days.

Noroviruses can survive exposure to up to 10 ppm free chlorine, and can therefore survive the usual chlorination processes used to treat public water supplies.

Thermal Inactivation

Noroviruses have been shown to remain infective when held at 60 °C for 30 min. The virus is able to survive some pasteurisation processes and has also caused illness after it was steamed in shellfish. It is inactivated by boiling.

Control Options

To reduce the risk of food-borne transmission of noroviruses, controls should focus on ensuring the use of potable water for food processing, strict hygiene control, and using shellfish from approved waters.

Processing

Food handlers or fruit pickers suffering from viral gastroenteritis should not return to work for at least 48–72 hours after symptoms have ceased. Effective training in adequate personal hygiene practices is essential. Thorough cleaning with an effective sanitiser should follow any episode of vomiting in a food processing environment.

Shellfish should be gathered from approved harvesting waters and the EFSA has recommended the introduction of control measures to avoid faecal contamination in mollusc production areas.

Product Use

Consumers should be advised not to eat raw shellfish and to ensure these products are thoroughly cooked prior to consumption. In addition consumers should be advised to thoroughly wash all fruits and vegetables that will be eaten raw or lightly cooked in potable water.

Legislation

There is no specific legislation in the EU or in the USA regarding levels of enteric viruses, including noroviruses, in foods. However, a 2011 EFSA report, *Foodborne Viruses: occurrence and control*, recommended the development of microbiological criteria for viruses in bivalve molluscs, unless they are labelled, ''to be cooked before consumption''. The report also suggested legislation to introduce virus microbiological criteria for the classification of high-risk bivalve mollusc production areas, when the molluscs will be consumed raw.

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1.2.7 PARVOVIRUSES

Hazard Identification

What are Parvoviruses?

The parvoviruses are very small, single-stranded DNA viruses belonging to the family *Parvoviridae*. These viruses have a smooth surface with no discernable features and were previously included in the group of viruses known as ''small round viruses'' (SRVs) or featureless viruses.

Data on these viruses as a cause of human gastroenteritis is limited, but it is known that parvoviruses may cause gastroenteritis in other animal species (*e.g.* canine parvovirus).

Occurrence in Foods

Data is very limited, although parvovirus or parvovirus-like particles have been linked to a number of outbreaks associated with the consumption of shellfish. Parvovirus-like particles similar to those found in patients have been detected in shellfish.

Hazard Characterisation

Effects on Health

Gastroenteritis caused by parvovirus has been described as ''winter vomiting virus'', suggesting similarities with norovirus infections. The virus causes mild, flu-like symptoms 4 to 14 days after infection; however complications such as joint pain and anaemia can occur. Those most at risk of developing complications from parvovirus infections are pregnant women and immunocompromised individuals.

During some outbreaks it has been found that large numbers of virus particles are excreted in the faeces of many patients. It is also known that the shedding of virus particles can continue for a number of weeks after symptoms subside. Low numbers of parvovirus-like particles can also be found in the faeces of healthy individuals.

Incidence and Outbreaks

A parvovirus serotype, known as the ''cockle agent parvovirus'' has been linked to a large outbreak (>800 cases) of gastroenteritis in the UK associated with the consumption of cockles.

Other parvovirus-like particles, the Parramatta agent and the Wollan/Ditchling group, have been linked to outbreaks of gastroenteritis in schools.

Sources

Parvoviruses causing gastroenteritis in humans are likely to be found in environments that are faecally contaminated. The cockle agent parvovirus was linked to cockles harvested during the winter, much closer to sewage outlets than was usual.

Growth and Survival Characteristics

Due to the infrequency with which parvoviruses are associated with gastrointestinal disease in humans there is very little data of the survival characteristics of these agents.

Control Options

To reduce the risk from viral gastroenteritis associated with the consumption of shellfish it is important to ensure that shellfish are harvested from approved waters and that these products are properly cooked prior to consumption.

Legislation

There is no specific legislation in the EU or in the USA regarding levels of enteric viruses, such as parvoviruses, in foods.

Sources of Further Information

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1.2.8 ROTAVIRUSES

Hazard Identification

What are rotaviruses?

Rotaviruses are non-enveloped, double-stranded RNA viruses, which are classified as belonging to the family *Reoviridae*, genus *Rotavirus*. There are seven described species or "serotypes" of rotavirus (known by the letters A–G). The name rotavirus is derived from the characteristic wheel like appearance of the viruses when viewed under an electron microscope. Groups A, B and C rotaviruses are known to infect humans, and of these, group A rotaviruses are the most significant. Group A rotaviruses are the leading cause of severe diarrhoea in infants and young children worldwide.

Although group A rotaviruses are a major cause of acute diarrhoea it is thought that only a small percentage (around 1%) of cases are actually food borne, the main route of transmission is person-to-person through the faecal–oral route.

Occurrence in Foods

Potentially an infected food handler could contaminate any food prepared and consumed without a subsequent heating step. Salads, cold foods (such as sandwiches and hors d'oeuvres), fruits and contaminated water (including ice cubes) have all been implicated in cases of food-borne rotavirus infections. Rotaviruses have also been detected in shellfish.

Hazard Characterisation

Effects on Health

In countries with a temperate climate, such as the UK and the USA, rotavirus infections usually occur in the winter and spring months, whereas in tropical regions infections occur throughout the year.

Rotaviruses are highly infectious and as few as 10 rotavirus A particles (possibly a single virus particle) can cause illness in a child. Although individuals of all ages are susceptible to rotavirus A infections, the disease usually occurs in infants and young children, and the most severe symptoms are seen in the very young, the immunocompromised and the elderly. Infection usually confers limited immunity to further rotavirus infections. When symptoms do occur in adults the disease is often very mild or even asymptomatic.

The incubation time is 1–3 days and initial symptoms include vomiting and watery diarrhoea for about 2–3 days, often leading to dehydration. The diarrhoea can sometimes persist for 5–8 days. Without electrolyte replacement and adequate fluids, severe, potentially fatal, dehydration can result.

Other symptoms include abdominal discomfort, headaches, chills and low-grade fever. In most cases the infection is self-limiting, and in developed countries most children make a full recovery.

During infection, affected individuals shed high numbers of virus particles in their stools (up to 10^{11} per g) and asymptomatic carriers of the virus also occur.

Incidence and Outbreaks

In developing countries rotaviruses cause an estimated 125 million cases annually in infants and young children. Some 18 million of these are severe cases resulting in nearly 1 million deaths each year.

In industrialised countries deaths from rotavirus infections are extremely rare. In 2006 the USA introduced a vaccine against rotavirus gastroenteritis and recommended its use in very young children. Rotavirus infections have decreased significantly since the introduction of the vaccine. In the pre-vaccine period, it was estimated that as many as 70 000 children (with an estimated 20–60 deaths in the 0–5 years age group) required hospitalisation annually as a result of the illness. In England and Wales it is estimated that 18 000 children are hospitalised each year, and in 2008 there were three deaths due to rotavirus infections. Most of these cases are not caused by food-borne infection, but in the USA it has been estimated that approximately 39 000 cases of viral diarrhoea annually are actually caused by food-borne rotaviruses.

Food-borne outbreaks of rotavirus infections have occasionally been documented in the literature. Suspected vehicles include sandwiches, lettuce, salads, cold foods, strawberry shortcake, potato stew and shepherd's pie. Contaminated water has been associated with outbreaks in many countries.

Sources

Infected individuals act as a reservoir for human rotaviruses. Individuals suffering from the disease, as well as asymptomatic cases, excrete high numbers viruses into the environment in their faeces. Most infections occur as a result of person-to-person transmission through the faecal–oral route. However the virus can contaminate environmental surfaces and objects and these can act as reservoirs for the disease, particularly in institutions such as hospitals and nursing homes.

Foods can be contaminated by infected food handlers, by the use of faecal matter to fertilise crops, or through the use of contaminated water for the irrigation of fresh produce.

Water contaminated with infected faeces can also act as a source of the virus and shellfish cultivated in contaminated water can accumulate rotavirus particles.

Survival Characteristics

Viruses, including rotaviruses, are unable to multiply outside of the host. However, rotaviruses can persist in the environment, and they are known to survive in river water at 20 °C and at 4 °C for several weeks. Rotaviruses can survive for some time on hard surfaces and can remain infective in anaerobically stored animal waste for up to six months. Bovine rotaviruses have been shown to survive processes used to produce soft cheese.

Rotaviruses are reported to be sensitive to drying and to extremes of pH.

Rotaviruses are relatively resistant to many disinfectants, but they are susceptible to 95% ethanol, 2% sodium hypochlorite (with a long contact time), and to 5% Lysol.

Thermal Inactivation

Rotaviruses are reported to be relatively heat sensitive. Although there is little data on the heat inactivation of these viruses, it is thought that normal cooking processes should inactivate them. A study found that rotavirus infectivity is reduced by 99% when heated at 50 °C for at least 30 min.

Control Options

Strategies to reduce the risk of food-borne outbreaks of rotavirus infections should focus on preventing foods from becoming contaminated by the use of clean water for the irrigation, washing and processing of foods, and preventing shellfish-harvesting areas from becoming contaminated with sewage.

Processing

Food handlers should implement frequent hand washing (rotaviruses are most effectively controlled using alcohol-based hand-cleaning agents) and the wearing of gloves, particularly at points in the food chain where foodstuffs that will receive no further cooking are handled. Food handlers suffering from viral gastroenteritis should be excluded from work and advised not to return for at least 48–72 hours after symptoms have ceased.

Product Use

Consumers should be advised not to eat raw or inadequately cooked shellfish.

Legislation

There is no specific legislation in the EU or in the USA regarding levels of enteric viruses, such as rotaviruses, in foods.

Sources of Further Information

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1.2.9 SAPOVIRUSES

Hazard Identification

What are Sapoviruses?

The sapoviruses are a group of single-stranded, positive-sense, RNA viruses recently classified in the family *Caliciviridae*, genus *Sapovirus*. Previously, these human caliciviruses were known as "Sapporo-like viruses" (SLVs), or referred to as classic, or typical caliciviruses. Sapoviruses can be distinguished from the other group of human caliciviruses, the noroviruses, by their six-pointed 'Star of David' morphological appearance when viewed with an electron microscope.

Sapoviruses are commonly associated with causing mild viral gastroenteritis in infants and children worldwide.

Occurrence in Foods

Recent studies in Japan have isolated sapoviruses from clams collected from supermarkets and fish markets, from oysters, as well as from environmental fresh waters during both summer and winter months. Sapovirus infections associated with the consumption of seafood have been reported.

Human caliciviruses, including sapoviruses, could potentially be present in any food or water supply where faecal contamination is present. Contaminated water supplies could result in the contamination of foods grown, irrigated, or washed with the water, such as shellfish, fruits and vegetables.

Hazard Characterisation

Effects on Health

The infective dose for caliciviruses, including sapoviruses, is low (estimated to be between 10 and 100 virus particles). Sapoviruses usually cause infections in infants and young children, although in neonates infections are often sub-clinical. It is thought that sapovirus infections in children may confer long-lived immunity against further infection. Occasionally, infections and outbreaks are reported amongst adults and the elderly and it is thought these illnesses are associated with weakened immunity. Although illness caused by the viruses can occur throughout the year, sapovirus infections peak in the winter months.

The incubation time for sapovirus infections is 1–3 days, and symptoms persist for about 4 days. Typically, the illness is characterised by watery stools, mild or acute diarrhoea, vomiting, nausea, stomach cramps and sometimes a low fever. Sapovirus infections are not well understood, but it is known that the infection is self-limiting, and individuals in developed countries usually make a full recovery. Deaths are very rare and occur mainly in those vulnerable to dehydration.

During infection individuals excrete very high numbers of the virus in their stools. In addition, asymptomatic carriers of these viruses can occur.

Incidence and Outbreaks

Transmission of sapoviruses generally occurs *via* the faecal–oral route. Secondary infections between close contacts (person-to-person transmission) such as in schools and childcare settings are also common. Most sapovirus infections occur as sporadic infections in young children and definite food vehicles have yet to be determined.

Food-borne outbreaks have occasionally been associated with sapoviruses, but they occur far less frequently than food-borne outbreaks associated with noroviruses. The data on food-borne sapovirus outbreaks is limited. Nevertheless, an outbreak in 1994 in Maryland, USA, and another in Japan amongst junior high school students in 2008, were thought to have been caused by food prepared by infected food handlers. An outbreak associated with the consumption of frozen stripped shellfish has also been described.

Sources

Humans are the reservoir for sapoviruses and infected individuals can excrete very high numbers of virus particles. Contaminated environmental sources such as sewage and water (both drinking and recreational) could also be potential sources of sapoviruses, as could foods contaminated by infected food handlers.

Survival Characteristics

Sapoviruses have not been as intensively studied as the noroviruses, and little is known about their survival characteristics. Like other viruses, they are unable to multiply outside the host, but they are thought to survive for some time in the environment.

High levels of chlorination are required to inactivate human caliciviruses in drinking water. Levels of around 10 ppm, or 10 mg l^{-1} of chlorine for more than 30 min have been reported as being required for adequate disinfection.

Thermal Inactivation

Human caliciviruses are thought to be inactivated by ‘adequate cooking processes’ (*e.g.* >1 min at 90 °C).

Control Options

The control of sapoviruses should focus on the implementation of strict personal hygiene by food handlers. Ready-to-eat foods that are handled but will receive no further cooking, such as sandwiches and salads, pose the greatest risk.

Legislation

There is no specific legislation in the EU or in the USA regarding levels of enteric viruses, such as sapoviruses, in foods.

Sources of Further Information

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1.2.10 ENTERIC PICORNAVIRUSES

Hazard Identification

What are Enteric Picornaviruses?

A number of different viruses are associated with food-borne illness and the most commonly reported types are covered in the preceding chapters. But there are several other types of virus that may occasionally cause gastroenteritis and for which food-borne transmission of infection is thought possible. Most of these are picornaviruses and are now classified in the family *Picornaviridae*.

The *Picornaviridae* are a diverse group of small, single-stranded RNA viruses, which cause a variety of diseases in humans, including poliomyelitis, meningitis, encephalitis, myocarditis and conjunctivitis, as well as respiratory and gastrointestinal infections. The family consists of 12 genera, of which three, *Enterovirus*, *Parechovirus* and *Kobuvirus*, include species/serotypes that may be involved in food-borne illness in humans.

Enterovirus

Enterovirus is a large genus and contains 10 species. Within these species, many different serotypes have been identified, of which at least 60 may infect humans.

Enteroviruses are extremely common causes of human viral infections, especially in children. Infections are often asymptomatic, but may also give rise to a variety of symptoms, including diarrhoea, flu-like symptoms and skin rashes. The incubation period is reported to be 7 to 14 days and symptoms are usually self-limiting, lasting a few days or weeks, although more serious illnesses, such as meningitis, can develop.

Transmission by the faecal–oral route is common, but the viruses are also often present in respiratory secretions. Food-borne transmission of infection has not been confirmed, but is thought possible. Enteroviruses have been isolated, along with other enteric viruses, from the stools of patients suffering gastroenteritis associated with the consumption of contaminated oysters in France.

Enteroviruses are often quite resistant to low and high pH, to drying and to cleaning chemicals, including detergents. They are not notably heat resistant and are likely to be destroyed by thermal processes designed to inactivate other food-borne viruses. Like other potentially food-borne viruses, they cannot replicate outside the host and will not grow in food or water.

Control measures should focus on good hygiene practice and the exclusion of infected individuals from food-handling areas.

Parechovirus

The genus *Parechovirus* contains only two species, human parechovirus and Ljungan virus, which was first isolated from rodents. Six serotypes of human

parechovirus have been identified, of which the best known are types 1 and 2 (formerly classified as echoviruses 22 and 23).

Human parechoviruses are a common cause of human viral infections, especially in children under five years of age. Studies have shown that 95–97% of adults are seropositive for antibodies to human parechoviruses, suggesting a very high incidence of infection in the general population. Symptoms are usually mild, with diarrhoea the most commonly observed, followed by respiratory symptoms. More serious illness may develop, affecting the central nervous system.

Transmission by the faecal–oral route is likely, but the viruses may also be present in respiratory secretions. Food-borne transmission of infection has not been confirmed, but is thought possible.

Like other picornaviruses, human parechoviruses may be quite resistant to low and high pH, to drying and to cleaning chemicals, including detergents. They are not notably heat resistant and are likely to be destroyed by thermal processes designed to inactivate other food-borne viruses. Like other potentially food-borne viruses, they cannot replicate outside the host and will not grow in food or water.

Control measures should focus on good hygiene practice and the exclusion of infected individuals from food-handling areas.

Kobuvirus

There are currently two confirmed species within the genus *Kobuvirus*, Aichi virus and bovine kobuvirus (found in cattle). Aichi virus was first identified in 1989 in the stools of patients in Japan suffering from gastroenteritis associated with the consumption of raw oysters. Three genotypes have been identified, referred to as A, B and C.

Although Aichi virus is rarely isolated in cases of gastroenteritis, several studies in different countries have shown that 80–95% of adults are seropositive for antibodies to the virus. This suggests a much higher incidence of infection in the population than has so far been recognised. It is thought that many infections are subclinical, but Aichi virus has been reported to be a cause of acute gastroenteritis in humans. Symptoms include diarrhoea, abdominal pain, nausea, vomiting and fever. More serious illnesses affecting the central nervous system are very uncommon.

The transmission of infection is thought very likely to occur mainly by the faecal–oral route. Food-borne transmission of infection by contaminated molluscan shellfish has been reported in Japan and France. Consumption of raw shellfish, such as oysters, from contaminated waters is considered to be an important risk factor for Aichi virus infection.

In common with other picornaviruses Aichi virus is likely to be quite resistant to low and high pH, to drying and to cleaning chemicals, including detergents. The virus is not notably heat resistant and is likely to be destroyed by thermal processes designed to inactivate other food-borne viruses.

Like other food-borne viruses, Aichi virus cannot replicate outside the host and will not grow in food or water.

Control measures should focus on good hygiene practice and the exclusion of infected individuals from food-handling areas. Consumers should be advised not to eat raw shellfish harvested from unapproved fisheries.

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CHAPTER 1.3

Parasites

1.3.1 PROTOZOA

1.3.1.1 *Cryptosporidium*

Hazard Identification

What is Cryptosporidium*?*

Cryptosporidium is a single-celled protozoan parasite belonging to the subclass Coccidia. Until recently, the only species thought to be important in human illness was classified as *Cryptosporidium parvum*. However, recent taxonomic studies have shown that several species can infect humans, including *C. hominis*, which is specific to humans, and *C. parvum*, which infects both humans and ruminants. Other species that have been reported to infect humans include *C. felis*, *C. canis*, *C. meleagridis*, *C. muris* and *C. suis*.

Cryptosporidium is an obligate parasite and requires a host in order to multiply. It was first discovered almost 100 years ago, but was not associated with human illness until 1976. It is a cause of gastrointestinal infection in humans and some other animals, especially calves and lambs, and is found worldwide.

Cryptosporidium has a complex lifecycle, most of which takes place within the gastrointestinal tract (mainly in the small intestine) of a single host. The transmissible stage in the cycle is a highly resistant, thick-walled spore, known as an oocyst.

Occurrence in Foods

Cryptosporidium is mainly associated with water that has been polluted by human or animal faeces, but oocysts have also been found in a number of unprocessed foods, notably raw milk, meat and shellfish and fresh fruit and vegetables. *Cryptosporidium* cannot grow in foods or in water and does not multiply in the environment outside of a suitable host.

The Food Safety Hazard Guidebook, 2nd Edition
Richard Lawley, Laurie Curtis and Judy Davis

Published by the Royal Society of Chemistry, www.rsc.org

Oocysts are easily destroyed by heat and *Cryptosporidium* is not normally associated with cooked and processed foods. Any food that may come into contact with contaminated water during production, and where there is no subsequent process that will destroy oocysts, is at risk from *Cryptosporidium* contamination. However, food is not considered to be a major vehicle for the transmission of the parasite. The person-to-person and animal-to-human (zoonotic) transmission routes are likely to be much more common.

Hazard Characterisation

Effects on Health

Cryptosporidium can cause an acute gastrointestinal infection in humans. It invades the epithelial cells lining the gut causing inflammation and loss of fluid. The incubation time for the infection is usually between 5–7 days, but it may vary from 2–14 days, possibly depending on the number of oocysts ingested. The main symptom is profuse watery diarrhoea, often accompanied by abdominal pain. Vomiting, fever and weight loss may also occur. Symptoms are most severe in the very young, the elderly and in immunocompromised adults, such as AIDS patients. In healthy adults, symptoms typically last for 2–4 days, but may last for up to 2–3 weeks in some cases. The infection is usually self-limiting and is resolved without medical treatment. However, in vulnerable individuals, infection can be more serious and long lasting, requiring hospital treatment, and deaths have been recorded. *Cryptosporidium* is also capable of invading other organs, such as the respiratory system, in some cases.

The infective dose is uncertain, but may be as low as 10 oocysts, or even fewer. A single oocyst is thought to be capable of causing disease in young lambs, and possibly also in very young children and immunocompromised adults. Infected individuals shed very large numbers of infectious oocysts in their faeces, and this may continue at a low level for several weeks after symptoms have subsided. This shedding of oocysts is the main reason why person-to-person and zoonotic transmission of the parasite are so common. Asymptomatic cases of infection have also been reported.

Incidence and Outbreaks

Cases of *Cryptosporidium* infection are not particularly common. For example, in England and Wales between 1989 and 2010, the number of reported cases each year generally ranged from 3000 to 6000, with a peak of nearly 8000 cases in 1989. The most recent data for the EU refers to 2008 and shows a total of 7032 reported cases of cryptosporidiosis from 21 countries. However, about 70% of these were from the UK, suggesting significant under-reporting in many other countries. The EU country with the highest reported incidence was Ireland with 9.4 cases per 100 000 people. The results also show that peaks of infection commonly occur in the autumn, or occasionally in spring. Cryptosporidiosis is a notifiable disease in the EU and in the USA.

There were 10 500 reported cases of cryptosporidiosis in the USA in 2008. The number of cases reported across the country rose dramatically between 2006 (6479) and 2007 (11 657), in part because of a number of outbreaks associated with recreational waters. A peak in the number of cases reported typically occurred in the summer and early autumn.

The incidence of cryptosporidiosis in New Zealand is reported to be relatively high (an average rate of 22.0 cases per 100 000 reported between 1997 and 2006), with a marked peak in the spring and notably higher reporting rates in rural areas.

There is little or no information about the proportion of reported cases that are food borne, but it is thought likely that the majority are caused by contact with infected animals, people, or contaminated water.

Most recorded outbreaks are associated with contaminated drinking water, or recreational waters. For example, in 1993 a water-borne outbreak occurred in Milwaukee in the USA, which affected more than 400 000 people and caused an estimated 69 deaths. Food-borne outbreaks have also been recorded, usually caused by an infected food handler, or by faecal contamination, either direct or through polluted water. Outbreaks have been linked to raw produce, chicken salad, green onions and raw milk. In the USA, there have been several outbreaks linked to unpasteurised apple cider. For example, in 2003, cider made from contaminated apples caused illness in 144 people. The cider had reportedly been treated with ozone, but this had clearly not been effective.

Sources

Cryptosporidium species are all obligate parasites and thus originate from the host animal. *C. hominis* is thought to primarily infect humans, while *C. parvum* infects humans and ruminants. The primary source of *Cryptosporidium* is therefore the faeces of infected humans and animals, which may contain up to 10^9 oocysts in a single bowel movement. Infected cattle are a particularly important reservoir of *C. parvum*. The oocysts are extremely infectious and may be transferred to food *via* an infected food handler, or through polluted water used for crop irrigation or processing.

Cryptosporidium oocysts are quite difficult to remove from water, even by modern water-treatment methods. Their small size (4–6 μm diameter) and resistance to chlorine enable them to pass through some water treatment plants, especially if they are present in high numbers. This can happen when heavy rains cause run-off from agricultural land used for grazing. Under these circumstances it may not be possible to guarantee that public water supplies are free from *Cryptosporidium* oocysts.

Stability in Foods

Cryptosporidium oocysts are very resistant to most environmental factors, with the exception of heat and desiccation. Oocysts can persist for months in water and in soil and have been shown to survive for hours on wet surfaces, including stainless steel. However, they are not resistant to drying and die rapidly on dry surfaces.

The oocysts are also remarkably resistant to many sanitisers and disinfectants, notably chlorine. One study reported survival for two hours on exposure to chlorine at 50 000 ppm. 18-Hour exposure to 4% iodophore and 10% benzalkonium chloride solutions has also been demonstrated to be ineffective in inactivating oocysts.

Cryptosporidium oocysts are not especially heat resistant and are destroyed by conventional milk pasteurisation. A temperature of greater than 73 °C will cause instantaneous inactivation of oocysts. Therefore most controlled cooking processes used in food production should destroy any viable oocysts in the product.

Oocysts can survive for short periods at temperatures below 0 °C, especially in water, but the commercial ice cream freezing process has been shown to cause inactivation and eventual die-off occurs at temperatures below − 15 °C.

Some loss of viability has been shown in acid conditions below pH 4.0. It has been reported that oocysts lost 85% of viability in 24 hours when contaminated water was used to brew beer and produce a carbonated beverage. Organic acids in fruit juice have been reported to inhibit the infectivity of oocysts, but viable *C. parvum* oocysts have been detected after 14 days suspended in media acidified with citric, lactic and acetic acids.

Control Options

Processing

Control measures for *Cryptosporidium* in food processing focus largely on the control of contamination in the water supply. Food processors using potable water from the public supply network should carry out a risk assessment on the consequences of mains water contamination and a "Boil Water Notice" issued by the water supplier. Where there is a high risk, as in the production of raw food products, such as fresh-cut produce and salads, it may be worthwhile considering the introduction of additional on-site water treatment measures, such as charcoal or membrane filtration. Treatment with biocides such as hydrogen peroxide and chlorine dioxide may be effective, but only at concentrations well above those usually used in water treatment.

Heat processing is an effective control against *Cryptosporidium* oocysts in food. Normal milk pasteurisation processes are effective, as are recommended "Listeria cook" processes for meat products (70 °C for at least 2 min). Reheating cooked foods to at least 74 °C will destroy oocysts immediately.

Freezing foods for at least seven days is an effective control, as is drying. Oocysts were reported to lose infectivity in seven days when stored at a water activity of 0.85 at 7 °C.

Hygiene

Infected food handlers are also a major *Cryptosporidium* contamination risk for foods that do not undergo any further processing, such as sandwiches and salads. Good personal hygiene practice, especially hand washing, is an essential

control and any staff suffering from gastroenteritis should be excluded from processing areas.

Legislation

Cryptosporidium is generally considered to be a water-borne pathogen rather than food borne. It may therefore be covered in drinking water regulations, as is the case in the UK, but is not usually mentioned specifically in food safety and hygiene law.

Sources of Further Information

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1.3.1.2 *Cyclospora*

Hazard Identification

What is Cyclospora*?*

Cyclospora is a single-celled protozoan parasite belonging to the subclass *Coccidiasina*. The only species known to cause human illness is *Cyclospora cayetanensis*. Other species have been found in a variety of animals, including chimpanzees and other non-human primates. However *C. cayetanensis* infections have only been found in humans and it is possible that humans are the primary host.

Cyclospora is an obligate parasite and requires a host in order to multiply. It was first discovered in 1881, but was not associated with human illness until the late 1970s. It is a cause of gastrointestinal infection (cyclosporiasis) in humans, and is endemic in some developing countries, notably in Central and South America and some parts of Asia.

Cyclospora has a complex lifecycle, most of which takes place within the gastrointestinal tract (mainly in the small intestine) of a single host. The transmissible stage in the cycle is a highly resistant, thick-walled spore, known as an oocyst.

Occurrence in Foods

Cyclospora was not considered to be a food-borne pathogen until 1996 when a large *C. cayetanensis* outbreak occurred in the USA. This was linked to imported raspberries from Guatemala. Until then, most reported cases in the USA were associated with foreign travel. Where *Cyclospora* is endemic, it is mainly associated with water that has been polluted by human or animal faeces. There has been very little attempted surveillance of *Cyclospora* oocysts in foods and effective test methods have been developed only recently. However, oocysts have been isolated from fresh basil implicated in a food-borne outbreak and epidemiological evidence from other outbreaks suggests that it may have been present in other fresh fruits and vegetables.

Cyclospora cannot grow in foods or in water and does not multiply in the environment outside of a suitable host. The parasite has not been reported to be associated with cooked and processed foods.

Contaminated water and food are thought to be the main routes for transmission of infection. Direct person-to-person transmission of *Cyclospora* is thought unlikely.

Hazard Characterisation

Effects on Health

Cyclospora cayenatensis can cause an acute gastrointestinal infection in humans. It invades the epithelial cells lining the gut, especially in the jejunum,

causing inflammation and loss of fluid. The incubation time for the infection is typically 5–7 days from ingesting sporulated oocysts, but it may vary from 1 to 14 days. The main symptom is watery diarrhoea, which may alternate with periods of constipation and persist for long periods (1–2 months in some cases). Other reported symptoms include abdominal pain, vomiting, fatigue, fever and weight loss. Diarrhoea is usually self-limiting in healthy adults, but may be more prolonged and debilitating in young children and the immunocompromised. Asymptomatic and mild cases of infection are reported to be common and immunity may be developed in areas where the disease is endemic.

The infective dose is uncertain, but is probably low. Infected individuals shed moderate numbers of oocysts in their faeces, but at this stage the oocysts are unsporulated and are not infectious. This is the main reason that person-to-person transmission is considered unlikely. Sporulation only takes place outside the body at higher concentrations of oxygen than those found in the gut and requires a period of 7–10 days at 30 °C. However, the process takes much longer at lower ambient temperatures. This may be why cyclosporiasis is not endemic in temperate regions.

Incidence and Outbreaks

Cases of cyclosporiasis are rare in developed countries, and until recently were generally associated with travel to countries where the disease is endemic, such as Peru, Haiti and Nepal. It is likely that *Cyclospora* is prevalent worldwide, but the incidence of disease is not known in most countries.

In England and Wales, approximately 60 cases of cyclosporiasis a year have been reported since the mid 1990 s, but many of these are known to have been acquired abroad. There is little published information on the incidence of the disease elsewhere in the EU and few documented reports of cases of food-borne infection. The lack of awareness of *Cyclospora cayetanensis* and the absence of surveillance suggests that the disease is likely to be substantially under-reported.

Surveillance for *Cyclospora* in the USA is more developed following several large outbreaks in the 1990 s. The overall incidence of cyclosporiasis in the USA in 2009 was estimated to be approximately 0.07 cases per 100 000 people. This equates to around 230 cases per year, but it is not known how many of these result from contaminated foods. There is usually a peak in reported cases in summer when high temperatures help the oocysts to sporulate. Cyclosporiasis is a notifiable disease in the USA.

Most recorded food-borne cyclosporiasis outbreaks have occurred in the North America, including the first recorded outbreak in 1996, which affected almost 1500 people in the USA and Canada and was linked to imported raspberries from Guatemala. Since then, there have been a further 10 or more outbreaks in the USA, almost all linked to contaminated produce, such as mesclun lettuce, fresh basil, snow peas and imported berries. In 2000 an outbreak affecting 34 people was reported in Germany associated with consumption of contaminated salad. These outbreaks are generally thought to be

caused by the use of contaminated water for irrigation rather than by infected food handlers.

Sources

Cyclospora cayetanensis is an obligate parasite and thus originates from the host animal. Humans may well be the primary host for the parasite and human faeces are therefore the main source of *Cyclospora cayetanensis* oocysts. The oocysts may be transferred to food crops *via* polluted surface water used for irrigation or to dilute pesticides for application by spraying. Once sporulation has taken place the oocysts become infectious if ingested.

Stability in Foods

Like the closely related *Cryptosporidium* oocysts, *Cyclospora* oocysts are reported to be resistant to most environmental factors, with the likely exception of heat and desiccation. However, there is little published information to confirm this.

The oocysts are quite resistant to chlorine and cases of cyclosporiasis have been associated with chlorinated water supplies in Nepal. It is likely that the normal chlorination levels used in water treatment would be insufficient to inactivate oocysts. It has also been reported that *Cyclospora* oocysts are very resistant to disinfectants commonly used in food processing, possibly in part because they are able to adhere strongly to the surface of fruits and vegetables.

There is no real evidence that *Cyclospora* oocysts are any more heat resistant than those of *Cryptosporidium* and it seems probable that they too are inactivated by milk pasteurisation and other cooking processes.

Cyclospora oocysts are larger than those of *Crytosporidium* (9–10 μm diameter) and are therefore more easily removed from water supplies by conventional treatment. However, their apparent resistance to chlorination means that there is a risk that they may pass into public water supplies if treatment, especially filtration systems, is not well controlled.

Control Options

Control measures for *Cyclospora* in food focus largely on Good Agricultural Practice in fruit and vegetable production in countries where the parasite is endemic and on ensuring that contaminated surface water is not used in irrigation or the application of pesticides and fertilizers. For example, the FDA has worked with Guatemalan raspberry growers since the 1996 outbreak to improve standards and has developed a code of practice that includes filtration of all water used in cleaning and sanitation. The expansion of supply chains for fresh fruit and vegetables into countries where *Cyclospora* is prevalent means that this approach is likely to become more important in the future to prevent food-borne outbreaks of cyclosporiasis.

Processing

Heat processing is probably an effective control against *Cyclospora* oocysts in food and normal milk pasteurisation processes are likely to inactivate them, as are cooking processes that raise the product temperature to 70 °C or more.

Freezing and drying of foods may also be effective controls, as is the case for *Cryptosporidium*.

Legislation

Cyclospora is not mentioned specifically in food safety and hygiene law in most countries. The USA government has adopted import restrictions for high-risk foods such as raspberries grown in Guatemala. Only growers approved by the FDA may export to the USA.

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1.3.1.3 *Entamoeba*

Hazard Identification

What is Entamoeba*?*

Entamoeba is a single-celled protozoan parasite belonging to the sub-phylum *Sarcodina*. The species important in human illness is *Entamoeba histolytica*, but at least five other species are also found in humans, notably *Entamoeba dispar*, which is morphologically indistinguishable from *E. histolytica*, but much more common and non-pathogenic. *E. histolytica* is also found in non-human primates and other mammals, including cats and dogs.

E. histolytica is an obligate parasite and requires a host in order to multiply. It has been recognised as a cause of gastrointestinal disease (amoebiasis) in humans for many years and is found worldwide, but is particularly prevalent in developing countries.

E. histolytica has a two-stage lifecycle, and exists in two forms. The active trophozoite stage exists and multiplies within the gastrointestinal tract of the host. Some of these form spore-like resistant cysts within the small intestine. Both forms may be excreted in the host's faeces, but the trophozoites die quickly and the transmissible stage in the cycle is the cyst.

Occurrence in Foods

E. histolytica is mainly associated with surface water that has been polluted by human faeces, but cysts may also be present in a number of unprocessed foods, including fruit and vegetables, if polluted water has been used for irrigation or processing. *E. histolytica* does not grow in foods or in water and does not multiply in the environment outside of a suitable host.

Cysts are destroyed by heat and *E. histolytica* is not normally associated with cooked and processed foods, unless re-contamination from an infected food handler has occurred. Any food that may come into contact with contaminated water or infected food handlers during production, and where there is no subsequent process that will destroy cysts, may be at risk from *E. histolytica* contamination. However, food is not considered to be a major vehicle for the transmission of the parasite. The water-borne and person-to-person transmission routes are thought to be much more common.

Hazard Characterisation

Effects on Health

E. histolytica can cause an acute gastrointestinal infection (amoebiasis) in humans, and may become invasive in a few cases. The trophozoites multiply in the gastrointestinal tract, particularly in the colon, and occasionally invade the cells of the intestinal mucosa by producing proteases. The trophozoites have also been reported to produce toxins. The incubation time for the infection is

very variable, but is usually between 1 to 4 weeks from ingestion of cysts. The majority of cases are asymptomatic, but about 10% of those infected suffer mild gastroenteritis symptoms of slight diarrhoea and abdominal discomfort. In some cases, more severe symptoms of acute colitis develop, characterised by bloody diarrhoea, high temperature, fever and severe lower abdominal pain. This condition is generally referred to as amoebic dysentery. Symptoms can be long lasting and may persist for several weeks, or even months. Very rarely, other tissues, notably the liver, may be invaded and abscesses can be formed. Chronic invasive amoebiasis is a serious disease and can be fatal. Immunocompromised individuals are particularly vulnerable to severe infections.

The infective dose is thought to be very low and, in theory, ingestion of a single cyst may be enough to cause amoebiasis. Infected individuals shed large numbers of infectious cysts in their faeces, and this may continue long after symptoms have subsided. Asymptomatic carriers have also been reported to shed cysts in their faeces over long periods, possibly several years in some cases.

Incidence and Outbreaks

E. histolytica is probably the most commonly reported intestinal parasite worldwide. It was previously estimated that approximately 500 million people worldwide were infected with *E. histolytica*, but it is now accepted that the majority of those people are carriers of non-pathogenic *E. dispar*. The true figure for the number of cases of infection with *E. histolytica* is now estimated to be about 50 million worldwide. The infection is also estimated to cause between 50 000 and 100 000 deaths each year, mostly in developing countries.

In England and Wales between 1990 and 2008, there was a downward trend in the number of confirmed cases of *E. histolytica* infection from a peak of 1017 cases in 1991 to just 68 in 2008. However, more recent figures showed a small rise, with 105 cases being reported in 2010. Most of these cases are thought to be associated with foreign travel.

Between 1990 and 1994 (the most recent national figures) approximately 3000 cases of amoebiasis were reported each year in the USA. The majority of these are associated with foreign travel or occurred in recent immigrants. The incidence is reported to be higher in states along the Southern border with Mexico.

There have been few documented outbreaks of amoebiasis in developed countries and none that were definitely food borne, despite the high incidence of the disease in many developing countries. A large outbreak associated with contaminated drinking water occurred in Chicago in 1933. This affected at least 1000 people with 58 deaths. Infected food handlers have been suspected of causing isolated cases of amoebiasis, but the incubation period for the infection is often too long to identify the source with much certainty. Food-borne outbreaks are probably quite common in developing countries where there is a

high incidence of the disease, but water-borne and person-to-person transmission are thought to be more important.

Sources

E. histolytica is an obligate parasite and thus originates from the host. The primary source of *E. histolytica* cysts is therefore the faeces of infected humans, many of whom do not display symptoms. Carriers may shed up to 15 million cysts each day in faeces. The cysts are infectious and may be transferred to food *via* an infected food handler, or through polluted water used for crop irrigation or processing.

E. histolytica cysts are larger than those of *Cryptosporidium* (10–15 μm diameter) and are not so difficult to remove from water using modern water-treatment methods, such as filtration. Amoebiasis is most often associated with conditions of poor sanitation and inadequate treatment of drinking water.

Stability in Foods

E. histolytica are relatively resistant to environmental factors, other than heat and desiccation. Cysts can remain infectious for some time in cool, moist conditions. However, there is relatively little published information on their survival and inactivation in foods.

E. histolytica cysts are not especially heat resistant and are reported to be destroyed by heating at temperatures above 50 °C and by conventional milk pasteurisation. Therefore most controlled cooking processes used in food production should destroy any viable cysts in the product.

The cysts are relatively resistant to chlorine at the levels used in conventional water treatment, but are reported to be destroyed by 1% solutions of sodium hypochlorite.

Control Options

Control measures for *E. histolytica* in food processing focus largely on the control of contamination in water and the management of infected food handlers.

Processing

Care should be taken to ensure that raw food ingredients and products that do not undergo further processing do not come into contact with contaminated surface water. In high-risk areas, fresh produce should be obtained from suppliers practicing Good Agricultural Practice. Fresh produce and other raw foods should only be washed/processed using potable quality water.

Heat processing is an effective control against *E. histolytica* cysts in food. Normal milk pasteurisation processes are effective, as are recommended

"Listeria cook" processes for meat products (70 °C for at least 2 min). Reheating cooked foods to at least 74 °C will destroy cysts immediately.

Hygiene

Infected food handlers are also a major *E. histolytica* contamination risk for foods that do not undergo any further processing, such as sandwiches and salads, and for the re-contamination of cooked foods. Good personal hygiene practice, especially hand washing, is an essential control and any staff suffering from gastroenteritis, especially following foreign travel, should be excluded from processing areas.

Legislation

E. histolytica is not usually mentioned specifically in food safety and hygiene law.

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1.3.1.4 *Giardia*

Hazard Identification

What is Giardia*?*

Giardia is a single-celled flagellate protozoan parasite belonging to the order *Diplomonadida*. The cells are unusual in having two nuclei. The species important in human illness is *Giardia intestinalis* (previously referred to as *G. lamblia*, or *G. duodenalis*). *G. intestinalis* is also found in a number of domestic and wild animals, including cattle, cats and dogs.

G. intestinalis is an obligate parasite and requires a host in order to multiply. It was first discovered in 1859, but was not confirmed as a human pathogen until the late 1970 s. It is a cause of gastrointestinal infection (giardiasis) in humans and some other animals, and is found worldwide.

G. intestinalis has a two-stage lifecycle, and exists in two forms. Pear-shaped flagellated trophozoites exist and multiply within the gastrointestinal tract of the host. Some of these form spore-like resistant cysts within the small intestine. Both forms may be excreted in the host's faeces, but the trophozoites die quickly and the transmissible stage in the cycle is the resistant, thick-walled cyst.

Occurrence in Foods

G. intestinalis is mainly associated with surface water that has been polluted by human or animal faeces, but cysts have also been found in a number of unprocessed foods, including root crops, lettuce, herbs and strawberries. *G. intestinalis* cannot grow in foods or in water and does not multiply in the environment outside of a suitable host.

Cysts are destroyed by heat and *G. intestinalis* is not normally associated with cooked and processed foods. Any food that may come into contact with contaminated water during production, and where there is no subsequent process that will destroy cysts, may be at risk from *G. intestinalis* contamination. However, food is not considered to be a major vehicle for the transmission of the parasite. The water-borne and person-to-person transmission routes are thought to be much more common. Animal-to-human (zoonotic) transmission may also occur, but the significance to human health of *G. intestinalis* in livestock and domestic animals is not clear.

Hazard Characterisation

Effects on Health

G. intestinalis can cause an acute gastrointestinal infection in humans, and children are especially vulnerable to infection. The mechanism by which it causes disease is unclear. The trophozoites attach to the epithelial cells lining the gut, but do not seem to invade the cells. They may produce a toxin in the

small intestine, but this has not been confirmed. The incubation time for the infection is usually 1–3 weeks from ingestion of cysts. The main symptom is diarrhoea, often accompanied by abdominal pain. Flatulence, fever and loss of appetite may also occur. In healthy adults, symptoms typically last for 1–2 weeks, but may last for up to 6 weeks in some cases. The infection is generally self-limiting in most cases, but drug treatment is sometimes required. However, in immunocompromised individuals, infection can be more serious and long lasting, requiring hospital treatment, and occasional deaths have been recorded. Complications of chronic giardiasis may include severe weight loss, the development of lactose intolerance and possibly reactive arthritis.

The infective dose is thought to be very low and ingestion of as few as 10 cysts (trophozoites are virtually non-infective) may be enough to cause giardiasis. Infected individuals shed very large numbers of infectious cysts in their faeces, and this may continue for months after symptoms have subsided. Asymptomatic cases of infection are quite common and asymptomatic carriers have been reported to continue shedding cysts for years.

Incidence and Outbreaks

G. intestinalis is probably the most commonly reported intestinal parasite in the developed world. In England and Wales between 1986 and 1996, the number of reported cases each year generally ranged from 5000 to 7000, but from 1996 to 2006 the number of confirmed cases fell and now averages around 3000 cases each year.

The most recent data for the EU refers to 2008 and shows a total of 167 414 reported cases of giardiasis from 22 countries. However, there are large differences between surveillance systems in different EU countries and there is likely to be significant under-reporting. The EU country with the highest reported incidence was Romania (691 cases per 100 000 people), which accounted for 87% of the reported cases, followed by Bulgaria (28 cases per 100 000 people), Estonia (20 cases per 100 000 people) and Sweden (17 cases per 100 000 people). The results also show that children aged 0–4 years were most commonly infected and that there are seasonal peaks of infection in spring and autumn. Giardiasis is a notifiable disease in much of the EU and in the USA.

There were 19 140 reported cases of giardiasis in the USA in 2008. This figure has been relatively stable in recent years. Most cases were reported from the Northern states and there was a peak in the summer and early autumn.

The incidence of giardiasis in New Zealand is reported to be relatively high (46.5 cases per 100 000 in 2000), with a peak of infection in the autumn.

There is little or no information about the proportion of reported cases that are food borne, but it is thought likely that the majority are caused by contact with contaminated water, infected people, and occasionally animals.

Most reported outbreaks of giardiasis are associated with contaminated surface water, or person-to-person transmission. Most of the documented outbreaks have been recorded in the USA, and outbreaks in the EU appear to be rare. Food-borne outbreaks have also been recorded in the USA, usually

caused by an infected food handler, or by faecal contamination, either directly or through polluted water. Outbreaks have been linked to salad, lettuce and tomatoes, noodle salad, canned salmon, cheese dip, sandwiches, fruit salad and ice.

Sources

G. intestinalis is an obligate parasite and thus originates from the host. The primary source of *G. inestinalis* is therefore the faeces of infected humans and animals, which may contain up to 10^9 cysts in a single day. The cysts are extremely infectious and may be transferred to food *via* an infected food handler, or through polluted water used for crop irrigation or processing.

G. intestinalis cysts are larger than those of *Cryptosporidium* (9–12 μm diameter) and are not so difficult to remove from water using modern water-treatment methods. They are also less resistant to chlorine, but are not inactivated by the concentrations normally used to treat water. They are much less likely to pass through water treatment plants into the public water supply system.

Stability in Foods

G. intestinalis cysts are generally resistant to environmental factors. Cysts can persist for months in cool, moist conditions and have been shown to survive for eight days on the leaves of herbs. However, there is little information on their survival and inactivation in foods.

The cysts are relatively resistant to some sanitisers and disinfectants, notably chlorine and ozone, but are reported to be inactivated by phenolic disinfectants.

G. intestinalis cysts are not especially heat resistant and are destroyed by conventional milk pasteurisation. A temperature of 60–70 °C for 10 min is reported to inactivate cysts completely. Therefore most controlled cooking processes used in food production should destroy any viable cysts in the product.

Oocysts can survive for significant periods at temperatures below 0 °C, especially in water, but frozen storage is reported to cause inactivation.

There is little information on the effect of pH, but it has been reported that cysts are resistant to low pH values down to about 3.0.

Control Options

Control measures for *G. intestinalis* in food processing focus largely on the control of contamination in water and the management of infected food handlers.

Processing

Care should be taken to ensure that raw food ingredients and products that do not undergo further processing do not come into contact with contaminated

surface water. Fresh produce should be obtained from suppliers practicing Good Agricultural Practice. Fresh produce and other raw foods should only be washed/processed using potable quality water.

Heat processing is an effective control against *G. intestinalis* cysts in food. Normal milk pasteurisation processes are effective, as are recommended "Listeria cook" processes for meat products (70 °C for at least 2 min). Reheating cooked foods to at least 74 °C will destroy cysts immediately.

Freezing foods for at least seven days is also an effective control.

Hygiene

Infected food handlers are also a major *G. intestinalis* contamination risk for foods that do not undergo any further processing, such as sandwiches and salads. Good personal hygiene practice, especially hand washing, is an essential control and any staff suffering from gastroenteritis should be excluded from processing areas.

Legislation

G. intestinalis is generally considered to be a water-borne pathogen rather than food borne. It may therefore be covered in drinking water regulations, as is the case in the UK, but is not usually mentioned specifically in food safety and hygiene law.

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1.3.1.5 *Toxoplasma*

Hazard Identification

What is Toxoplasma*?*

Toxoplasma is a single-celled protozoan parasite belonging to the subclass *Coccidia*. The species of significance to human health and food safety is *Toxoplasma gondii*.

Toxoplasma is an obligate parasite and requires a host in order to multiply. It has been known as the cause of a disease (toxoplasmosis) in humans for many years. *Toxoplasma* is able to infect humans, most other mammals and also birds, and has a worldwide distribution. However, the definitive hosts for *Toxoplasma gondii* are members of the cat family, including domestic cats.

Toxoplasma has a very complex lifecycle, consisting of several stages and forms, and a wide range of intermediate host species, including humans. There are two transmissible stages in the cycle. One is a resistant, thick-walled spore, known as an oocyst, which is only present in the faeces of cats and becomes infective following sporulation in the environment. The second transmissible stage is microscopic infective tissue cysts, which are found in the muscles of a number of intermediate hosts.

Occurrence in Foods

Toxoplasma oocysts may be present on raw foods, such as home-grown fresh produce, that have been contaminated by cat faeces. Contaminated water has also been implicated as a source of infection and it has been suggested that shellfish may retain oocysts when growing in contaminated sea water.

However, the presence of tissue cysts in meat is probably of more significance from a food safety point of view. Infective tissue cysts have been found in a wide range of domestic and wild species, but infected pork is considered to be particularly important in the transmission of toxoplasmosis to humans. Tissue cysts have also been found in sheep and goat meat, rabbit, horse and deer meat and in poultry, but have rarely been observed in meat from cattle. Beef and veal are considered to be much less significant than pork as a source of infection, but there is some uncertainty about their true importance. The number of tissue cysts in the meat of infected animals is generally low and has been estimated as approximately one cyst per 100 g of meat.

Unpasteurised goats' milk has been implicated as a source of toxoplasmosis, but there are no reports of cows' milk causing infection.

Oocysts are destroyed by heat and *Toxoplasma* is not normally associated with cooked and processed foods, although raw and undercooked meats containing tissue cysts carry a high risk of infection. Cured pork has also been identified as a risk factor in epidemiological studies. The main routes of transmission are from animal to human (zoonotic), either by ingestion of

oocysts through direct contact with cat faeces, contaminated water, or food, or by ingestion of tissue cysts in raw or undercooked meat from an infected animal. Infection can also occur by handling infected meat and subsequent ingestion of tissue cysts. Direct person-to-person transmission has not been reported. *Toxoplasma* cannot grow in foods or in water and does not multiply in the environment outside of a suitable host.

Hazard Characterisation

Effects on Health

Toxoplasma gondii infection in humans is thought to be very common, but is usually asymptomatic. On ingestion of sporulated oocysts, or viable tissue cysts, an invasive stage of the parasite, known as tachyzoites, are eventually released in the gut and enter the body through the wall of the intestine. They then migrate through the body and invade various tissues, subsequently multiplying and forming cysts. This process is not usually noticed by the host and no clinical symptoms are reported, but in about 15% of cases, invasion of the tissues is accompanied by self-limiting mild flu-like symptoms and swelling of the lymph nodes. In a very few cases, more serious symptoms may develop, including visual impairment and brain damage, sometimes proving fatal. Where symptoms do occur, the incubation time is generally from 3–25 days.

Certain specific groups of the population are at risk of serious disease from infection by *Toxoplasma*. Infection in pregnant women may result in the tachyzoites crossing the placenta and invading the developing foetus. This infection can cause the death of the foetus in 3–4% of cases and often leads to long-term disease (congenital toxoplasmosis) in the rest. This may take various forms, most commonly visual impairment or blindness, but also including mental retardation, convulsions, and in a few cases, hydrocephalus. In some countries, including France and Austria, pregnant women are routinely screened for *Toxoplasma gondii* infection.

Immunocompromised individuals are also at serious risk from toxoplasmosis, particularly those suffering from AIDS. In these cases the brain and central nervous system are often affected and symptoms may include encephalitis, but other organs may also be affected. Between 10 and 30% of AIDS patients with toxoplasmosis are estimated to die from the infection.

There is also some epidemiological evidence that infection with *Toxoplasma gondii* may be involved in behavioural changes in humans and may have a role in the development of some psychotic illnesses, particularly schizophrenia.

The infective dose is uncertain, but is probably quite low.

Incidence and Outbreaks

Toxoplasma gondii is one of the commonest parasitic infections in humans, and it has been estimated that at least a third of the world's population has been exposed to the parasite. Up to 34% of adults in the UK are estimated to carry

antibodies to *Toxoplasma gondii*, with the estimates for other EU countries ranging from 50–80%. The figure for the USA is thought to be around 23%. The vast majority of these cases are asymptomatic. Recent studies using data from the USA and the EU have estimated that 50–60% of cases may be associated with the food chain. The number of food-borne cases is probably higher in countries where raw or rare-cooked meat is a regular part of the diet.

The incidence of clinical toxoplasmosis is much lower. In the UK for example, 133 cases were reported in 2008. In the EU there were 1788 cases reported in 16 countries in 2008, with the highest incidences being reported in Lithuania (3.5 cases per 100 000 people) and Slovakia (3.2 per 100 000) the overall incidence for the EU is estimated to be 0.76 per 100 000. However, it is likely that there is considerable under-reporting of the disease.

Estimates for the incidence of acute toxoplasmosis in the USA suggest that as many as 1.5 million people each year suffer symptoms. It is also estimated that there are between 400 and 4000 cases of congenital toxoplasmosis each year.

It is difficult to identify food-borne outbreaks of toxoplasmosis because of the relatively long incubation time and the high proportion of asymptomatic cases. However, outbreaks have been reported in a number of countries, including the UK, the USA, Australia, Korea and Brazil, usually associated with raw, or undercooked meat. A large water-borne outbreak, in which more than 100 people suffered acute toxoplasmosis, was reported in Canada in 1994–1995. The outbreak was caused by a contaminated water supply that was chlorinated but not filtered.

Sources

Toxoplasma gondii is an obligate parasite and thus originates from the host animal. The only source of infectious oocysts is the faeces of members of the cat family, with domestic cats being the commonest source in most parts of the world. Infected cats shed very large numbers of oocysts in their faeces, but usually only for short periods (1–2 weeks). The oocysts persist in the environment for long periods and may be present in surface water and on fruit and vegetables grown in contaminated soil. Insect activity may also help to distribute the oocysts from contaminated soil.

The tissue cysts can be present in the flesh of any infected mammal and also in poultry. The most important source of tissue cysts for human infection is considered to be pig meat, but all other food animals are also potential sources of infection, although beef and veal are considered to present a much lower risk.

Stability in Foods

Toxoplasma oocysts are relatively resistant to most environmental factors. Oocysts have been reported to remain infectious for up to 400 days in water and also persist for long periods in soil. Sporulated oocysts are inactivated by freezing at $-21\,^{\circ}C$ for 28 days and unsporulated oocysts are also inactivated at

this temperature. Sporulated oocysts are reported to be gradually inactivated by drying.

The oocysts are relatively resistant to some sanitisers and disinfectants, and may not be inactivated by levels of chlorine normally used in drinking water treatment. *Toxoplasma* oocysts are not reported to be especially heat resistant and are likely to be destroyed by conventional milk pasteurisation.

Tissue cysts in meat are able to survive at refrigeration temperature (4 °C) for several weeks, but are not heat resistant and will be destroyed by proper cooking processes. Cysts in pork are reported to be killed in 44 s at 55 °C and in 6 s at 61 °C and a *D*-value of 1 s at 67 °C has been reported. However, rare-cooked meats may not achieve an internal temperature sufficient to kill all cysts.

Tissue cysts are also inactivated by freezing at temperatures of less than 10 °C and are destroyed by irradiation at a dose of 1 kGy. The cysts are thought to have some susceptibility to curing agents, such as salt, used in meat, but raw cured pork has been identified as a risk factor for human infection.

Control Options

Control of *Toxoplasma* in food is achieved principally by implementing good practice in meat production and by proper cooking of high-risk meats, such as pork.

Primary Production

Infection of pigs and other food animals by *Toxoplasma gondii* can be controlled to some extent by minimising potential exposure to cat faeces using best practice biosecurity measures. However, this is difficult to achieve for animals kept outdoors.

Fruit and vegetable growers should also adopt measures to exclude cats from fields where produce for human consumption is grown.

Processing

Good hygiene practice at slaughter and in meat processing is important to prevent cross-contamination between infected carcasses and *Toxoplasma*-free animals, since the cysts can be carried on the skin or on soiled equipment and utensils.

Tissue cysts in meat, especially in pork and mutton, are destroyed by heat and ideally all meat should be cooked to an internal temperature of at least 70 °C to ensure inactivation of cysts. Inactivation of cysts can also be achieved by freezing meat at −12 °C or less.

Fruit and vegetables should be washed thoroughly before consumption to remove occysts.

Hygiene

Good personal hygiene practice, especially hand washing, is an essential cross-contamination control when handling raw meat and is also important when preparing fruit and vegetables.

Vulnerable consumers, such as pregnant women and the immunocompromised should avoid direct contact with raw meat, especially pork.

Legislation

Toxoplasmosis is a notifiable disease in some developed countries, but *Toxoplasma gondii* is not usually mentioned specifically in food safety and hygiene law.

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1.3.2 NEMATODES

1.3.2.1 Anisakids

Hazard Identification

What are Anisakids?

The anisakids are a family of parasitic marine nematode worms that can cause a potentially serious infection (anisakiasis or anisakidosis) and allergic (hypersensitivity) reactions in humans following consumption of infected seafood. The principal species identified in human infection is *Anisakis simplex* (whale worm or herring worm), but the closely related species *Pseudoterranova decipiens* (seal worm or cod worm) may also be found in humans. Other related marine nematodes, such as *Contracaecum* species and *Hysterothylacium* species, have been implicated in human infections, but these have only very rarely been reported in developed countries.

Anisakids are found in the marine environment worldwide and have a very complex lifecycle involving a number of hosts. Humans are only an incidental host to the infective third stage (L3) larvae, which may occur in the viscera and muscle tissue of infected fish. The larvae rarely reach the adult stage in humans and are eventually expelled from the gut, or die in the tissues.

Occurrence in Foods

The infective L3 larvae of *Anisakis simplex* and other species occur in the viscera and muscle tissue of infected fish as small, but visible cysts containing the coiled, 2–3 cm long larva. There is evidence that the larvae migrate from the viscera into the muscle tissue when the intermediate host dies. A number of food fish species are known to act as intermediate hosts, including whitefish such as cod, whiting and haddock, herring, monkfish, mackerel and salmon. Some species of squid may also contain live larvae. Where infection is heavy, it may be obvious on examination of the fish flesh, especially in whitefish, but for fish with pigmented flesh the presence of the larvae may be much less obvious.

Fresh fish is the principal vehicle for *A. simplex* infection in humans, especially if it is eaten raw or undercooked. The larvae die quite quickly in fish that is frozen and do not survive effective cooking, and so processed fish and seafood products present a negligible risk of infection. However, the larvae may survive in some fermented, lightly salted, or cold-smoked and marinated fish products, such as pickled herrings and gravadlax. The growing trend for consumption of raw and lightly cooked fish, such as sushi and sashimi, in the West is thought to be increasing the likelihood of human infection with anisakid worms.

Wild fish are considered to carry a much higher risk of infection than farmed fish. Surveys of fish on sale in markets around the world generally show that a

significant proportion (approximately 10–30%) is infected with live L3 *A. simplex* larvae. However, a survey of Norwegian farmed salmon found no infected fish, even though the parasite is quite common in wild salmon. This may be because farmed fish are not able to feed on infected intermediate hosts, such as copepods and other small invertebrates.

Hazard Characterisation

Effects on Health

The gastrointestinal tract of humans resembles that of marine mammals (the definitive hosts) sufficiently for ingested live *A. simplex* and other anisakid larvae to survive for a short time, but most ingested larvae die in the gastrointestinal tract. However, in some cases they may cause a potentially serious acute infection known as anisakiasis, or anisakidosis. This occurs when the L3 larvae burrow into the wall of the digestive tract in the stomach or intestine and occasionally penetrate the gut wall completely, entering the body cavity. This process is often accompanied by severe abdominal pain, nausea and vomiting and the larvae may sometimes be coughed up. Symptoms usually occur within a few hours of ingestion. An inflammatory response is also produced, which occasionally leads to the formation of an abscess (eosinophilic granuloma) surrounding the worm. When this occurs in the intestine, symptoms similar to those of Crohn's disease (abdominal pain, diarrhoea and bleeding) may develop after 7–14 days. Abdominal pain can persist for several weeks until the larvae in the gut are expelled, or those that have penetrated the tissues die. In severe cases, the pain is extreme and may require surgical removal of the larvae.

Ingestion of the L3 larva of *A. simplex* can also cause a hyperimmune allergic reaction in some individuals. This may be associated with symptoms such as skin rashes (urticaria), asthma and even anaphylactic shock in a few cases. Cases of illness involving simultaneous infection and allergy (gastroallergic anisakiasis) have also been reported. Allergic reactions have been reported following exposure to very small quantities of *A. simplex* allergens, even in the absence of viable larvae.

Incidence and Outbreaks

The reported prevalence of anisakiasis has been increasing over the last 30 years, probably because of better diagnostic testing and a growing demand for raw and lightly cooked fish products in many developed countries. About 90% of the cases reported are from Japan, where approximately 2000 people suffer from the symptoms of anisakiasis each year. The annual number of cases in the EU is estimated to be about 70, with the highest incidences being recorded in Spain, the Netherlands and Germany. According to the USA Food and Drug Administration (FDA), about 10 cases of anisakiasis are

reported in the USA each year. Outbreaks have been reported in Japan, the Netherlands and Spain.

Although the global incidence of anisakiasis is quite low, there is evidence that exposure to the parasite is much higher in some countries. Many individuals who ingest *A. simplex* larvae do not develop acute symptoms, but may develop specific antibodies to the larvae. A survey of over 34 000 people with skin rashes, or symptoms of seafood allergy, in Japan found that almost 30% had antibodies specific to *A. simplex* in their blood. Similar findings have been reported from Spain. This appears to indicate a more widespread exposure in the population and suggests that allergy caused by *A. simplex* L3 larvae may be more common than expected. However, *A. simplex* allergy is highly cross-reactive with other allergies and is difficult to diagnose.

Sources

The definitive hosts for the adult worms are marine mammals, including whales and dolphins (*Anisakis*) and seals (*Pseudoterranova*), but the various larval stages infect intermediate hosts, including copepods and other small invertebrates, fish and squid. The adult worms live in the gut of marine mammals and eggs are expelled in the faeces. Free-swimming larvae hatch from the eggs once in the marine environment and may be eaten by small crustaceans. The larvae then develop into L3 third-stage larvae, which are infective to fish and squid that feed on the infected crustaceans. The larvae penetrate the gut of the infected fish and grow in the viscera, but appear to migrate to muscle tissue when the host dies. The lifecycle is then completed when the infected fish are consumed by marine mammals.

Anisakids are found in sea water worldwide, but are less common in fish populations in areas where marine mammals are rare. The rate of infection may also be seasonal and may be affected by water temperature. They do not occur in fresh water.

Stability in Foods

Infective L3 larvae are able to survive in the flesh of dead fish for some time, but are killed by freezing. They are not heat resistant and are killed by temperatures above 60 °C. However, they may survive cold-smoking, marinating and fermentation processes applied to fish.

A. simplex allergens are reported to be more heat stable than the larvae and may survive both cooking and freezing.

Control Options

The principal control for anisakid infections in wild fish is visual inspection. The larvae can be seen by ‘candling’ or inspection on a light table, but this is less effective for fish such as salmon that have pigmented flesh. It is

possible to physically remove the larvae, but obviously infected fish should not be consumed. Inspection cannot be guaranteed to detect all larvae in infected fish.

Processing

Since the larvae may migrate from the viscera of infected fish into the muscular tissue after death, it is important to ensure that fish are gutted as soon as possible after capture to minimise this migration.

Fish that will be eaten raw or lightly cooked should be frozen at −20 °C or less for at least 24 hours to kill the larvae. This should also apply to fish intended for to be cold-smoked, fermented, or marinated before consumption.

Hot-smoking processes where an internal temperature of at least 60 °C is attained will destroy the larvae, as will cooking to a temperature of 70 °C for at least two minutes.

However, the relative stability of *A. simplex* allergens means that neither cooking nor freezing can be relied upon as a control measure and cooked and frozen fish may still cause an allergic reaction.

Legislation

In the EU, legislation measures to protect consumers against anisakiasis are contained in European Commission (EC) Regulation No. 853/2004, which includes requirements for fishery products consumed raw or lightly processed. This legislation requires inspection of fish for parasites, and the removal of obviously infected fish from sale. Fish to be eaten raw must be frozen at −20 °C or less for at least 24 hours, as must certain species intended for cold-smoking, marinating or salting.

In the USA, the FDA Food Code recommends blast freezing to –35 °C followed by storage at −20 °C or less for at least 24 hours, or complete freezing to −20 °C for seven days, for fish intended for consumption without cooking.

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1.3.2.2 *Trichinella*

Hazard Identification

What is Trichinella*?*

Trichinella is a genus of parasitic nematode worms that can cause a potentially serious infection (trichinellosis or trichinosis) in humans following consumption of infected meat. *Trichinella* was first described as a cause of disease in man as early as 1865. Up to ten species (or genotypes) have been described, at least seven of which can infect man, but the principal species identified in human infection, and the species of most concern to the food industry is *Trichinella spiralis*. The other recognised species identified in human cases are *T. britovi*, *T. pseudospiralis*, *T. nativa*, *T. murrelli*, *T. papuae* and *T. nelsoni*, but these are less commonly found than *T. spiralis* and are usually associated with wild animals.

Trichinella species are found worldwide and infect a wide variety of animal hosts, mostly carnivorous and omnivorous wild mammals, especially those that scavenge, such as foxes, bears, pigs and wild boar. Rodents, such as rats and mice, are also thought to play an important role as hosts in areas where the infection is endemic. The entire lifecycle normally occurs within a single host species and consists of an adult worm and two larval stages. Humans are not definitive hosts, but may become infected by ingesting the infective second stage larvae, which may occur in cysts in the striated muscle tissue of infected animals.

Occurrence in Foods

The infective second stage larvae of *Trichinella* occur in the muscle tissue of infected animals as very small, but detectable, cysts containing the larva. *T. spiralis* cysts are found in highest numbers in the diaphragm and tongue of the infected animal but can also occur in the skeletal muscles. Historically, infected pork from pigs fed with feed containing animal waste was the principal source of *Trichinella* infection in the EU and North America, but successful controls in pork production have greatly reduced the prevalence of infection in commercial herds. The prevalence in commercial pig herds in the EU has been estimated at fewer than 1 in 100 000 animals, with some variation between countries. In the USA, the prevalence of infection in commercial production has been reduced from an estimated 1.41% in 1900, down to 0.013% (13 in 100 000 animals) in 1995.

However, there is still some risk from home-raised pigs and from pigs that are allowed to forage for food in the natural environment, which may include organically produced pigs. There is also a significant risk of infection from wild animals, especially wild boar in parts of the EU and bears in the USA. Imported horsemeat is also now a very significant source of infection in parts of the EU, especially France and Italy.

Raw, or undercooked meat is the principal vehicle for *Trichinella* infection in humans. The larvae do not survive effective cooking, and properly cooked pork and other meats present a negligible risk of infection. However, the larvae may survive in raw cured meats and some *Trichinella* species larvae are not killed by freezing. Therefore lightly processed and frozen pork or wild game products may still carry the risk of infection.

Hazard Characterisation

Effects on Health

The severity of trichinellosis infection in humans is highly variable. It may be asymptomatic in some cases, while in others complications may prove fatal. The severity of infection seems to be correlated with a number of factors, including the *Trichinella* species involved, the number of encysted larvae ingested and the strength of the immune response in the patient. The minimum infective dose is uncertain but has been estimated at between 100 and 300 live larvae.

After ingestion the larvae are released from the cysts by stomach acid and digestive enzymes and invade the lining of the small intestine, where they develop into adults. This process may be accompanied by gastrointestinal symptoms, including abdominal pain, vomiting and diarrhoea. Onset of symptoms typically occurs 24–48 hours after ingestion but may take longer. After about seven days the adult females release live larvae that migrate through the tissues to the striated muscles where they form cysts. This stage usually takes 4–8 weeks to complete and produces a different range of symptoms, including swelling of the face and around the eyes, fever, muscle pain, conjunctivitis and rashes. The production of the cysts usually causes muscle pain and weakness, but once it has been completed, the symptoms largely disappear.

However, in some cases potentially serious neurological and/or cardiovascular complications may occur, producing a variety of symptoms, such as headache, apathy, dizziness, chest pains and an irregular heartbeat. Rarely, complications may be fatal, especially in elderly people.

Incidence and Outbreaks

It has been suggested that as many as 11 million people worldwide could be affected by trichinellosis and an estimated 10 000 cases occur every year. However, the incidence of the disease in most EU and North American countries has been decreasing for many years. For example, in the USA between 1947 and 1951, the average number of reported cases each year was 393 and 57 people died from the disease. But from 2002 to 2007 the annual average was only 11 cases, with no deaths. In the EU, there has been a general downward trend in the incidence of trichinellosis over the last 12 years, and the number of reported cases has been stable since 2000. However, incidence varies considerably between different countries. In 2008, 670 confirmed cases were

reported in 13 countries, with the highest numbers being recorded in Romania (75%), Bulgaria (10%), Lithuania (4.7%) and Spain (4%). Many countries reported no cases, including the UK and Sweden. Elsewhere, relatively high incidences have been reported in Argentina (600 cases per year).

Many outbreaks of trichinellosis have been reported all around the world. In the EU there have been significant outbreaks in the last 20 years. Most of these have occurred in Spain, France, Italy and Germany and were caused either by horsemeat imported from third countries, wild boar, or non-intensively raised pigs. An outbreak affecting 124 people in Poland in 2003 was believed to have been caused by wild boar meat and a large outbreak in Romania in 2008, in which 108 people needed hospital treatment, was associated with pork sold without veterinary control. However, 52 cases reported in Germany in 1998–1999 were linked to commercially produced raw sausages and minced meat.

Outbreaks in the USA have also been reported. In 1990, 105 people were affected in two outbreaks associated with raw sausages made from commercially produced pork. However, since that time, most outbreaks have involved foods prepared from wild game meat, including wild boar and bear.

Sources

Two distinct cycles for *Trichinella* are recognised by epidemiologists. The natural, or sylvatic cycle occurs in wild animals, especially carnivores that scavenge or exhibit cannibalistic behaviour. In this cycle, a number of the recognised *Trichinella* species are involved. The parasites develop in one host and infective encysted larvae are passed to another when infected tissues are consumed. In the domestic cycle, *Trichinella* (most commonly *T. spiralis*) circulate in farm raised pigs that are fed with feed containing infected animal tissue, or are allowed to come into contact with other infected animals.

The domestic cycle is now much less important in developed countries than was once the case, following improvements in pig husbandry and in statutory controls. For example, in the USA between 1997 and 2001, 72 cases of trichinellosis were reported and only 12 of these were associated with commercial pork products. The remaining cases were caused by consuming wild game, or pork raised under unregulated conditions. In the EU, the most important sources of trichinellosis are now wild boar meat, and horsemeat imported from the Eastern EU. Some EU countries, including the UK, Ireland and Sweden, have not reported cases of human trichinellosis caused by locally produced meat products for at least 20 years.

Stability in Foods

The encysted larvae of *Trichinella* species are extremely persistent in the live host and may survive for many years in striated muscle tissue. Encysted larvae of *T. spiralis* are not resistant to freezing and are killed by rapid freezing and storage at $-20\,^{\circ}C$ or below for at least 48 hours. However, this may not be the case for other species of *Trichinella*. Infective *Trichinella* species larvae have been found in frozen bear meat after storage for more than two years.

The larvae may also be able to survive some curing processes used for pork products. They are not heat resistant and are killed by temperatures above 60 °C for 2 min.

Control Options

The principal control for *Trichinella* in commercial meat products is inspection by a recognised direct detection method, usually tissue digestion followed by microscopic examination of the remaining sediment. This is mandatory for pork, horsemeat and game in the EU and in other developed countries. Infected meat is designated unfit for human consumption.

Primary Production

Improved animal husbandry has been very effective in reducing *Trichinella* infection in commercial pig herds. Measures include ensuring that all pig feed is adequately heat-processed to destroy infective larvae, effective separation of pigs from rodents and other potentially infected animals and good on-farm hygiene practices.

Processing

The larvae of *T. spiralis* can be destroyed by freezing, cooking and by some curing procedures. The USDA has produced specific freezing and cooking times and temperatures for pork products and has also specified curing methods. Freezing times and temperatures are dependent on the size of the pieces of meat involved, but for cooking processes, fresh pork should reach a minimum internal temperature of 71 °C. The EU has also specified several freezing treatments that can be used to kill *T. spiralis* larvae in meat. These are detailed in the relevant legislation (see below).

Freezing cannot be relied upon to destroy the larvae of other *Trichinella* species that may be found in game meat and horses.

Legislation

In EU legislation, measures to protect consumers against trichinellosis are contained in a EC Regulation No. 2075/2005. This covers inspection of meat at slaughter, detection methods and freezing procedures.

The USA Code of Federal Regulations contains similar requirements and includes recommendations for freezing, cooking and curing of pork products.

Many countries have introduced legislation regulating aspects of animal husbandry, meat inspection and pork processing designed to protect consumers from trichinellosis.

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1.3.3 OTHER PARASITES

There are a large number of parasites that can cause human infection and many have the potential to be food borne. However, most of these are now found only in tropical and sub-tropical regions, or in areas where standards of sanitation are poor. They are rarely found in developed countries, where infection is only likely to occur in people who have travelled to areas where these parasites are endemic. The preceding sections have dealt with those parasites that are known to present a food-borne risk to public health in developed countries, but there are certain other species that may present a food safety risk as a consequence of the growing globalisation of food supply chains.

Brief details are given below of some parasites that may have food safety significance. All are known to infect humans and may occur as contaminants in certain food commodities.

Protozoa

Balantidium coli

Balantidium coli is a large (70 μm diameter) ciliate protozoan parasite that is normally associated with pigs, although other mammals, including rodents and non-human primates, may also act as reservoirs of infection. It occurs worldwide, but is most commonly reported in areas where pigs are raised in unsanitary conditions. Balantidiasis is endemic in some countries, such as Bolivia and the Philippines.

The infective stage in the lifecycle is a cyst, which is passed in the host's faeces and may be present as a contaminant in polluted water or on food that has been contaminated by human or animal faeces. After ingestion, the cysts rupture to release trophozoites that colonise the large intestine and may invade the wall of the colon. Most cases of human infection are asymptomatic, but where symptoms occur, they generally include persistent diarrhoea, abdominal pain and weight loss. The illness resembles amoebic dysentery and can be severe, or even fatal in some cases.

Control of *Balantidium coli* infection can be achieved by effective water sanitation measures and good food hygiene practices.

Sarcocystis

Sarcocystis species are coccidian parasites that have a complex lifecycle requiring two hosts, one a definitive predatory host and the other an intermediate prey species host. A number of species associated with specific hosts have been described and several of these can infect humans, including *S. hominis* and *S. suihominis*. *Sarcocystis* species have a widespread distribution and are common parasites in commercially raised cattle and pigs.

Humans can become infected by ingestion of infective oocysts excreted in the faeces of the definitive host, or by consumption of the meat of an intermediate host containing encysted larvae (sarcocysts). Consumption of raw or undercooked pork or beef containing sarcocysts may result in gastrointestinal illness with symptoms including nausea, diarrhoea and abdominal pain lasting for 24–48 hours. In rare cases the parasites may invade the body causing a variety of more serious symptoms, including inflammation of muscular and vascular tissue, abortion and congenital disorders. Human outbreaks have been recorded in the EU and North America.

Controls include inspection of meat for the presence of sarcocysts, and effective cooking of beef and pork before consumption.

Nematodes

Ascaris

Ascaris lumbricoides is a very common nematode parasite, for which humans are the host. Infection is endemic in many developing countries, and it is estimated that 25% of the world's population may carry the infection.

The adult worms live in the intestine of the host and produce eggs that are passed out of the body in faeces. The eggs may be ingested in polluted water, or on foods contaminated with human faeces by irrigation or washing with polluted water. The ingested eggs hatch in the intestine and the larval stages may migrate to other tissues, including lungs and liver before they return to the intestine and mature. Many infections are asymptomatic, but the intestinal mucosa may be irritated, causing diarrhoea and affecting protein uptake. Very young children may suffer from diarrhoea and stunted growth if infected soon after birth. When tissue invasion occurs, infection of the liver or lungs can produce a severe acute illness.

Control of *Ascaris* infection can be achieved by proper water sanitation and good hygiene practice in food preparation.

Trematodes (Flukes)

Fasciola hepatica

Fasciola hepatica is a parasitic liver fluke that commonly infects cattle and sheep in many developing countries. This parasite has a complex lifecycle involving a larval stage in water snails. It also causes human infection in areas where water sanitation is inadequate, especially in parts of South America and North Africa. Large outbreaks have also occurred in the Middle East. Cases may sometimes occur in developed countries following consumption of contaminated fresh produce, especially watercress and other green vegetables grown in or near contaminated water. There may be some risk from imported salad greens.

Humans become infected when they ingest cysts in contaminated water or food. The cysts hatch and develop into adult flukes that inhabit the liver. Symptoms of infection include fever, abdominal pain and weight loss and there is come evidence for a link with liver tumours.

Control of *Fasciola hepatica* can be achieved largely by adequate water sanitation.

Paragonimus (Lung Fluke)

There are at least nine species of *Paragonimus* lung flukes that can infect the lungs of humans and other animals, including pigs, dogs and cats. They have a widespread distribution and a complex lifecycle with at least two intermediate hosts, including freshwater snails and crabs, or crayfish. They may also infect other animals that feed on crustaceans.

Humans usually become infected by eating raw, or undercooked, crustaceans, but wild boar meat has also been implicated in human infection in Japan. Infection is usually followed by gastrointestinal symptoms of diarrhoea, fever and abdominal pain. Later, coughing and chest pains may occur as the immature worms pass through the diaphragm and into the lungs. If large numbers of worms are ingested, they can cause chronic lung disease and may enter the central nervous system. In rare cases, infection can be fatal.

Paragonimus is quite resistant and is not destroyed by salting or pickling, but control can be achieved by adequate cooking of crabs and crayfish.

Cestodes (Tapeworms)

Taenia

Taenia species are tapeworms that parasitise a number of animals. Humans are the definitive hosts for two species, *Taenia solium* (the pork tapeworm) and *Taenia saginata* (the beef tapeworm), and are commonly infected by both. Other species have been reported to infect man on rare occasions. The intermediate hosts for *T. saginata* are cattle and pigs act as the intermediate host for *T. solium*, although some other species may be infected. Both species have a widespread distribution and human infection is common in areas where sanitation is inadequate. *T. solium* is rare in countries where pork is not eaten for religious reasons. It has been estimated that as many as 50 million people worldwide could be infected by both species each year.

Intermediate hosts of *Taenia* species become infected by the ingestion of eggs in human faeces. These hatch in the gut, producing larvae that migrate to the muscles and other tissues and form persistent cysts (cysticerci). Humans become infected by eating raw, or undercooked meat from an infected animal and ingesting viable cysticerci. Once in the human gut, these develop into the long-lived adult, which grows to a length of several metres and produces a continuous supply of eggs in the faeces. Infection may be asymptomatic, or

may be accompanied by a range of symptoms, such as abdominal pain, constipation, or diarrhoea.

Humans can also serve as the intermediate host for *T. solium* if eggs are ingested. This can have serious, or even fatal, consequences as the larvae encyst in the tissues. Cysticercosis can affect the eyes and the brain and may cause various neurological symptoms, including severe pain, convulsions and paralysis. It has been estimated that cysticercosis may cause as many as 50 000 deaths each year worldwide.

Control of *Taenia* species in most developed countries has been achieved by improved sanitation and animal husbandry practices, together with effective meat inspection and adequate cooking processes, especially for pork.

Diphyllobothrium

Diphyllobothrium species are usually associated with freshwater fish and are often referred to as the fish tapeworms. *Diphyllobothrium latum* is the species most commonly associated with humans, who are one of the definitive hosts for the parasite, along with other fish eating mammals such as bears. It has a complex lifecycle, often involving several intermediate hosts, including copepods, small freshwater fish and larger predatory fish, such as pike and perch. It is common in some temperate regions of the Northern hemisphere, such as the Great Lakes of North America, the Baltic and Russia. Infection is most common in countries where raw freshwater fish is eaten, such as Finland and Japan.

Humans become infected by eating raw, or undercooked, fish infected with *D. latum* larvae (plerocercoids). The plerocercoids develop into adult worms in the human gut and can grow very large (up to 10 m in length). Infection is often asymptomatic, but may be accompanied by various symptoms, such as weight loss, abdominal pain and a type of anaemia. In some individuals, multiple infections with many worms can occur. Symptoms are more likely in these cases.

Control of *Diphyllobothrium* can be achieved by proper cooking of freshwater fish to kill the plerocercoids before consumption.

Echinococcus

There are four recognised species of *Echinococcus*, small tapeworms that normally parasitise members of the dog family. Two of these are of importance in human health in developed countries. *E. granulosus* is a tapeworm of dogs that can cause potentially serious disease (echinococcosis, or hydatid disease) in humans. Intermediate hosts are usually cattle, sheep and other grazing animals. The definitive host of *E. multilocularis* is the fox and the intermediate hosts are usually rodents. This species causes rare, but highly pathogenic alveolar echinococcosis in humans. *E. granulosus* is prevalent in many parts of the world, especially areas where animals are grazed, but *E. multilocularis* is largely restricted to the Northern hemisphere. Human echinococcosis is regularly reported in the Southern EU, notably in Spain, Greece, Italy and Portugal and

is also quite common in some parts of the Eastern EU. Cases are also occasionally reported in North America.

Humans become infected when they ingest the eggs of the tapeworm in contaminated water, or on unprocessed vegetables. The eggs hatch in the gut, releasing a larval stage called an oncosphere that penetrates the gut wall and migrates to other tissues, especially the liver and lungs. Once in place the oncospheres form cysts that gradually enlarge and produce daughter cysts. Symptoms are slight at first, but as the cysts grow, their size may eventually cause pain and other symptoms. Hydatid cysts caused by *E. granulosus* may finally rupture, causing hypersensitivity reactions, including anaphylactic shock, and the dissemination of new cysts. In alveolar echinococcosis caused by *E. multilocularis*, the cysts invade the tissues, usually the liver, in the same way as a slow-growing destructive tumour. Alveolar echinococcosis is normally fatal if not treated.

Control of *Echinococcus* species can be achieved by the proper destruction of the viscera of infected intermediate host species and by effective hygiene measures when washing and preparing vegetables. There is some concern that growing red fox numbers in the EU may cause an increase in cases of alveolar echinococcosis.

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CHAPTER 1.4

Prions

Hazard Identification

What are Prions?

The term prion (pronounced ''pree-on'') is now used as a generic term for a small group of small glycosylated proteins found mainly in the brain-cell membranes of humans and other mammals. The name was first used by Stanley Prusiner in 1982 to describe the infective agent for a group of invariably fatal diseases known as transmissible spongiform encephalopathies (TSEs), so called because of the 'sponge-like' appearance of the brain in the later stages of the disease. The word prion was derived from the term ''proteinaceous infectious particle.'' The role of prions in human disease is still the subject of some controversy, but the consensus of scientific opinion is that abnormal forms of these proteins can act as unconventional infective agents that can replicate without associated DNA or RNA, and are therefore not a form of life in the accepted sense.

Normal non-infective prions are benign, and like other proteins in that they have a three-dimensional α-helical structure. Infective prions differ in that their structure is flattened into a form referred to as a β-sheet. These abnormal proteins are much less soluble than the normal version and much more resistant to enzymes. The hypothesis for prion infectivity proposes that when these abnormal proteins reach the brain, they are able to cause the normal proteins to change their shape, so that they too assume a β-sheet structure. These altered prions then also become infective, resulting in a progressive change in conformation of the normal prion proteins in brain-cell membranes. This leads to a change in brain structure and function that are characteristic of TSEs. The evidence for this hypothesis is strong and growing steadily.

A number of spongiform encephalopathies have been described, affecting a wide range of animals, including humans, cattle, sheep and goats (scrapie), deer

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(chronic wasting disease), elk, cats and mink. Most of these conditions occurring in humans, such as classic Creutzfeldt–Jakob disease (CJD) are considered to be inherited genetic diseases, or caused by sporadic mutations. However, a few are thought to be transmissible by ingestion of an infective agent (probably a prion), and it is these TSEs that are of concern in food safety terms.

By far the best known and the most significant of these is variant Creutzfeldt–Jakob disease (vCJD), a condition first described in 1996, which is now widely considered to be the human form of bovine spongiform encephalopathy (BSE, or ''mad cow disease''), a TSE found in cattle. The hypothesis is that human cases of vCJD may have been caused by the ingestion of infective prions in meat from BSE-infected cattle in the food chain. The possibility that some prions are able to cross the species barrier is a major concern.

This section will focus on BSE and vCJD as there is no evidence to suggest that the causative agents of other TSEs have caused disease in humans.

Occurrence in Foods

The infective prion thought to be the causative agent for foodborne vCJD in humans is present in certain tissues of cattle suffering from BSE. High levels of BSE prions are known to occur in the central nervous system, particularly in the spinal cord and the brain. Lower levels are also considered to be present in other tissues, such as the tonsils, eyes, large and small intestines, mesentery, skull and vertebral column. These tissues are now known collectively as ''specified-risk material'' (SRM) and are not allowed to enter the food supply in many countries. Before the introduction of BSE controls, some of these materials were present in meat products, such as mechanically recovered meat (MRM), used in some low-grade beef products, including pies and burgers. BSE prions have not been detected in bovine milk.

Hazard Characterisation

Effects on Health

The disease vCJD differs markedly from classic CJD in terms of age of those affected and length of the illness. vCJD affects younger individuals, with the average age being 29 years (classic CJD is 65 years). For vCJD, the usual duration of the illness until death is on average 14 months, whereas for classic CJD it is much shorter, usually 4.5 months on average.

The minimum infectious dose of BSE prions needed to cause vCJD in humans is unknown. However, it is known that the infectious oral dose of the BSE agent for cattle is ≤ 1 g homogenised infected brain tissue, but it is difficult to establish the effect of the species barrier has on the infectivity of the agent from cattle to humans. These experiments cannot be conducted for obvious reasons and the infectivity of various bovine materials is still the subject of investigation.

The incubation period for vCJD is also unknown but has been suggested to vary from a few years to more than 25 years (average 15 years). It is thought that some individuals have a genetic factor that may make them more susceptible to infection and to rapid onset of the disease. All of those who have so far died of vCJD in the UK were found to have this factor.

Early symptoms of the disease include psychiatric symptoms such as behavioural changes, depression or schizophrenia-like psychosis. About 50% of affected individuals experience unusual sensory symptoms, *e.g.* stickiness of the skin. As the disease progresses, patients experience unsteadiness and difficulty walking, as well as involuntary movements. Eventually the patient is totally immobile and mute. There is no cure for vCJD and the prognosis for all patients displaying clinical symptoms of this progressive disease is eventual death.

Incidence and Outbreaks

Although the disease was first described in 1996, the first patient to develop the disease of what is now known as vCJD became ill in 1994. The majority of cases of vCJD worldwide are in individuals who live in, or have lived in, the UK, reflecting the fact that the UK is the country where the population has had the highest exposure to BSE prions.

By March 2011 the UK had reported 172 cases of vCJD, 168 of whom have died. Elsewhere in the world, a further 48 cases of vCJD have been reported, of whom 47 have died. Some of these cases had a history of visiting or living in the UK. France has the second highest number of reported cases of vCJD in the world (25 as of March 2011) and imported relatively large quantities of cattle products from the UK before the introduction of import restrictions.

Sources

The first outbreak of BSE in cattle was recognised in the UK in 1986, but the first cases probably occurred at least a year earlier. The original source of the disease has been suggested as being scrapie-infected meat-and-bone meal (MBM) used as a protein supplement in cattle feed, but this has not been confirmed. However, it is thought that the practice of feeding MBM made from infected cattle to young calves may have amplified the outbreak and accelerated its spread. It is estimated that a total of more than two million cattle in the UK have been infected with BSE, and that at least 750 000 of these were slaughtered and potentially consumed by the UK population between 1980 and 1996, when BSE controls were introduced.

Since the first identification of BSE in the UK in the 1980s, other countries have also reported BSE in cattle, in many cases probably caused by the importation of contaminated feed or infected animals. An additional 24 countries have reported BSE in cattle to date, including many EU countries, Japan, Israel, Canada and the USA. However the current incidence of BSE in these countries is far lower than that reported in the UK.

Growth and Survival Characteristics

Infective prions are only capable of 'replicating' in the tissue of the host. However, they have been found to be extremely resistant to a wide range of environmental factors, including heat, chemical sterilants, extremes of pH and radiation. For example, the long-term infectivity of prions in rendered MBM made from diseased animals is a demonstration of their stability.

Thermal Resistance

The heat resistance of infective prions is considerable. At high temperatures, the survival of infectivity is greater in dry conditions (dry heat at $<300\,^{\circ}C$ cannot be guaranteed to inactivate infective prions), but experiments have shown that if large amounts of infective material are present, a heat treatment of 133 °C for 20 min under 3 bar of pressure may still be inadequate even at high moisture contents. A heat treatment of 140 °C for 30 min at 3.6 bar pressure has been suggested as an alternative.

Control Options

The control of vCJD in humans is inextricably linked to the control of BSE in cattle. Attempts to eradicate the disease in cattle in affected countries have focussed on banning the use of protein derived from ruminants in all farmed animal feed. This was introduced in 1988 and enhanced in 1994 and 1996, and has been very successful in restricting the spread of the disease in cattle in the UK, with the result that there were only 11 cases of BSE-infected cattle reported in the whole of 2010. At the peak of the epidemic, more than 850 cows were diagnosed with BSE every week.

The other main thrust of vCJD/BSE control is to protect consumers from exposure to BSE-infected materials. To this end, since 1989, a wide range of statutory controls have been introduced in the UK and other affected countries designed to prevent SRM from entering the food chain. Between 1996 and 2005, cattle of more than 30 months of age were also banned from the food chain in the UK, following the discovery that older animals are more likely to develop the disease. A comprehensive programme of BSE testing at slaughter has now been introduced in the EU, and older animals that test negative can be allowed to enter the food chain.

A very wide range of BSE controls (usually mandatory) have been implemented in affected countries. These are beyond the scope of this book and readers are referred to the web links below and to their national food safety and animal health authorities for specific details of controls that apply at each stage in the meat supply chain.

Legislation

A substantial and ever-changing raft of legislation designed to control BSE in cattle and to protect the public from exposure to BSE-infected materials has

been introduced in the EU, North America and other affected countries. The specific BSE legislation is beyond the scope of this book and readers are referred to some of the web links below and to their national food safety and animal health authorities for information on current legislation.

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Section 2: Chemical Hazards

CHAPTER 2.1

Biological Toxins

2.1.1 FUNGAL TOXINS

2.1.1.1 Aflatoxins

Hazard Identification

What are Aflatoxins?

The aflatoxins are a group of chemically similar toxic fungal metabolites (mycotoxins) produced by certain moulds of the genus *Aspergillus* growing on a number of raw food commodities. Aflatoxins are highly toxic compounds and can cause both acute and chronic toxicity in humans and many other animals. Their importance was first established in 1960 when 100 000 turkeys and other poultry in the UK died in a single event. The cause of this was eventually traced to a toxic contaminant in groundnut meal used in the bird's feed. The contaminant was later named aflatoxin.

The aflatoxins consist of about 20 similar compounds belonging to a group called the difuranocoumarins, but only four are naturally found in foods. These are aflatoxins B_1, B_2, G_1 and G_2. Aflatoxin B_1 is the most commonly found in food and also the most toxic. When lactating cattle and other animals ingest aflatoxins in contaminated feed, toxic metabolites can be formed and may be present in milk. These hydroxylated metabolites are termed aflatoxin M_1 and M_2 and they are potentially important contaminants in dairy products.

Occurrence in Foods

Aflatoxins may be present in a wide range of food commodities, particularly cereals, oilseeds, spices and tree nuts. Maize, groundnuts (peanuts), pistachios, Brazil nuts, chillies, black pepper, dried fruit and figs are all known to be high-risk foods for aflatoxin contamination, but the toxin has also been detected in

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many other commodities. Milk, cheese and other dairy products are also known to be at risk of contamination by aflatoxin M. The highest levels are usually found in commodities from warmer regions of the world where there is a great deal of climatic variation.

It is important to recognise that, although it is primary food commodities that usually become contaminated with aflatoxins by mould growth, these toxins are very stable and may pass through quite severe processes. For this reason they can be a problem in processed foods, such as peanut butter.

Hazard Characterisation

Effects on Health

At high enough exposure levels, aflatoxins can cause acute toxicity, and potentially death, in mammals, birds and fish, as well as in humans. The liver is the principal organ affected, but high levels of aflatoxin have also been found in the lungs, kidneys, brains and hearts of individuals dying of acute aflatoxicosis. Acute necrosis and cirrhosis of the liver is typical, along with haemorrhaging and oedema. LD_{50} (lethal dose) values for animals vary between 0.5 and 10 mg per kg of body weight.

Chronic toxicity is probably more important from a food safety point of view, certainly in more developed regions of the world. Aflatoxin B_1 is a very potent carcinogen and a mutagen in many animals, and therefore potentially in humans, and the liver is again the main target organ. Ingestion of low levels over a long period has been implicated in primary liver cancer, chronic hepatitis, jaundice, cirrhosis and impaired nutrient conversion. Aflatoxins may also play a role in other conditions, such as Reye's syndrome and kwashiorkor (a childhood condition linked to malnutrition). Less is known about the chronic toxicity of aflatoxin G_1 and M_1, but these are also thought to be carcinogens, though probably a little less potent than B_1.

Little is known about the level of dietary exposure to aflatoxins necessary to affect health, especially in humans, and diagnosis of chronic toxicity is very difficult. It is generally agreed that the best approach is to minimise the levels in all foods as far as is technically possible and to assume that any dietary exposure is undesirable.

Incidence and Outbreaks

The incidence of chronic aflatoxicosis in humans is unknown and is almost impossible to estimate because the symptoms are so difficult to recognise. However, human liver cancer is quite common in parts of the world where aflatoxin contamination of food is likely and there may be a link, although this remains unproven.

Acute human aflatoxicosis is rare, especially in developed countries, where contamination levels in food and monitored and controlled. However, there have been outbreaks in some developing countries, notably in sub-Saharan Africa, where maize and groundnuts can be an important part of the diet and

where the climate is suitable for rapid mould growth on crops in the field and in storage.

A notable outbreak occurred in India in 1974 when almost 400 people became ill with fever and jaundice after eating maize contaminated with between 0.25 and 15 mg kg^{-1} aflatoxin and more than 100 died. Major outbreaks have also occurred in Kenya, the largest in 2004 when 317 people were affected and 125 died, probably as a result of eating contaminated maize.

Sources

Aflatoxins are produced by at least three *Aspergillus* species. These are *A. flavus*, *A. parasiticus* and the much more rare *A. nomius*. These moulds are able to colonise a wide range of crops both in the field as non-destructive pathogens and in storage and can grow and produce aflatoxins at quite low moisture levels (approximate minimum $A_w = 0.82$) and over a broad temperature range (13–37 °C).

Their growth is strongly influenced by climate and, although they are found all over the world, they are more common in tropical regions with extreme variations in temperature, rainfall and humidity. *A. flavus* invasion of groundnut crops in the field is known to be favoured by drought stress and maize crops are vulnerable if damaged by insect pests.

Mould growth and aflatoxin production during storage of crops is also important, especially if drying is inadequate, or storage conditions allow access for insect or animal pests.

Stability in Foods

Aflatoxins are quite stable compounds and survive relatively high temperatures with little degradation. Their heat stability is influenced by other factors, such as moisture level and pH, but heating or cooking processes cannot be relied upon to destroy aflatoxins. For example, roasting green coffee at 180 °C for 10 min gave only a 50% reduction in aflatoxin B_1 level.

The stability of aflatoxin M_1 in milk fermentation processes has also been studied and although appreciable losses do occur, significant quantities of the toxin were found to remain in both cheese and yoghurt.

Aflatoxins can be destroyed by alkaline and acid hydrolysis and by the action of oxidising agents. However, in many cases, the resulting by-products also carry a risk of toxicity, or have not been identified.

Control Options

The ability of aflatoxin-producing fungi to grow on a wide range of food commodities and the stability of aflatoxins in foods mean that control is best achieved by measures designed to prevent the contamination of crops in the field and during storage, or detection and removal of contaminated material from the food supply chain.

Pre-harvest

Pre-harvest control of aflatoxins is best achieved through general Good Agricultural Practice (GAP) to include such measures as:

- Land preparation, crop waste removal, fertiliser application and crop rotation
- Use of fungus- and pest-resistant crop varieties
- Control of insect pests
- Control of fungal infection
- Prevention of drought stress by irrigation
- Harvesting at the correct moisture level and stage of maturity

Post-harvest Handling and Storage

The most important and effective control measure in post-harvest handling and storage is the control of moisture content and hence, the water activity of the crop. Ensuring that susceptible crops are harvested at a safe moisture level, or are dried to a safe level immediately after harvest is vital to prevent mould growth and aflatoxin production during storage. The safe moisture level varies between crops—for maize it is approximately 14% at 20 °C, but for groundnuts it is much lower, about 7%. These moisture levels must be maintained during storage and transport.

It is also important to ensure that the moisture content does not vary too much in a bulk-stored crop. Small localised 'wet spots' can develop mould growth and these can extend to neighbouring areas as the fungus produces metabolic water during respiration. Insect and animal pest damage can also act as focal points for fungal growth.

Decontamination

Physical separation of contaminated material can be an effective means of reducing aflatoxin levels in contaminated commodities. For example, colour sorting is often used to remove mouldy peanuts from bulk shipments. Density segregation, mechanical separation and the removal of fines and screenings from grain and nut shipments can also be effective measures.

Chemical decontamination methods have been investigated, especially for material used in animal feed, but most of the methods investigated are impractical, or produce toxic by-products. So far, an ammoniation process has shown the most promise and has been successfully used to remove aflatoxins from feed in the USA.

Biological decontamination has also been considered, and a single bacterial species, *Flavobacterium aurantiacum*, has been shown to remove aflatoxin B_1 from peanuts and corn.

Although decontamination methods for aflatoxin M_1 in milk and dairy products have also been investigated, most of these are not practical for the

dairy industry. The only really effective control is to minimise the contamination of materials used in animal feed for dairy cows.

Testing

Many countries monitor imported commodities that are susceptible to aflatoxin contamination, such as pistachios and Brazil nuts, by sampling and analysis. A number of analytical methods have been developed based on TLC, HPLC and ELISA and there are also rapid screening kits available. However, moulds and aflatoxins in bulk food shipments tend to be highly heterogeneous in their distribution and it is essential to ensure that an adequate sampling plan is used to monitor imported materials.

In some commodities, such as figs, aflatoxins fluoresce strongly under UV light and this can be used as a rapid screening test for high concentrations.

Legislation

Around 100 countries around the world have regulations governing aflatoxins in food and most include maximum permitted, or recommended levels for specific commodities.

EU

The EU sets limits for aflatoxin B_1 and for total aflatoxins (B_1, B_2, G_1 and G_2) in nuts, dried fruits, cereals and spices. Limits vary according to the commodity, but range from 2–12 μg kg^{-1} for B_1 and from 4–15 μg kg^{-1} for total aflatoxins. There is also a limit of 0.050 μg kg^{-1} for aflatoxin M_1 in milk and milk products. Sampling and analytical methods are also specified.

Limits of 0.10 μg kg^{-1} for B_1 and 0.025 μg kg^{-1} for M_1 have been set for infant foods.

USA

USA food safety regulations include a limit of 20 μg kg^{-1} for total aflatoxins (B_1, B_2, G_1 and G_2) in all foods except milk and a limit of 0.5 μg kg^{-1} for M_1 in milk. Higher limits apply in animal feeds.

Others

Both Australia and Canada set limits of 15 μg kg^{-1} for total aflatoxins (B_1, B_2, G_1 and G_2) in nuts. This is the same as the international limit recommended for raw peanuts by the Codex Alimentarius Commission.

More information can be found at the FAO web link below.

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2.1.1.2 Citrinin

Hazard Identification

What is Citrinin?

Citrinin is a toxic fungal metabolite (mycotoxin) produced by some moulds of the genera *Penicillium*, *Aspergillus* and *Monascus* growing on certain food commodities, especially cereals and fruit. It was first isolated from a culture of *Penicillium citrinum* in 1931. Citrinin exhibits a number of toxic effects in animals and its presence in food is undesirable.

Citrinin is a relatively small molecule ($C_{13}H_{14}O_5$, CAS No. 518-75-2) and is slightly soluble in water.

Occurrence in Foods

Citrinin has been found in a range of cereals, including rice, wheat, barley, maize, rye and oats. Co-occurrence with ochratoxin A in cereals has been reported. It has also been found in wheat flour and there is some evidence that it may survive to some extent in processed cereal products. Citrinin has also been found in peanuts and in mouldy fruit.

Citrinin also occurs in some fermented foods that are susceptible to surface mould growth, such as cheeses and fermented sausages. There is evidence that it may penetrate two or more centimetres into cheese showing surface mould growth. Recently citrinin has also been found in certain vegetarian foods that have been coloured with pigments derived from *Monascus* species fungi.

It is likely that the occurrence of citrinin in foods is under-reported, since it is not often looked for and has a tendency to partially degrade during analysis.

Hazard Characterisation

Effects on Health

Most of the information on the toxicity of citrinin is derived from animal studies and there is little or no experimental, or epidemiological, data on acute or chronic toxicity in humans.

At relatively high doses, citrinin is acutely nephrotoxic in mice and rats, rabbits, pigs and poultry causing swelling and eventual necrosis of the kidneys and affecting liver function to a lesser extent. LD_{50} values (lethal dose) are variable, but values of about 50 mg per kg of body weight have been reported for oral administration in rats.

The International Agency for Research on Cancer (IARC) has reviewed the available data and concluded that there is limited evidence for carcinogenicity in animals.

It has been proposed that citrinin may be implicated in human disease, such as ‘yellow rice’ disease in Japan and Balkan Endemic Nephropathy, when present with other mycotoxins, especially ochratoxin A.

Sources

Citrinin is produced by at least 12 species of *Penicillium*, including *P. citrinum*, some strains of *P. camembertii* (used in cheese production) and *P. verrucosum*, which also produces ochratoxin A. Some *Aspergillus* species, such as *A. terreus* and *A. niveus* are also reported to produce citrinin and the toxin has also been detected in cultures of *Monascus ruber* and *Monascus purpureus*, used to make red pigments.

P. citrinum has been isolated from a very wide range of food commodities worldwide. It is able to grow in a temperature range of 5–37 °C and at water activity values as low as 0.80.

Stability in Foods

Citrinin is not particularly stable and is degraded by heat and by alkaline conditions. There is little published information on the fate of citrinin during food processing, but it seems unlikely that it persists in significant amounts in bakery products and other processed cereal foods. However, there is some evidence that toxic breakdown products may be formed when citrinin degrades in wet environments.

Citrinin is unlikely to survive the brewing process and more than 90% is reported to be destroyed during barley germination, with the remainder being degraded during mashing.

Citrinin produced by mould growth on cheese appears to be quite stable with more than 50% still being present after storage for eight days.

Control Options

There are few specific documented control measures for citrinin, but its co-occurrence with ochratoxin in cereals means that the pre- and post-harvest control measures recommended for ochratoxin may also provide indirect control of citrinin.

Processing

Control of citrinin in fermented foods, such as cheese and sausage can be achieved by good hygienic practice to prevent surface contamination and growth of toxin-producing mould species. Where potentially citrinin-producing species of *Penicillium* or *Aspergillus* (*e.g. P. camembertii*) are used in the production of fermented foods, it is important to select non-toxin-producing strains as starter cultures.

Cheese that has undergone surface mould spoilage is often trimmed to remove mould growth before sale, but it is important to remember that some citrinin may still be present in the surface layers of trimmed cheese.

Testing

Quantitative analysis of citrinin in agricultural products down to levels of about 10 ppb can be achieved using HPLC methods, but it is important to

ensure that degradation does not occur during analysis. There are also screening methods based on ELISA techniques.

Legislation

There are no current specific regulations setting mandatory or recommended maximum limits for citrinin in food or feed.

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On the Web

European Mycotoxin Awareness Network. http://www.mycotoxins.org/

2.1.1.3 Cyclopiazonic Acid

Hazard Identification

What is Cyclopiazonic Acid?

Cyclopiazonic acid ($C_{20}H_{20}N_2O_3$, CAS No. 18172-33-3) is a toxic fungal metabolite (mycotoxin) produced by some moulds of the genera *Penicillium* and *Aspergillus* growing on a wide range of food commodities. As it can be produced by *Aspergillus flavus*, it has the potential to co-occur with aflatoxins, but there is comparatively little data about its occurrence in foods. At high concentrations it exhibits a number of toxic effects in animals and its presence in food is undesirable.

Cyclopiazonic acid (CPA) is an indole tetramic acid with a molecular mass of 336. Cyclopiazonic acid imine occurs as a related metabolite in culture, but this is considered to be much less toxic than the parent compound.

Occurrence in Foods

CPA acid has been detected at levels of up to 10 mg kg^{-1} in a wide variety of food and feed commodities, including maize and other cereals, pulses, peanuts, cheese, ham and sausages, tomatoes, milk, hay and mixed animal feeds. It has also been found to co-occur with aflatoxins in some samples of peanuts.

Natural occurrence in foods and the potential for human exposure from the diet appear to be quite low.

Hazard Characterisation

Effects on Health

Most of the information on the toxicity of CPA is derived from a limited number of animal studies and there is little or no experimental, or epidemiological, data on acute or chronic toxicity in humans. Its significance for human health is therefore still unclear.

CPA is a specific inhibitor of the sarcoplasmic reticulum calcium pump in skeletal muscle cells, interfering with muscle contraction/relaxation. It is reported to be neurotoxic when injected intraperitoneally into rats and an LD_{50} (lethal dose) of 2.3 mg per kg of body weight has been observed. However, higher oral doses appear to be necessary to cause significant toxic effects, and an LD_{50} (lethal dose) of 36–63 mg per kg of body weight has been reported for rats when CPA was administered by feeding. A number of toxic effects have been observed, notably lesions in the liver, kidneys and spleen, with varied symptoms, including diarrhoea, dehydration, hypokinesis, convulsion and death. It may also be toxic to poultry, but interpretation of published studies is complicated by the possible presence of other mycotoxins.

CPA displays some mutagenic activity and it may also contribute to overall toxicity when it co-occurs with aflatoxins.

It has been proposed that CPA is implicated in 'Kodua' poisoning in India, a neurological condition associated with eating mouldy millet. Symptoms include somnolence, tremors and giddiness.

Sources

CPA is produced by several species of *Penicillium*, including *P. cyclopium*, *P. commune* and *P. camembertii*. Some strains of *Aspergillus flavus* and *A. versicolor* have also been demonstrated to produce the toxin.

The species known to be capable of producing CPA have a widespread distribution, are able to colonise a very wide range of food commodities and can grow over a wide range of temperatures and water activities. There is therefore a potential for CPA to be produced in a number of foods intended for human consumption. Furthermore, one CPA-producing species, *P. camembertii*, is used in the production of some types of cheese as a surface-ripening agent.

Stability in Foods

Relatively little is known about the stability of CPA during food processing. It has been found to be quite stable on dry-cured ham and in milk stored at chill temperatures. It also survives spray-drying processes used in milk powder production. Approximately 40% of CPA was lost during the manufacture of condensed milk using temperatures of 100 °C.

Control Options

There are few specific documented control measures for CPA, but its co-occurrence with other aflatoxins means that the pre- and post-harvest control measures recommended for aflatoxins may also provide indirect control of CPA.

It is important to consider the possible production of CPA when selecting cultures of *P. camembertii* for cheese manufacture. Although many strains appear to have the potential to produce the toxin, not all are reported to do so on cheese, and it is important to choose a non-toxin-producing strain.

Some mould species that cause mould spoilage of stored foods such as dry-cured hams and fermented sausage products may be capable of producing CPA. For this reason it is preferable to control mould growth on the surface of these foods.

Legislation

There are no current specific regulations setting mandatory or recommended maximum limits for CPA in food or feed.

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On the Web

European Mycotoxin Awareness Network. http://www.mycotoxins.org/

2.1.1.4 Deoxynivalenol

Hazard Identification

What is Deoxynivalenol?

Deoxynivalenol (DON) is a toxic fungal metabolite (mycotoxin) produced by certain plant pathogenic moulds, especially *Fusarium* species, infecting cereal crops in the field. DON is also known as vomitoxin and is one of a large group of chemically related mycotoxins called the trichothecenes. DON is toxic to humans and livestock, is quite common in some food commodities and can occur at high levels. For these reasons it is of concern from a food safety point of view.

The trichothecenes are a group of around 150 compounds characterised as tetracyclic sesquiterpenes. DON ($C_{15}H_{20}O_6$, CAS No. 51481-10-8) belongs to the B group of trichothecenes and has a molecular mass of 296. It is soluble in water and extremely stable.

Occurrence in Foods

DON is almost exclusively associated with cereals, particularly in temperate regions, and it is a common contaminant in wheat, barley, oats, rye, maize and rice. The level of contamination varies widely between regions and from year to year, but where cereals become infected with DON-producing *Fusarium* species, more than 50% of grain samples may show contamination and levels have been reported to reach approximately 9000 $\mu g\ kg^{-1}$ for barley, 6000 $\mu g\ kg^{-1}$ for wheat, 5000 $\mu g\ kg^{-1}$ for rice and 4000 $\mu g\ kg^{-1}$ for maize.

DON has also been found in processed foods, especially those produced from cereals. Foods reported to be contaminated have included flour, bread, breakfast cereals, noodles, infant foods, malt and beer. DON contamination does not seem to be a problem in foods of animal origin, despite the fact that it is a frequent contaminant of animal feed. One reason for this may be that the presence of high levels of DON in feed tends to lead to feed refusal by livestock. Furthermore, lower levels are metabolised and eliminated rapidly in food animal species. Only trace amounts have been reported in eggs and milk.

The main contributor to DON in the diet in the EU is wheat ($\sim$80%), whereas in the Far East, rice is equally important.

Hazard Characterisation

Effects on Health

DON is associated with acute toxicity in both animals and humans, but its effects are difficult to quantify because it often co-occurs with other *Fusarium* mycotoxins, especially nivalenol and zearalenone. Trichothecenes in general are known to interfere with protein synthesis, but the main effects of DON now appear to be related to its role as a pro-inflammatory agent.

Pigs are particularly sensitive to DON in feed and acute toxicity is characterised by vomiting—the source of the synonym vomitoxin. At lower levels, a variety of symptoms have been reported, including feed refusal and reduced weight gain. Acute toxicity in humans has also been reported, with symptoms including vomiting, diarrhoea, abdominal pain, headache and fever. These symptoms can develop within 30 min and are difficult to distinguish from some types of bacterial food poisoning, particularly that caused by preformed emetic toxins of *Bacillus cereus*. However, it should be noted that the role of DON in these cases is uncertain, as other mycotoxins are almost always present. Recovery is usually quite rapid and no deaths have been reported.

Long-term chronic toxicity from low levels of DON in the diet has been investigated in animals. Studies show changes in some blood parameters and suggest adverse effects on the immune system. However, there is no evidence of carcinogenicity, or of mutagenic or teratogenic effects. Based on the data available from animal studies, the EU Scientific Committee on Food established a temporary tolerable daily intake (TDI) for DON of 1 μg per kg of body weight for humans in 2002. Although this is in line with TDIs established by other authorities, dietary surveys suggest that some EU consumers could have an intake quite close to this figure.

Incidence and Outbreaks

There are a number of documented outbreaks of food poisoning caused by foods contaminated with DON. For example, in India in 1987, approximately 50 000 people were ill with mild gastrointestinal symptoms after eating bread made from rain-damaged wheat. Samples of the wheat showed that DON was present at levels from 340–8400 μg kg^{-1}, but several other trichothecenes were also present at lower concentrations.

Sources

The principal sources of DON in cereals are the *Fusarium* species *F. graminearum* and *F. culmorum*. Both of these species are considered to be field fungi and are pathogenic to cereals, causing *Fusarium* head blight in wheat and *Gibberella* ear rot in maize. Distribution of the two species is influenced by temperature, and *F. graminearum* is found mainly in warmer regions.

DON is produced in the crop prior to harvest, rather than during storage, and contamination in wheat is directly related to the incidence of *Fusarium* head blight, which is itself related to moisture levels at flowering. Rainfall at this time is a critical factor for the incidence of the disease, but the amount of rainfall does not appear to be important. The disease causes shrivelling of the wheat seeds and DON is typically produced on the surface of infected grains. However, where high levels are produced, it may be more evenly distributed in the wheat kernel itself.

Stability in Foods

DON is extremely heat stable and is not destroyed by temperatures of 120 °C. It therefore survives most cooking processes and significant quantities are reported to remain even in baked products cooked at 200 °C. It has also been shown to survive autoclaving and extrusion processes.

The toxin is unstable under alkaline conditions. Production of maize flour for tortillas by first boiling maize in calcium hydroxide (nixtamalization) has been shown to reduce DON levels by approximately 80%.

Control Options

Since DON production occurs mainly in the field, the most successful controls are applied at the pre-harvest stage.

Pre-harvest

GAP measures designed to reduce *Fusarium* infection in cereal crops are also effective in limiting the formation of DON. Control measures include the following.

- Land preparation, crop rotation and crop debris removal to reduce the inoculum of *Fusarium* in the field
- Use of fungus-resistant crop varieties
- Control of infection by appropriately timed application of effective fungicides
- Harvesting at the correct moisture level and stage of maturity

Post-harvest Handling and Storage

Further production of DON after harvest can be prevented by rapid drying to a water activity value of 0.8, and by implementing good storage practice.

Decontamination

Physical decontamination methods can be an effective means of reducing DON levels in contaminated grain. These include gravity separation and grain washing, although this process produces large amounts of effluent. The milling process also reduces DON concentrations in wheat flour by removing the generally more heavily contaminated bran, but the effectiveness of this depends on the distribution of the toxin in the grain.

Chemical decontamination methods, such as treatment with sodium bisulphite, have been investigated, but are not yet developed for commercial use.

Heat treatments are not usually effective.

Testing

Some countries monitor cereals for DON contamination by sampling and testing using analytical methods, such as LC with UV detection, or GC-MS. Sensitive ELISA methods and lateral flow test strips have also been developed for screening purposes and commercial kits are available. However, as with other mycotoxins, the distribution of DON in bulk commodities may be highly heterogeneous and it is essential to ensure that an adequate representative sampling plan is used.

Legislation

At least 40 countries around the world have introduced mandatory or guideline levels for DON in foods, mostly since the late 1990 s when the toxin became a cause for concern.

EU

The EU sets a maximum level for DON of 1250 μg kg^{-1} in most unprocessed cereals, but the permitted level in unprocessed durum wheat, oats and maize is 1750 μg kg^{-1}. Up to 750 μg kg^{-1} is allowed in pasta and in cereals, flour and bran for direct human consumption. The limit for bread, biscuits, breakfast cereals and cereal snacks is 500 μg kg^{-1}. A limit of 200 μg kg^{-1} has been set for foods intended for babies and young children.

USA

USA food safety regulations include a limit of 1000 μg kg^{-1} for DON in finished wheat products for human consumption. Higher limits apply in animal feeds.

Others

The Canadian authorities have introduced a limit of 2000 μg kg^{-1} for DON in domestic raw soft wheat and 1200 μg kg^{-1} for soft wheat flour. The limit for flour used in infant food is 600 μg kg^{-1}.

A number of other countries, such as China, have introduced a limit of 1000 μg kg^{-1} for DON in wheat and maize flour.

More information can be found at the FAO web link below.

Sources of Further Information

Published

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2.1.1.5 Ergot

Hazard Identification

What is Ergot?

The term ergot refers to fungal structures (sclerotia) produced by certain species of *Claviceps* fungi that infect cereals and wild grasses. These sclerotia are hard black masses of fungal hyphae that act as a resistant resting stage for the fungus and are visible on the grain ears of infected cereals. Ergots contain a number of different types of alkaloids, which can produce toxic effects in animals and humans. The effects of these alkaloids have been known for hundreds of years and they were the main cause of outbreaks of a toxic condition known as "St Anthony's Fire", which occurred regularly in Europe during the Middle Ages.

There are at least 40 different ergot alkaloids, but the most important are ergotamine, ergometrine, ergosine, ergocristine, ergocryptine, ergocornine and their related -inines. These compounds are derivatives of the hallucinogenic drug lysergic acid (LSD), or of isolysergic acid (-inines). In addition, some *Claviceps* species produce clavine alkaloids, such as agroclavine, which are also toxic and are derivatives of dimethylergoline.

Occurrence in Foods

Ergot can occur in all common cereals, including wheat, barley, oats, rye, millet, sorghum, maize and rice, but rye is more susceptible to infection than other cultivated crops. Ergot contamination in cereals is usually expressed in terms of the percentage, by weight, of sclerotia present in the grain, rather than as ergot alkaloids. However, some studies have measured the levels of individual and total ergot alkaloids in contaminated grain. For example, the concentrations of total ergot alkaloids in sclerotia from rye and wheat have been reported to be 700 mg kg^{-1} and 920 mg kg^{-1} respectively. A survey of cereal products on the market in Switzerland showed levels of total ergot alkaloids between 4.2 μg kg^{-1} (wheat flour) and 139.7 μg kg^{-1} (rye flour). The daily intake of total ergot alkaloids by human beings in Switzerland was estimated to be 5.1 μg per person.

There is no evidence that ergot alkaloids transfer and accumulate in the tissues of animals fed on contaminated cereals and they have not been found in milk or eggs.

Hazard Characterisation

Effects on Health

Acute toxicity (ergotism) in humans is now rare, but it is still occasionally reported in livestock.

There is little information on the toxicity of individual ergot alkaloids, but in practice affected humans and animals are likely to be exposed to complex mixtures of varying composition. For this reason, the range of toxic effects and

symptoms is quite broad, and different animals display widely different symptoms.

In general, two main types of ergotism, gangrenous and convulsive, can occur in animals. In the first type ergot alkaloids affect blood circulation, causing vasoconstriction, which may lead to a dry gangrene in the extremities, especially the limbs. Cattle affected in this way tend to become lame and may develop gangrene in their ears and tail, as well as the feet. Convulsive ergotism results from the neurotoxic activity of ergot alkaloids and symptoms include feed refusal and dizziness, as well as convulsions.

Symptoms of St Anthony's Fire in humans have been documented for centuries, and include gangrene, burning sensations (hence the name) and hallucinations. The disease was often fatal.

Little is known about the long-term effects of low levels of ergot alkaloids in the diet, or the potential carcinogenicity of these compounds.

Incidence and Outbreaks

Outbreaks of ergotism are rare in recent times and no documented outbreaks have occurred in the EU since 1928. However, a serious outbreak of gangrenous ergotism did occur in Ethiopia in 1978, when 93 cases were reported, along with a further 47 related deaths. The outbreak was caused by a high level of ergot-infected wild oats in the local barley crop, and 0.75% ergot was reported in the implicated grain.

Outbreaks of ergotism have also been reported in India, most recently in 1975, caused by consumption of infected millet, but the symptoms were mainly nausea and vomiting followed by drowsiness, and no deaths occurred. These outbreaks were found to be related to clavine alkaloids, such as agroclavine, present in implicated grain at levels of 15–199 mg kg^{-1}.

Sources

The principal source of ergot alkaloids in cereals is the ascomycete fungus *Claviceps purpurea*. The clavine alkaloids are produced mainly by a different species, identified as *Claviceps fusiformis*, which is primarily a parasite of pearl millet in tropical regions. Ergot alkaloids have been isolated from other fungi, including some *Penicillium* and *Aspergillus* species, but their significance for human health is unknown.

When *Claviceps purpurea* spores infect a susceptible host, the fungus invades the developing grains in the floret, then destroys and replaces them. Eventually the hard, dark sclerotia, or ergots, are formed and are easily visible as dark purple bodies up to 20×6 mm in size. At this stage, the ergot alkaloids begin to accumulate in the sclerotia.

Cereals are more susceptible to infection in wet weather, which favours the germination of sclerotia in the soil and the production of fruiting bodies and airborne ascospores. Cool, wet conditions during flowering of cereals and grasses favour the invasion of the florets, whereas hot, dry conditions inhibit

infection. If weather conditions or other factors result in prolonged flowering periods, infection becomes more likely.

Stability in Foods

The heat stability of the ergot alkaloids is quite variable, but the most pharmacologically active forms tend to be less stable than the inactive isomers. Heat processes such as baking produce a significant reduction (50% or more) in the concentration of the most important ergot alkaloids.

Beer made from ergot-contaminated grain has been reported to contain only low levels (10 ng ml^{-1}) of ergot alkaloids.

Control Options

Ergot infection occurs entirely in the field, and there are control options that can be applied at the pre-harvest stage. Decontamination is also an important control.

Pre-harvest

Control measures include the following.

- Land preparation (*e.g.* deep plowing), crop rotation with non-susceptible crops and crop debris removal to reduce the inoculum of ergot
- Plant only ergot-free seed
- Effective control of wild grasses in and around the crop

Decontamination

Physical decontamination methods, such as grain cleaning, can achieve considerable reduction in ergot contamination. However, where small pieces of sclerotia, similar in size to individual grains, are present, they may not be removed effectively.

Testing

The presence of ergot in foodstuffs can be detected by analysis for ricinoleic acid, which is diagnostic for ergot in the absence of other sources, such as castor oil. This marker compound can be detected using a GC-LC method.

Methods for detection of specific ergot alkaloids in cereals have also been developed, using a variety of techniques, including HPLC, GC-MS and TLC.

Legislation

Most regulations for the control of ergot in foods specify a recommended, or mandatory limit based on the percentage by weight, or number, of ergots in grain, rather than ergot alkaloid concentration. These limits are most often applied to animal feed. In general, feed containing >0.1% of ergot is not suitable for livestock, but many countries have developed higher voluntary standards.

Australia and New Zealand have set a maximum level of 0.5% ergot kernels by weight for cereal grains used in human food and Canada has also set various tolerances for different cereal foods and pulses based on percentage by weight. Canada is also unusual in having specific limits for ergot alkaloids in animal feed. Switzerland has set a maximum level for FB_1 and FB_2 in maize of 1000 μg kg^{-1}.

More information can be found at the FAO web link below.

Sources of Further Information

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2.1.1.6 Fumonisins

Hazard Identification

What are Fumonisins?

The fumonisins are a group of at least 15 chemically related toxic fungal metabolites (mycotoxins) produced by certain mould species of the genus *Fusarium*, which may colonise cereals, especially maize, in the field. They were first identified as recently as 1988, although their effects had been noted many years before. Fumonisins are known to cause adverse health effects in livestock and other animals and are considered to be potentially toxic to humans. They have been found in maize and maize products worldwide. For these reasons they are of concern from a food safety point of view.

The fumonisins are polar compounds based on a long hydroxylated hydrocarbon chain containing methyl and amino groups. They are quite stable compounds and are divided into five groups, A, B, C, P and H, according to their chemical structure. The most widespread fumonisins in nature are the B group, and of these the most important and probably the most toxic is fumonisin B_1 (FB_1), although fumonisins B_2, B_3 and B_4 have also been found in food commodities. The chemical formula of FB_1 is $C_{34}H_{59}NO_{15}$ (CAS No. 116355-83-0) and its molecular mass is 721.

Occurrence in Foods

The fumonisins were initially thought to be confined to maize and maize products, but more recently they have also been found in other food commodities, such as rice, sorghum, asparagus and mung beans. Contamination levels in maize can vary considerably from year to year, and are strongly influenced by climatic conditions. High levels of fumonisins are associated with hot and dry weather, followed by a period of high humidity. Surveys of maize harvested in Iowa, in the USA, showed that the average concentration of FB_1 from 1988–1991 was >2000 μg kg^{-1}, but from 1992–1996 it was <450 μg kg^{-1}. Similar variation has been found elsewhere. The mean level of FB_1 in sound maize traded around the world in any given year has been estimated to vary from 200 to 2500 μg kg^{-1}. However, it should be noted that much higher levels may be present in visibly mouldy maize. For example, a sample tested in Italy in 1994 recorded a level FB_1 and FB_2 of 300 000 μg kg^{-1}. Detectable levels in other crops are much less common.

Fumonisins have also been found in processed foods, especially those produced from maize, such as maize meal and cornstarch, popcorn, maize-based breakfast cereals and snack products, polenta and beer. Levels are usually much lower than those found in unprocessed maize. Foods of animal origin do not seem to be a significant source of fumonisins.

A mean daily intake for FB_1 in the EU diet has been estimated at 0.2 μg per kg of body weight. By far the main contributors to fumonisins in the diet worldwide are maize and maize products.

Hazard Characterisation

Effects on Health

The acute toxicity of fumonisins in animals is relatively low in comparison with other mycotoxins, such as aflatoxins, although it is important to note that they may be present at very high levels in mouldy maize. Exposure to fumonisins in mouldy feed is associated with diseases in some livestock, especially horses and pigs. Horses exposed to fumonisins in feed over a period can develop a fatal disease known as equine leucoencephalomalacia (ELEM), which causes neurotoxic effects, liver damage and degeneration in the brain. A minimum dose of FB_1 of 200–440 μg per kg of body weight per day is reported to be sufficient to cause ELEM in horses. Pigs may suffer from pulmonary oedema and develop respiratory problems. An outbreak of human gastrointestinal disease in India was reported to be associated with mouldy sorghum or maize containing FB_1 at a level of 64 000 μg kg^{-1}, but other mycotoxins were probably also present.

Toxicity testing in animals shows that the liver and kidneys are the main targets for fumonisin toxicity, especially in rodents. Cardiovascular effects have also been reported. The basis for the toxicity of fumonisins is thought to be interference with the synthesis of complex glycol-sphingolipids, which has effects on cell growth, development and function. The long-term chronic toxicity and carcinogenicity of FB_1 has been investigated in animals. Studies show adverse effects on the liver and kidneys of rats and mice and the development of cancers at higher levels (2500–7000 μg per kg of body weight).

Epidemiological studies have suggested links between consumption of fumonisin-contaminated maize and high incidences of oesophageal cancer in humans, but these studies are considered inconclusive. FB_1 is classified as by the IARC as "possibly carcinogenic to humans (IARC Group 2B)". Based on the data available from animal studies, the EU Scientific Committee on Food established a tolerable daily intake (TDI) for FB_1 of 2 μg per kg of body weight for humans.

Sources

The only known source of fumonisins in maize and other crops are *Fusarium* species fungi. The two species most associated with FB_1 and FB_2 production in maize are *F. verticillioides* (older synonym *F. moniliforme*) and *F. proliferatum*. However, other species, such as *F. nygamai*, *F. napiforme*, *F. anthophilum* and *F. dlamini* are also reported to produce fumonisins and are associated with food grains.

F. verticillioides is considered to be the main cause of Fusarium kernel rot in maize, a disease that occurs predominately in warm, dry weather. High levels of FB_1 can accumulate in infected maize grains under these conditions, especially in maize that has been damaged by insects. *F. verticillioides* is very common in tropical and sub-tropical regions, but less so in cooler climates. It is able to grow over a fairly wide temperature range (2–35 °C) and FB_1 and FB_2 production occur at water activity levels down to about 0.90. Most toxin production in maize occurs in the field, or during the early stages of drying, rather than during storage.

Stability in Foods

Fumonisins are fairly heat stable and significant destruction occurs only when temperatures above 150 °C are reached. They therefore survive many cooking processes, but are less heat stable under alkaline conditions. Production of maize flour for tortillas by first boiling maize in calcium hydroxide (nixtamalization) has been shown to reduce fumonisin levels considerably, but the hydrolysed breakdown products formed are also thought to be toxic.

FB_1 has been shown to survive fermentation and brewing processes and has been detected in beer.

Control Options

Since fumonisin production occurs almost entirely in the field, the most effective controls are applied at the pre-harvest stage.

Pre-harvest

GAP measures designed to reduce *Fusarium* infection in cereal crops are also effective in limiting the formation of fumonisins. Control measures include the following.

- Land preparation, crop rotation and crop debris removal to reduce the inoculum of *Fusarium* in the field
- Use of fungus-resistant crop varieties
- Control of infection by appropriately timed application of effective fungicides
- Effective control of insect crop pests
- Harvesting at the correct moisture level and stage of maturity

Post-harvest Handling and Storage

Further production of fumonisins during storage can be prevented by rapid drying to a water activity value of 0.8 immediately after harvest, and by implementing good storage practice.

Decontamination

Physical decontamination methods, such as separation of screenings, can be an effective means of reducing fumonisin levels in contaminated maize. However, fumonisins also occur in whole undamaged grains. Milling processes also reduce fumonisin concentrations in maize flour by removing the generally more heavily contaminated bran and germ, but the effectiveness of this depends on the distribution of the toxin in the grain. In wet milling processes, significant quantities of fumonisins leach out of the grain into the steep water.

Chemical decontamination methods for FB_1, such as a modified nixtamalization process and ammoniation, have been investigated, but are not yet developed for commercial use.

Heat treatments are not usually effective, unless high temperatures (>150 °C) are used.

Testing

Some countries monitor cereals for FB_1 and FB_2 contamination in maize and maize products by sampling and testing using analytical methods, usually based on HPLC. ELISA methods for FB_1 and FB_2 have been developed for screening purposes and commercial kits are available. However, as with other mycotoxins, the distribution of fumonisins in bulk commodities may be highly heterogeneous and it is essential to ensure that an adequate representative sampling plan is used.

Legislation

Very few countries outside the EU and North America have introduced mandatory or guideline levels for fumonisins in foods.

EU

The EU has set maximum levels for FB_1 and FB_2 in combination. The maximum level for unprocessed maize (other than maize intended to be processed by wet milling) is 4000 μg kg^{-1}, for maize and maize-based foods intended for direct human consumption it is 1000 μg kg^{-1}, and for maize-based breakfast cereals and snacks it is 800 μg kg^{-1}. The limit for maize-based foods for infants and young children is 200 μg kg^{-1}.

USA

USA food safety regulations include maximum guidance levels for FB_1, FB_2 and FB_3 in combination for maize products. These vary from 2000 to 4000 μg kg^{-1} depending on the product. Much higher levels are allowed in animal feeds.

Others

Switzerland has set a maximum level for FB_1 and FB_2 in maize of 1000 μg kg^{-1}.

More information can be found at the FAO web link below.

Sources of Further Information

Published

"The Mycotoxin Factbook: food & feed topics", ed. Barug, D., Bhatnagar, D., Van Egmond, P., Van Der Kamp, J.W., Van Osenbruggen, W.A. and Visconti, A., Wageningen Academic Publishers, Wageningen, 2006.

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2.1.1.7 Moniliformin

Hazard Identification

What is moniliformin?

Moniliformin is a toxic fungal metabolite (mycotoxin) produced by some moulds of the genus *Fusarium* growing on certain food commodities, especially cereals. It was originally reported to be produced by *Fusarium moniliforme* (now re-classified as *F. verticillioides*), which also produces fumonisins, but these reports are now discounted. Although comparatively little is known about the occurrence of moniliformin, it exhibits a number of toxic effects in animals and its presence in food is undesirable.

Moniliformin is an ionic compound with a four-carbon ring structure and occurs as sodium or potassium salts of 1-hydroxycyclobut-1-ene-3,4-dione. It is soluble in water.

Occurrence in Foods

Moniliformin appears to be relatively uncommon in food commodities, but it has been reported in cereals, including wheat, rye, rice and especially maize. Levels of up to 12 mg kg^{-1} were reported in maize intended for human consumption in South Africa, and up to 4.6 mg kg^{-1} moniliformin was reported in 60% of samples of milled maize imported into the UK for use in animal feed. Moniliformin has also been found in Polish cereals showing mould damage.

It has recently been detected in asparagus spears and in rotting apples.

Little is known about the occurrence of moniliformin is processed foods, but it was detected in corn tortillas at levels of up to 0.1 mg kg^{-1}. Similar levels have also been reported in other maize-based foods, such as polenta.

Natural occurrence in foods and the potential for human exposure from the diet appear to be quite low.

Hazard Characterisation

Effects on Health

Most of the information on the toxicity of moniliformin is derived from a limited number of animal studies and there is little or no experimental, or epidemiological, data on acute or chronic toxicity in humans. Its significance for human health is therefore still unclear.

The toxicity of moniliformin is based on its ability to inhibit mitochondrial pyruvate and ketoglutarate oxidation. But relatively high doses appear to be necessary to cause significant toxic effects on mammals, and an oral LD_{50} (lethal dose) of 25–50 mg per kg of body weight has been reported for rodents. Birds are reported to be more sensitive to moniliformin (LD_{50} of 4 mg kg^{-1} for day-old chicks). The main effect of acute toxicity is intestinal haemorrhage, but chronic toxicity mainly affects the heart. The interpretation of animal studies

based on the feeding of contaminated maize is complicated by the likely presence of other *Fusarium* mycotoxins.

There is no significant evidence for carcinogenicity, but the amount of reported data is quite limited.

It has been proposed that moniliformin may be implicated in human disease, notably Keshan disease, a cardiomyopathy endemic in certain parts of China. However, it is likely that other factors, such as selenium deficiency, are also involved in this condition.

Sources

Moniliformin is reportedly produced by several species of *Fusarium*, including *F. avenaceum*, *F. subglutinans* and some strains of *F. proliferatum* and *F. oxyporum*, at least in laboratory culture. Erroneous reports of production by *F. moniliforme* are now thought to be the result of working with mixed cultures of more than one species.

F. subglutinans is thought to be a producer of moniliformin in the field and this species has a global distribution. It has been isolated from maize in the EU, North and South America, Asia and Australia and is also a pathogen of pineapples and bananas. *F. avenaceum* is also found worldwide, but is rarely isolated from food commodities and is not regarded as a major pathogen of cereals. It has been reported to cause occasional spoilage in fruits and vegetables, such as apples and tomatoes. It is able to grow in a temperature range of -3 to 35 °C and at water activity values as low as 0.90.

Stability in Foods

Relatively little is known about the stability of moniliformin during food processing, but like many mycotoxins, it is thought to be quite heat stable. It has been reported to survive autoclaving of creamed corn at 121 °C for 65 min, and roasting corn meal at 218 °C for 15 min gave a 45% reduction. Significant concentrations have also been shown to survive in the manufacture of corn chips from spiked maize. Moniliformin is less stable at alkaline pH, and production of tortillas using nixtamalization processes gave a 70% reduction.

Control Options

There are few specific documented control measures for moniliformin, but its co-occurrence with other *Fusarium* mycotoxins in cereals means that the pre- and post-harvest control measures recommended for fumonisins may also provide indirect control of moniliformin.

Legislation

There are no current specific regulations setting mandatory or recommended maximum limits for moniliformin in food or feed.

Sources of Further Information

Published

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Scientific information on mycotoxins and natural plant toxicants – EFSA external report. http://www.efsa.europa.eu/en/supporting/doc/24e.pdf

European Mycotoxin Awareness Network. http://www.mycotoxins.org/

2.1.1.8 Ochratoxins

Hazard Identification

What are Ochratoxins?

Ochratoxins are a small group of chemically related toxic fungal metabolites (mycotoxins) produced by certain moulds of the genera *Aspergillus* and *Penicillium* growing on a wide range of raw food commodities. Some ochratoxins are potent toxins and their presence in food is undesirable.

The ochratoxins are pentaketides made up of dihydroisocoumarin linked to β-phenylalanine. The most important and most toxic ochratoxin found naturally in food is ochratoxin A (OTA). The only other ochratoxin found in food is ochratoxin B, which is rare and much less toxic. Other structurally related ochratoxins include ochratoxin C, α and β. These have been isolated from fungal cultures, but are not normally found in foods. The remainder of this section therefore refers specifically to OTA.

Occurrence in Foods

In surveys, OTA has been found in a very wide range of raw and processed food commodities all over the world. It was first reported in cereals, but has since been found in other products, including coffee, dried fruits, wine, beer, cocoa, nuts, beans, peas, bread and rice. It has also been detected in meat, especially pork and poultry, following transfer from contaminated feed.

OTA levels in different food products vary, but are generally low in properly stored commodities (mean value $<1\,\mu g\,kg^{-1}$ for cereals from temperate regions). However, much higher concentrations can develop under inadequate storage conditions. Levels of up to 6000 $\mu g\ kg^{-1}$ and 5000 $\mu g\ kg^{-1}$ have been reported in Canadian wheat and UK barley respectively, but the concentrations found are usually below 50 $\mu g\ kg^{-1}$. The major contributors to OTA in the diet in the EU are cereals and wine. Coffee was thought to be important in this respect, but is now considered less significant. Pork products have also been suggested as a significant dietary source.

Hazard Characterisation

Effects on Health

OTA is a potent nephrotoxin and causes both acute and chronic effects in the kidneys of all mammalian species tested. The sensitivity of different species varies, but a level of 200 $\mu g\ kg^{-1}$ in feed over three months is sufficient to cause acute damage to the kidneys of pigs and rats. There are no documented cases of acute OTA toxicity in humans.

OTA is also genotoxic (damages DNA) and teratogenic (damages the foetus) and is considered a probable carcinogen, causing renal carcinoma and other cancers in a number of animal species, although the mechanism for this is

uncertain. It is also reported to have adverse effects on the immune system in some species. The evidence for carcinogenicity in humans is not conclusive, but in view of the evidence for other mammalian species, the presence of OTA in food and feed must be considered undesirable. Some toxicologists suspect that OTA may be a very significant food contaminant from a public health point of view.

OTA has been detected in human blood and breast milk, demonstrating dietary exposure. Daily intakes have been estimated at between 0.2 and 4.7 ng per kg body weight. In 2006, the EFSA derived a tolerable weekly intake (TWI) of 120 ng per kg body weight for OTA in the diet, based on the latest scientific evidence.

Sources

In tropical and sub-tropical regions, OTA is produced mainly by *Aspergillus* species, particularly the widespread *A. ochraceus*. But in temperate climates (Canada, Northern EU and parts of South America), the main producer is *Penicillium verrucosum*.

OTA production by *A. ochraceus* is favoured by relatively high temperatures (13 °C to 37 °C), but *P. verrucosum* grows and produces the toxin at temperatures as low as 0 °C. *A. ochraceus* is able to produce OTA at water activities down to 0.80, while the lower limit for significant toxin production by *P. verrucosum* is thought to be about 0.86, although growth can occur at lower values. Both are considered to be storage fungi, rather than field contaminants or plant pathogens, and toxin production occurs mainly when susceptible commodities are stored under inappropriate conditions, particularly at high moisture levels.

Stability in Foods

OTA is a relatively heat-stable molecule and survives most cooking processes to some extent, although the reduction in concentration during heating depends on factors such as temperature, pH and other components in the product. For example, heating wet wheat at 100 °C for 2.3 hours gave a 50% reduction in OTA concentration, but in dry wheat, the same reduction took 12 hours.

Processes such as coffee roasting and baking of cereal products and biscuits can produce significant losses in OTA levels, but processes like pasta manufacture produce little reduction. OTA also survives brewing and winemaking and can be found in a variety of processed consumer food products.

OTA is destroyed by acid and alkaline hydrolysis and by the action of some oxidising agents.

Control Options

The ability of OTA-producing fungi to grow on a wide range of food commodities and the persistence and ubiquity of OTA in the food chain mean that control is best achieved by measures designed to prevent the contamination of foods using HACCP-type techniques. Detection and removal of

OTA-contaminated material from the food supply chain is also important for imported products.

Pre-harvest

Both *A. ochraceus* and *P. verrucosum* are considered to be storage fungi rather than field fungi. Pre-harvest controls are therefore limited to harvesting susceptible crops at the correct moisture level and stage of maturity.

Post-harvest Handling and Storage

For cereals, the most important and effective control measure in post-harvest handling and storage is the control of moisture content and hence, the water activity of the crop. Ensuring that susceptible crops are harvested at a safe moisture level, or are dried to a safe level immediately after harvest is vital to prevent mould growth and OTA production during storage. In tropical and sub-tropical climates stored grains must be dried rapidly to an A_w value of below 0.8 and this level must be maintained throughout storage to prevent *A. ochraceus* growth. In temperate regions a target moisture content of 18% for grain drying is recommended, together with rapid cooling of grain if hot-air drying is used. This should be followed by further drying down to a moisture level of 15% (UK Code of Good Storage Practice).

Other important cereal storage factors are effective cleaning of grain stores and handling equipment between crops, and fumigation to prevent insect infestation. In tropical regions, the use of controlled atmosphere storage to control insects may also help to inhibit mould growth.

Rapid and effective drying is also important in the control of OTA production in other commodities, especially coffee. For dried fruits, minimising mechanical and insect damage during handling and storage helps to prevent the entry of moulds into the fruit before drying.

Monitoring raw material quality is the most effective control for processed foods. Any ingredient that displays visible mould growth should not be used. Testing for the presence of OTA in susceptible materials, such as barley for brewing, may be necessary in some cases.

Decontamination

Physical separation of contaminated material can be an effective means of reducing OTA levels in contaminated commodities. Mouldy grain should not be used for food, or for animal feed.

There has been little practical evaluation of chemical decontamination methods for OTA to date, but an ammoniation process has been shown to be effective for cereals.

Testing

Some countries monitor imported commodities that are susceptible to OTA contamination, such as grains and coffee beans, by sampling and analysis. A number of analytical methods have been developed based on TLC, HPLC and ELISA and there are also rapid screening kits available. However, moulds and mycotoxins in bulk food shipments tend to be highly heterogeneous in their distribution and it is essential to ensure that an adequate sampling plan is used to monitor imported materials.

Legislation

A number of countries, particularly in the EU, have regulations governing OTA in food and feed and most include maximum permitted, or recommended levels for specific commodities.

EU

The EU has set limits for OTA in cereals, dried vine fruits, roasted coffee beans and ground coffee, soluble coffee, wine and grape juice. Limits vary according to the commodity, but range from 2–10 $\mu g\,kg^{-1}$. The limit for unprocessed cereals is 5.0 $\mu g\,kg^{-1}$, but for processed cereal products intended for direct human consumption it is 3.0 $\mu g\,kg^{-1}$. The limit for dried vine fruits is 10 $\mu g\,kg^{-1}$. There is also a limit of 0.50 $\mu g\,kg^{-1}$ for OTA in processed cereal-based foods for infants and young children.

In 2010, additional limits were set for OTA in spices and liquorice products. The maximum permitted level for spices, including chilli powder, paprika, pepper, nutmeg, and turmeric, is set at 30 $\mu g\ kg^{-1}$ until mid 2012, when it will be reduced to 15 $\mu g\ kg^{-1}$. The limit for liquorice root is 20 $\mu g\ kg^{-1}$ and for liquorice extract it is 80 $\mu g\ kg^{-1}$.

Others

Switzerland applies a limit of 5.0 $\mu g\ kg^{-1}$ for all foods except cereal-based infant foods, where the limit is 0.5 $\mu g\ kg^{-1}$, and Turkey has set limits of between 3.0 and 10 $\mu g\ kg^{-1}$ for various food commodities.

Few other countries outside the EU have imposed limits for OTA, but a number have proposals to do so. Uruguay sets a limit of 50 $\mu g\ kg^{-1}$ for rice, cereals and dried fruits and Canada sets a limit of 2000 $\mu g\ kg^{-1}$ for OTA in pig and poultry feed.

More information can be found at the FAO web link below.

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2.1.1.9 Patulin

Hazard Identification

What is Patulin?

Patulin is a toxic fungal metabolite (mycotoxin) produced by certain moulds of the genera *Penicillium*, *Aspergillus* and *Byssochlamys* growing on certain food commodities, especially fruit. Patulin exhibits a number of toxic effects in animals and its presence in food is undesirable.

Chemically, patulin is a polyketide lactone. It is a relatively small molecule ($C_7H_6O_4$, CAS No. 149-29-1) and is soluble in water.

Occurrence in Foods

Patulin occurs most often in apples that have been spoiled by mould growth, or in products made from spoiled apples, such as apple juice, pies and conserves. It has also been found in other fruits, including pears and grapes, in vegetables and in cereal grains and cheese.

Apples and apple products are considered to be by far the most significant contributor to patulin in the diet. Contaminated apple juice usually contains patulin at levels below 50 $\mu g\,l^{-1}$, but much higher levels (up to 4000 $\mu g\,l^{-1}$) have been reported occasionally.

Hazard Characterisation

Effects on Health

Most of the information on the toxicity of patulin is derived from animal studies and there is little or no experimental, or epidemiological, data on acute or chronic toxicity in humans.

At relatively high doses, patulin is acutely toxic in mice and rats, causing gastrointestinal lesions, distension and haemorrhage in the stomach and small intestine. However, it is possible that these effects are due to the selective antibiotic action of patulin against gram-positive bacteria, which may give gram-negative intestinal pathogens an advantage. LD_{50} values (lethal dose) of 20–100 mg per kg of body weight have been reported for patulin administered orally to mice and rats. These levels are much higher than those likely to be encountered in human diets. Relatively high doses of patulin have also been shown to be immunotoxic and neurotoxic in animals.

Of more concern from a food safety point of view are longer term chronic effects. It has been suggested that patulin could be a carcinogen at low levels in the diet, but the IARC has reviewed the available data and concluded that there is no convincing evidence of carcinogenicity in animals or in humans, other than at extremely high doses.

Data from feeding experiments have been used to derive a no-observed-effect level (NOEL) of 43 μg per kg of body weight per day and a provisional

maximum tolerable daily intake (PMTDI) for humans of 0.4 μg per kg of body weight. This is well above the maximum daily intake levels estimated for adults and children (0.1 and 0.2 μg per kg of body weight respectively).

Sources

Patulin is produced by certain species of *Penicillium*, *Aspergillus* and *Byssochlamys*, notably *Penicillium expansum* and *Aspergillus clavatus*. *P. expansum* is the most significant producer of patulin, as it is a common cause of rot in apples. Patulin production by *P. expansum* has been reported over a temperature range 0–25 °C and over a pH range in apple juice of 3.2–3.8.

Stability in Foods

Patulin is relatively heat stable and is not destroyed by pasteurisation of apple juice at 90 °C for 10 s. However, it is broken down in fruit juice and other foods in the presence of sulphur dioxide used as a preservative. It does not appear to survive fermentation processes and is not usually found in alcoholic drinks, such as cider, but the toxicity of its breakdown products is uncertain.

Patulin produced by mould growth on cheese is inactivated by interaction with high cysteine levels.

Control Options

Patulin is only considered to be a significant problem in apples and apple products, especially apple juice.

Pre-harvest

GAP measures designed to minimise insect and bird damage to apples can help to prevent mould infection and patulin production before harvest.

At harvest, rotten and damaged apples should be discarded, as these are much more likely to contain patulin.

Post-harvest

Control in harvested apples is best achieved by good storage practice designed to ensure hygienic conditions in apple stores and to minimise physical damage that might promote fungal infection and rotting. Storage at temperatures of less than 10 °C is also a useful control measure.

Processing

Physical separation of mouldy and damaged apples before processing will help to reduce patulin levels in apple juice and other apple products. This can be

done by hand, or by using water flumes or high-pressure water jets. Washing of apples can also help to reduce patulin levels.

Testing

Monitoring of patulin levels in susceptible products, such as apple juice, by sampling and analysis can be valuable—the test method of choice being HPLC with UV detection. In the UK, significant reductions in patulin levels in apple juice have been achieved since regular monitoring was implemented in 1992.

Legislation

Although patulin is now considered to be a less significant food safety hazard than previously, a number of countries have introduced regulations specifying maximum permitted levels in susceptible products.

EU

The EU has set a maximum limit for patulin of 50 μg kg^{-1} in fruit juices and in drinks containing apple juice or derived from apples. For solid apple products, such as apple puree, the limit is 25 μg kg^{-1}. A lower limit of 10 μg kg^{-1} has been set for certain foods intended for infants.

USA

The FDA has set an upper limit of 50 μg kg^{-1} for patulin in apple juice and apple juice concentrates.

Others

The Codex Alimentarius Commission has also set a recommended upper limit of 50 μg kg^{-1} for patulin in apple juice and apple ingredients in other beverages. More information can be found at the FAO web link below.

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2.1.1.10 Sterigmatocystin

Hazard Identification

What is Sterigmatocystin?

Sterigmatocystin is a toxic fungal metabolite (mycotoxin) produced by some moulds of the genus *Aspergillus* growing on certain food commodities, such as maize. Sterigmatocystin is a potent carcinogen in animals and its presence in food is undesirable.

Chemically, sterigmatocystin is closely related to, and is a precursor of, the aflatoxins. It consists of a xanthone nucleus attached to a bifuran structure. It is only slightly soluble in water. It is one of a group of at least seven related compounds, others of which may also occur naturally.

Occurrence in Foods

Sterigmatocystin has been reported in mouldy cereals, particularly maize, peanuts and pecans, green coffee beans, spices and cheese. It appears to be much less common and less widely distributed than aflatoxins, although low levels in foods may be under-reported because sensitive analytical techniques have only recently become available. However, it has hardly ever been detected in surveys of good quality food commodities, even with the use of reliable analytical methods.

Sterigmatocystin has very rarely been detected in naturally contaminated processed foods, but it has been reported to be present in quite high levels in bread and cured meats inoculated with toxin-producing mould cultures.

Hazard Characterisation

Effects on Health

Most of the information on the toxicity of sterigmatocystin is derived from animal studies and there is little or no experimental, or epidemiological, data on acute or chronic toxicity in humans.

The toxicity of sterigmatocystin is very similar to that of aflatoxin B_1, causing liver and kidney damage and diarrhoea, although its acute toxicity is lower for most species. Cattle ingesting feed containing about 8 mg kg^{-1} sterigmatocystin were reported to have developed bloody diarrhoea and loss of milk production.

Chronic toxicity is probably more important from a food safety point of view. Sterigmatocystin is a potent carcinogen, mutagen and teratogen in many animals, and therefore potentially in humans, and the liver is again the main target organ. It is classified by the IARC as "possibly carcinogenic to humans (IARC Group 2B)". However, it is considered a less potent carcinogen than aflatoxin B_1, although levels as low as 15 μg per day caused liver cancer when fed to rats.

Based on data from animals, the California Department of Health has derived a "no significant risk" intake level for humans of 8 μg per kg of body weight per day.

Sources

Sterigmatocystin is produced by a number of different *Aspergillus* species, notably *A. versicolor* and *A. nidulans*. Some other moulds, including *Chaetomium* species, are also reported to be sterigmatocystin producers. The toxin is produced primarily on stored products that undergo mould spoilage rather than on crops in the field.

A. versicolor is quite widely dispersed and has been isolated from a number of foods, such as fruits and dried meats, in which sterigmatocystin itself has not been found. It is able to grow in a temperature range of 9–39 °C and at water activity values as low as 0.80.

Stability in Foods

There is little published information on the stability of sterigmatocystin in foods, but its chemical similarity to the aflatoxins suggests that it likely to be similarly heat stable and persistent.

Control Options

As sterigmatocystin is produced mainly in stored cereals and other foods that undergo mould spoilage, effective control can be achieved by applying good storage practice and by ensuring that moisture levels in cereals are low enough to prevent mould growth.

Most of the sterigmatocystin in contaminated rice is reported to be removed during the milling stage.

Legislation

There are no current specific regulations setting mandatory or recommended maximum limits for sterigmatocystin in food or feed. However, some Eastern EU countries did set limits in legislation prior to becoming members of the EU. For example, the Czech Republic set maximum limits of 5 or 20 μg kg^{-1}, depending on the nature of the product.

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European Mycotoxin Awareness Network. http://www.mycotoxins.org/

2.1.1.11 Trichothecenes

Hazard Identification

What are Trichothecenes?

The trichothecenes are a large group of around 150 chemically related toxic fungal metabolites (mycotoxins) produced by moulds, especially *Fusarium* species, which may colonise cereals and other crops in the field. Several of the trichothecenes are known to be acutely toxic to humans and livestock. They have been found in a number of food commodities and can be present at high levels. For these reasons they are of concern from a food safety point of view.

The trichothecenes are characterised as tetracyclic sesquiterpenes. They are chemically stable and persistent compounds and are divided into two groups, A and B, according to their chemical structure. The most commonly reported group-A trichothecenes in foods are T2 toxin ($C_{24}H_{34}O_9$, CAS No. 21259-20-1) and HT-2 toxin ($C_{22}H_{32}O_8$, CAS No. 26934-87-2), while group B trichothecenes include deoxynivalenol (DON), which is covered elsewhere, and nivalenol ($C_{15}H_{20}O_7$, CAS No. 23282-20-4). The remainder of this chapter refers to T-2 and HT-2 toxins and, to a lesser extent, nivalenol.

Occurrence in Foods

Trichothecenes are mainly associated with cereals, and have been found to occur in wheat, barley, oats, rye, maize and rice. Oats and, to a lesser extent barley, are most likely to be contaminated with T-2 and HT-2 toxins. Their presence has also been reported in other commodities, such as soya beans, potatoes, sunflower seeds, peanuts and bananas. The frequency of contamination in cereals varies from year to year, but surveys in the EU have shown that T-2 toxin was present in 11% of cereal samples, while HT-2 toxin occurred in 14% of samples. The level of contamination found for T-2 and HT-2 toxins in cereals is usually low ($<100\ \mu g\ kg^{-1}$), but high levels do occur in a small number of samples. For T-2 toxin, levels have been reported to reach $820\ \mu g\ kg^{-1}$ in wheat from Asia, $1700\ \mu g\ kg^{-1}$ in oats from the EU and $2400\ \mu g\ kg^{-1}$ in maize from the USA. A level of $2000\ \mu g\ kg^{-1}$ of HT-2 toxin has been reported in oats from the EU. High levels of both toxins may occasionally be present in the same samples.

Trichothecenes have also been found in processed foods, especially those produced from oats and other cereals. Foods reported to be contaminated have included bread, breakfast cereals, noodles, and beer. Foods of animal origin do not seem to be a significant source of trichothecenes in the human diet.

Daily intakes for T-2 and HT-2 toxins in the EU have been estimated at 7.6 ng per kg of body weight and 8.7 ng per kg of body weight respectively. The main contributors to trichothecenes in the diet in the EU are oats, barley and wheat, but it is probable that other crops, such as rice and maize are more significant in other regions.

Hazard Characterisation

Effects on Health

Trichothecenes are associated with acute toxicity in both animals and humans and T-2 toxin, HT-2 toxin and nivalenol are all acutely toxic to mice at much lower concentrations than DON. The toxicities of T-2 and HT-2 toxins are generally considered in combination, largely because T-2 toxin is rapidly converted to HT-2 toxin and other metabolites in the gut. Trichothecenes in general are known to inhibit protein synthesis, and are immunosuppressive at low concentrations.

Acute toxicity in animals is characterised by haemorrhaging in the gastrointestinal tract and severe gastroenteritis, which may eventually be fatal. Other symptoms include necrotic lesions in the mouth and on the skin and degeneration of the bone marrow and lymph nodes. Acute toxicity in humans has also been reported, with symptoms including nausea and vomiting, dizziness, diarrhoea, abdominal pain and distension, throat irritation and chills. In some suspected outbreaks a high mortality rate was recorded, but in others no deaths occurred. It should be noted that the role of individual toxins in these cases is usually uncertain, as other mycotoxins are almost always present. T-2 and HT-2 toxins are thought to be the most significant in most cases, but the role of other trichothecenes, such as DON and nivalenol may also be important.

Long-term chronic toxicity from low levels of T-2 and HT-2 toxins in the diet has been investigated in animals. Studies show adverse effects to the immune system, leading to changes in the white blood cell count and, in some cases, decreased resistance to microbial infection. Other effects in animals include reduced feed intake and weight gain. However, there is little evidence of carcinogenicity, and T-2 and HT-2 toxins are not considered likely to be potent carcinogens. Based on the data available from animal studies, the EU Scientific Committee on Food established a temporary tolerable daily intake (TDI) for T-2 and HT-2 toxins (alone or in combination) of 0.06 μg per kg of body weight for humans. A temporary TDI of 0–0.7 μg per kg of body weight was established for nivalenol.

Incidence and Outbreaks

There are a number of documented outbreaks of food-poisoning-like illness caused by foods contaminated with trichothecenes. For example, a series of outbreaks of a condition termed alimentary toxic aleukia were reported in the former Soviet Union during the 1940 s and 1950 s and caused widespread disease with many deaths. These outbreaks were associated with consumption of over-wintered wheat and subsequent analysis of fungi isolated from wheat samples showed that some could produce T-2 toxin and other trichothecenes.

There have also been reported outbreaks affecting hundreds of people in China and India. These were associated with eating contaminated rice. T-2 toxin at concentrations of 180–420 μg kg^{-1} was found in rice from one Chinese

outbreak, but it seems likely that other trichothecenes were involved in some of these cases.

Sources

The principal sources of trichothecenes in cereals and other crops are *Fusarium* species fungi. Group-A trichothecenes are produced by mainly saprophytic species such as *F. poae*, *F. sporotrichioides*, *F. langsethiae* and *F. acuminatum*, whereas group B trichothecenes are produced by cereal pathogens such as *F. graminearum* and *F. culmorum*. All of these are common soil fungi and may colonise or infect cereals in the field.

F. sporotrichioides and *F. langsethiae* are the most important producers of T-2 and HT-2 toxins in cereals in temperate regions and are able to grow at low temperatures (−2 °C to 35 °C). However, they cannot grow at water activities of below 0.88. Most toxin production by these species occurs in water-damaged grains that have either remained in the field for long periods, especially in cold weather, or become damp during storage. T-2 and HT-2 toxins are typically produced on the surface of infected grains. However, where high levels are produced, it may be more evenly distributed in the kernel.

Stability in Foods

Trichothecenes are extremely heat-stable and are not destroyed by temperatures of 120 °C. They therefore survive most cooking processes and T-2 and HT-2 toxins are reported to be relatively stable even in baking processes. Some natural degradation seems to occur in grain in the field or during storage, but the mechanism for this is uncertain.

Control Options

Since trichothecene production occurs in the field and during storage, controls are applied at both the pre-harvest and post-harvest stages.

Pre-harvest

GAP measures designed to reduce *Fusarium* infection in cereal crops are also effective in limiting the formation of trichothecenes. Control measures include the following.

- Land preparation, crop rotation and crop debris removal to reduce the inoculum of *Fusarium* in the field
- Use of fungus-resistant crop varieties
- Control of infection by appropriately timed application of effective fungicides
- Harvesting at the correct moisture level and stage of maturity

Post-harvest Handling and Storage

Further production of trichothecenes after harvest can be prevented by rapid drying to a water activity value of 0.8, and by implementing good storage practice.

Decontamination

Physical decontamination methods, such as gravity separation, can be effective means of reducing trichothecene levels in contaminated grain. The milling process also reduces trichothecene concentrations in wheat flour by removing the generally more heavily contaminated bran, but the effectiveness of this depends on the distribution of the toxin in the grain.

Chemical decontamination methods for T-2 toxin, such as treatment with calcium hydroxide monomethylamine, have been investigated, but are not yet developed for commercial use.

Heat treatments are not usually effective.

Testing

Some countries monitor cereals for T-2 and HT-2 toxin contamination by sampling and testing using analytical methods, such as LC or GC-MS. HPLC methods have also been developed for some group B trichothecenes. Sensitive ELISA methods for T-2 and HT-2 toxins are available for screening purposes and commercial kits are available. However, as with other mycotoxins, the distribution of trichothecenes in bulk commodities may be highly heterogeneous and it is essential to ensure that an adequate representative sampling plan is used.

Legislation

Very few countries around the world have introduced mandatory or guideline levels for trichothecenes, other than DON, in foods.

EU

The EU has not yet set maximum levels for T-2 and HT-2 toxins. However, the public health value of a combined maximum level for cereals and cereal products is due for review after the completion of an appropriate risk assessment. A number of Eastern EU countries did set limits for T-2 toxin in cereals (typically 100 $\mu g\ kg^{-1}$) in national legislation prior to EU accession.

USA

USA food safety regulations include a limit for DON in finished wheat products for human consumption, but not for other trichothecenes.

Others

The Russian Federation and the Ukraine have both set a limit of 100 μg kg^{-1} for T-2 toxin in cereals.

The Canadian authorities have introduced a limit of 1000 μg kg^{-1} for T-2 toxin in pig and poultry feed and 100 μg kg^{-1} for HT-2 toxin in cattle and poultry feed.

More information can be found at the FAO web link below.

Sources of Further Information

Published

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2.1.1.12 Zearalenone

Hazard Identification

What is Zearalenone?

Zearalenone is a toxic fungal metabolite (mycotoxin) produced by certain mould species of the genus *Fusarium* colonising cereal crops in the field and during storage. Zearalenone is an oestrogenic mycotoxin well known as a cause of hormonal effects in livestock, especially pigs and sheep. It is also commonly found in a wide range of food commodities and can be found in processed, ready-to-eat foods. For these reasons it is of concern from a food safety point of view.

Zearalenone ($C_{18}H_{22}O_5$, CAS No. 17924-92-4) is characterised chemically as a phenolic resorcyclic acid lactone and has a molecular mass of 318. It is only slightly soluble in water and is quite stable. Several closely related metabolites of zearalenone have been identified in fungal cultures, notably α- and β-zearalenols, but the presence and significance of these compounds in foods is uncertain.

Occurrence in Foods

Zearalenone has been found worldwide in a range of cereals and other crops, including wheat, barley, maize, rice, oats, sorghum and some legumes. It may also be present in certain vegetable oils and high levels have been reported in bananas grown in India. The level of contamination in cereal crops varies widely depending on climatic conditions. For example, zearalenone was found in 11–80% of wheat samples collected randomly in Germany between 1987 and 1993. The mean yearly contents were 3–180 μg kg^{-1} and the highest level found was 8000 μg kg^{-1}. There is evidence that cereal crops produced by 'alternative' or 'ecological' cultivation methods may develop higher levels of contamination than those produced by conventional methods.

Zearalenone has also been found in processed foods, especially those produced from cereals, although levels are usually low. Foods reported to be contaminated have included wheat and corn flour, bread, breakfast cereals, noodles, biscuits, snacks and corn beer. The metabolite β-zearalenol may be produced from zearalenone by yeast fermentation and so may occur in beer. Contamination with zearalenone does not seem to be a major problem in foods of animal origin. It has been found to be excreted into the milk of lactating cows, along with α- and β-zearalenols, but only when very high oral doses (6000 mg) were used.

Average dietary intakes of zearalenone in humans have been estimated at 1.5 μg per day for the EU diet and 3.5 μg per day for the Middle Eastern diet. Cereals are the major contributor of zearalenone in the diet, but some vegetable oils, especially corn germ oil and wheat germ oil, also contribute to zearalenone exposure. It has been estimated that vegetarian diets in the EU could result in a two-fold increase in zearalenone exposure over conventional diets.

Hazard Characterisation

Effects on Health

The acute toxicity of zearalenone is low and its toxic effects are related to the potent oestrogenic activity of the toxin itself and its metabolites. Zearalenone is metabolised in the gut of animals, especially pigs and potentially humans, forming α- and β-zearalenols. These metabolites are then conjugated with glucuronic acids and may be more potent oestrogens than zearalenone itself.

Zearalenone has been shown to cause hormonal effects on the reproductive systems of pigs and sheep, which appear to be more sensitive than other animal species. Feeding zearalenone to female pigs at levels of up to 0.25 mg kg^{-1} produced slight inflammation of external sexual organs. Effects of higher doses (50 mg kg^{-1}) in the diet of pigs included abortion and stillbirths, while more moderate doses (10 mg kg^{-1}) caused reduced litter sizes and birth weights. Sheep are similarly affected and zearalenone is reported to be a cause of infertility in flocks in New Zealand. Dairy cows are also reported to develop reproductive abnormalities when the toxin is present in the diet.

There is some evidence for similar effects in humans. Zearalenone was suspected as a cause of an outbreak of early secondary breast development affecting girls from six months to eight years old in Puerto Rico between 1978 and 1981. A similar incident was reported in Hungary in 1997. A high incidence of precocious puberty was reported in the Italian district of Viareggio in 2010, which may have been related to elevated levels of serum zearalenone.

There is only very limited evidence for the carcinogenicity of zearalenone. It has been evaluated by the IARC as "not classifiable as to its carcinogenicity in humans (IARC Group 3)". Based on the data available from studies in pigs, the EU Scientific Committee on Food established a temporary TDI for zearalenone of 0.2 μg per kg of body weight for humans.

Sources

The principal sources of zearalenone in cereals are *Fusarium* species, particularly *F. graminearum*, but also *F. culmorum*, *F. equiseti*, *F. verticillioides* and *F. crookwellense*. These species are considered to be field fungi and are pathogenic to cereals, causing diseases such as, *Fusarium* head blight in wheat and *Gibberella* ear rot in maize. The same species also produce other mycotoxins, such as deoxynivalenol, and infected cereals may be contaminated with more than one *Fusarium* toxin.

Zearalenone is produced in the crop prior to harvest, and can continue to be produced during storage in moist grain. Important factors influencing the degree of mould growth and toxin production in crops in the field include high rainfall and high humidity, but toxin production appears to be particularly favoured by wet, cool weather.

Stability in Foods

Zearalenone is heat stable and is not destroyed by temperatures of 120 °C except at higher pH values. It therefore survives most cooking processes and significant quantities (60–80%) are reported to remain even in baked bread and biscuits.

Moderate amounts of zearalenone also appear to survive fermentation processes, such as brewing.

Control Options

Since zearalenone production occurs both in the field and during storage, therefore controls should be applied pre-harvest and post-harvest.

Pre-harvest

GAP measures designed to reduce *Fusarium* infection in cereal crops are also effective in limiting the formation of zearalenone. Control measures include the following.

- Land preparation, crop rotation and crop waste removal to reduce the inoculum of *Fusarium* in the field
- Use of fungus-resistant crop varieties
- Control of infection by appropriately timed application of effective fungicides
- Harvesting at the correct moisture level and stage of maturity

Post-harvest Handling and Storage

Further production of zearalenone after harvest can be prevented by rapid drying to a water activity value of 0.8 immediately after harvest, and by implementing good storage practice.

Decontamination

Physical decontamination methods, including gravity separation, can be effective means of reducing zearalenone levels in contaminated grain. The milling process has also been shown to reduce zearalenone concentrations in corn flour and grits by around 80–90% by removing the more heavily contaminated bran.

Heat treatments are not usually effective.

Testing

In some countries cereals are monitored for zearalenone contamination by sampling and testing using various analytical methods, such as HPLC with UV detection. ELISA methods have also been developed for screening purposes but are less sensitive. As with other mycotoxins, the distribution of zearalenone in

bulk may be highly heterogeneous and it is essential to ensure that an adequate representative sampling plan is used.

Legislation

Few countries outside the EU have yet introduced mandatory or guideline levels for zearalenone in foods.

EU

The EU sets a maximum level for zearalenone of 100 μg kg^{-1} in most unprocessed cereals, but the permitted level in unprocessed maize is 350 μg kg^{-1}. Maize intended for direct human consumption and maize-based snacks and cereals are permitted to contain a maximum of 100 μg kg^{-1}, and the limit for other cereals, flour and bran for direct human consumption is 75 μg kg^{-1}. The limit for bread, cereal snacks, biscuits, pastries and breakfast cereals (excluding maize-based products) is 50 μg kg^{-1}. A limit of 20 μg kg^{-1} has been set for foods intended for babies and young children.

Others

Chile has set a limit for zearalenone of 200 μg kg^{-1} for all foods, while Indonesia requires the toxin to be "not detectable" in maize, and Iran has a limit of 200 μg kg^{-1} for most cereals.

The Canadian authorities have introduced a limit of 3000 μg kg^{-1} for zearalenone in pig feed.

More information can be found at the FAO web link below.

Sources of Further Information

Published

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2.1.1.13 Other Mycotoxins

Introduction

Many toxic fungal metabolites (mycotoxins) have been identified and characterised, but relatively few of these are currently thought to be important from a food safety perspective. The preceding sections have dealt with the most significant food-borne mycotoxins, but there are a number of others that may be relevant to food safety. Some are very uncommon, or usually co-occur with other mycotoxins, and others have been very little studied, so that their public health significance is uncertain.

Brief details are given below of some mycotoxins that may have food safety significance. Most of these are thought to cause toxic effects in animals and may occur naturally in certain food commodities. They therefore have the potential to affect human health.

Aflatrem

Aflatrem is one of a group of related mycotoxins known as tremorgens. These compounds can cause a range of neurological symptoms in animals, including tremors, seizures and even death. Their presence in mould contaminated feed has been implicated in a disease of cattle known as ''staggers syndrome''.

Chemically, aflatrem is an indole-diterpene with a molecular mass of 502. It is a potent tremorgen, and is of importance in food safety because it is produced by *Aspergillus flavus*, which also produces aflatoxins. It may therefore co-occur with aflatoxins in a wide range of food commodities. Aflatrem probably contributes to the overall toxicity of aflatoxins, but its precise significance to human health is uncertain. Control measures designed to prevent aflatoxin formation are also likely to be effective against aflatrem.

Alternaria Toxins

Mould species belonging to the genus *Alternaria*, notably *Alternaria alternata*, are able to attack a range of fruit and vegetable crops at the pre- and post-harvest stages. They also produce a number of toxic metabolites under certain conditions, but most do not seem to occur naturally in foods. Those that do include alternariol, alternariol monomethyl ether, altenuene, altertoxin I, and tenuazonic acid, of which tenuazonic acid is probably the most important and the most toxic. A few rare isolates also produce Alternaria alternata toxin (AAT), a highly toxic compound related to the fumonisins.

Alternaria toxins exhibit a range of acute and chronic toxic effects in animals, especially poultry and rabbits, and have also been implicated in human illness. Tenuazonic acid inhibits protein synthesis and most alternaria toxins are cytotoxic. The altertoxins are also mutagenic.

A. alternata and its toxins have been isolated from cereals, sunflower seeds, olives and a number of fruits and vegetables. It is an important pathogen of

tomatoes and also attacks peppers and apples. Alternaria toxins are normally only found in visibly mouldy food commodities and rarely occur naturally in human food. Therefore the potential for human exposure is thought to be very limited.

Aspergillus Clavatus Toxins

Aspergillus clavatus is a mould species normally found in soil. It is capable of producing a number of toxins in culture, including agroclavine (an ergot alkaloid), cytochalasin E and K and several tremorgens. *A. clavatus* grows well in malting barley and is the cause of condition known as ''malt worker's lung'', but it does not seem to produce significant quantities of mycotoxins naturally in barley. Nevertheless, it has been implicated in the intoxication of cattle consuming mouldy grain.

Citreoviridin

Citreoviridin consists of a lactone ring conjugated to a furan ring and has a molecular mass of 402. It is produced by some species of *Penicillium*, notably *P. citreonigrum* and *P. ochrosalmoneum*. It is a neurotoxin and causes a number of severe symptoms in mice and other animals, including vomiting, convulsions, paralysis and respiratory arrest. Historically, citreoviridin was recognised as the cause of a condition known as ''acute cardiac beriberi'' in Japan, which was linked to the consumption of mouldy ''yellow rice''. The banning of this food in 1910 has eradicated the disease from Japan.

P. citreonigrum is not common, but is widespread, especially in the temperate rice growing regions. It grows in rice after harvest, but only dominates within a narrow moisture range around 15%. *P. ochrosalmoneum* is also rare, but has been isolated from unharvested maize in the USA and may produce citreoviridin naturally in maize under certain conditions.

Other Fusarium Toxins

In addition to the important mycotoxins described elsewhere, species of the genus *Fusarium* produce a number of other less well known and less studied toxic metabolites. Some of these have the potential to affect human health.

Beauvericin is a cyclic hexadepsipeptide produced by *F. subglutinans*, *F. proliferatum* and several other species and has been shown to be toxic to human cells in culture. It has been detected in wheat infected with Fusarium head blight and also in maize, but the extent of human exposure is not known.

Enniatin is also a cyclic hexadepsipeptide and is produced by *F. avenaceum*. It too has been found in wheat infected with Fusarium head blight, but its toxicity and the potential for human exposure are uncertain.

Fusaproliferin is a sesterterpene produced by *F. subglutinans* and *F. proliferatum*. It has been shown to be cytotoxic to some human and animal cell lines and may occur in infected maize.

Gliotoxin

Gliotoxin is a potent immunosuppressive agent produced by the pathogenic mould species *Aspergillus fumigatus* and some other *Aspergillus* and *Penicillium* species. It may have a role in the development of human aspergillosis infections, but there is limited evidence that it is occasionally produced in mould infected cereals.

Mycophenolic acid

Mycophenolic acid is another immunosuppressant produced by some species of *Penicillium*, including *P. roqueforti*. It has been detected in mould-ripened cheese. It has been demonstrated to be toxic at quite high concentrations in rodents and primates and may also be mutagenic.

β-Nitropropionic Acid

This toxin is a toxic metabolite of *Aspergillus oryzae* used in the production of soy sauce. *A. oryzae* has been shown to produce β-nitropropionic acid in cooked potatoes and in ripe bananas. It is a neurotoxin and can cause toxic effects in livestock fed with contaminated feed. It has also been implicated in cases of human illness in China.

Penicillic Acid

Penicillic acid is a toxic metabolite of several species of *Penicillium* and of *Aspergillus* species, including *A. ochraceus*, which also produces ochratoxin A. It can cause liver cancers in some animal species and has been isolated from maize, dried beans and tobacco. It has also been reported to have been detected in fermented sausage.

Phomopsins

Phomopsins are produced by the fungus *Phomopsis leptostromiphoris*, which is an important pathogen of lupins. These toxins may be present at significant levels in lupin seeds used to produce animal feed, but also now increasingly used as an ingredient in human foods. The phomopsins are potent liver toxins and carcinogens in rats and other animals. Their significance in human health in not known, but their presence in foods is considered undesirable and of concern, especially as they are stable compounds likely to survive cooking processes. Australian legislation sets a maximum level of 5 μg kg^{-1} for phomopsins in lupin seeds and lupin seed products.

PR-toxin

PR-toxin is a toxic metabolite of *Penicillium roqueforti*. It is lethal to rats, mice and cats and is reported to cause toxic effects in the lungs, brain, liver and

kidney. It has been detected at low levels in blue cheeses and mouldy cereal grains. It is not particularly stable in cheese and degrades to other less toxic compounds quite rapidly. Adverse health effects associated with consumption of blue cheese containing PR-toxin have not been reported.

Penitrem A

Penitrem A is a potent neurotoxin produced primarily by *Penicillium crustosum*, which is a common and widespread food and feed spoilage mould. It is a tremorgen and has been associated with outbreaks of tremorgenic disease in cattle, sheep and horses. Its significance for human health is so far uncertain. *P. crustosum* can cause spoilage in a variety of foods, including maize, nuts, cheese, cured and processed meat products, cakes and biscuits, and fruit. Most strains can potentially produce penitrem A at high levels, but only at high moisture levels. This may explain the comparatively few reports of animal and human poisoning caused by this toxin.

Roquefortines

Roquefortines A, B and C are reported to be produced by several *Penicillium* species, including *Penicillium roqueforti*, used in the production of some blue cheeses. They are indole compounds and have been reported to be toxic to rats, mice and poultry at relatively high levels. Their significance for human health is so far uncertain. Roquefortines have been detected in blue cheese, but only at low levels, and adverse health effects associated with consumption of blue cheese containing these compounds have not been reported.

Satratoxins

The satratoxins are trichothecene mycotoxins produced by fungi of the genus *Stachybotrys*, notably *Stachybotrys chartarum*. These fungi are widespread and have been isolated mainly from environmental samples, especially from water-damaged buildings, but also from mouldy cereals. The satratoxins are potent toxins that inhibit protein synthesis in mammalian cells. They have been linked with a disease of horses associated with consumption of mouldy hay and straw and also with illness in other animals. Their food safety significance is uncertain.

Viomellein, Vioxanthin and Xanthomegnin

These toxins are produced by some *Penicillium* species, such as *P. cyclopium* and *P. viridicatum* and also by *Aspergillus* species, including *A. ochraceus*. They are known to co-occur with other mycotoxins, especially ochratoxin A, and are nephrotoxic. They may be involved in kidney disease of animals, such as pigs, caused by ochratoxin A, but their food safety significance is not known.

Walleminol A

Walleminol A is a toxic metabolite of the xerophilic mould species *Wallemia sebi*, which is known to grow on a wide range of foods, including cereals, pulses, dried fruits, cakes, confectionary and conserves. Walleminol A has been shown to be toxic to animal cells, but its significance for human health and its occurrence in foods have not yet been investigated.

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2.1.2 PLANT TOXINS

2.1.2.1 Cucurbitacins

Hazard Identification

What are Cucurbitacins?

Courgettes (zucchini), together with many closely related species of the *Cucurbitacea* family, including cucumber and squash, produce an intensely bitter group of compounds known as cucurbitacins. Some wild-type squashes are so bitter that they become almost inedible to humans and most animals. Some can even kill small animals.

The cucurbitacins are highly oxygenated triterpenoid compounds and are divided into twelve different categories according to their structure. They are potent toxins with natural insecticidal and/or fungicidal properties.

Occurrence in Foods

Natural production of cucurbitacins occurs in members of the cucumber family. As well as cucumbers, these include courgettes, marrows, melons and squashes. The compounds are responsible for the bitter taste that is sometimes evident in cucumbers and courgettes.

The varieties of courgette and squash that are grown commercially and domestically in the garden have been selected for low levels of these bitter compounds, although one notable exception to this is bitter melon, which is used in Asian cuisine, where the bitterness is a prized part of the flavour. Larger courgettes and marrows will have higher levels of cucurbitacins than smaller fruit. Natural cross-pollination with wild varieties may also increase the bitterness of cultivated varieties.

Hazard Characterisation

Effects on Health

Cucurbitacins are toxic at high levels, but they are so bitter that it is almost impossible for anyone to eat sufficient quantity of the toxins to cause significant harm. Cucurbitacin-B, for example, has an oral LD_{50} in the mouse of 5 mg per kg of body weight. Theoretically, this means that a dose of 300 mg could be sufficient to kill a human.

In New Zealand, in the early summer of 2001, there was a series of cases of severe stomach cramps associated with eating courgettes. So many cases were reported that the health authorities instigated an official investigation. Many of those who became ill reported eating bitter-tasting courgettes. The summer had been unusually wet, which favoured fungal infection, and it is likely that increased fungal infection led to up-regulation of the genes involved in cucurbitacin production, thus increasing the toxin levels in the courgettes.

Because of their extreme bitter taste, ingestion of cucurbitacins is usually limited and symptoms of intoxication are generally mild. Stomach cramps, nausea, vomiting and diarrhoea have all been reported. However, a fairly recent event illustrates how toxic these substances can be. *The Times* of India (10th July 2010) reported the case of a 60-year-old man who died after drinking a bottle of bitter gourd juice (lauki) on an empty stomach. His wife complained of vomiting blood and severe diarrhoea, but survived. The scientist was reported to have been drinking the juice for at least four years, but had complained that the bottle concerned was especially bitter tasting.

Sources

The natural production of cucurbitacins, which occurs in members of the *Cucurbitacea* family, is controlled by the plants so that they are produced only when they are needed. The gene that codes for curcubitacin production is switched on only when climatic conditions are favourable for insect infestation or fungal infection. Their concentration therefore varies according to weather and the potential for fungal infestation or insect attack.

Commercially grown cucumbers, courgettes and related vegetables have been selected for low levels of the bitter cucurbitacins. However, even carefully selected varieties will produce high levels of the toxins when environmentally stressed, or when conditions are ripe for fungal infection or insect infestation.

Stability in Foods

Cucurbitacins are heat resistant and only slightly soluble. They are therefore neither destroyed, nor removed by cooking of courgettes and other food plants.

Control Options

There is little that can be done to reduce the level of cucurbitacins once the plant has started to produce them. Their heat stability and poor solubility mean that cooking the vegetables in water has little effect. It is thought that cutting off the end of the courgette, nearest to the blossom, can reduce some of the bitterness. The preferred control options are to ensure that the plants are watered carefully during growth, and to harvest the crop as early as possible.

Legislation

There is no specific legislation governing cucurbitacin levels in foods. Plants of the Cucurbita family are included in the European Food Safety Authority (EFSA) ''Compendium of Botanicals that have been reported to contain toxic, addictive, psychotropic or other substances of concern'', published in the EFSA Journal in 2009.

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2.1.2.2 Cyanogenic Glycosides

Hazard Identification

What are Cyanogenic Glycosides?

Cyanogenic glycosides are chemical compounds that occur naturally in many plants, including species of *Prunus* (wild cherry), *Sambucus* (elderberry), *Manihot* (cassava), *Linum* (flax), *Bambusa* (bamboo) and *Sorghum* (sorghum). Chemically, they are defined as glycosides of the α-hydroxynitriles. These compounds are potentially toxic as they are readily broken down by enzymic hydrolysis to liberate hydrogen cyanide when the plant suffers physical damage.

Occurrence in Foods

There are approximately 25 known cyanogenic glycosides, and a number of these can be found in the edible parts of some important food plants. These include amygdalin (almonds), dhurrin (sorghum), lotaustralin (cassava), linamarin (cassava, lima beans), prunasin (stone fruit) and taxiphyllin (bamboo shoots). Table 2.1.1 below summarises some of the main food sources of cyanogenic glycosides and their estimated potential yield of hydrogen cyanide released on hydrolysis.

Table 2.1.1 Cyanogenic food sources and their approximate hydrogen cyanide yield.[a]

Food source	*Cyanogenic glycoside*	*Hydrogen cyanide yield/mg per 100 g fresh weight*
Almond bitter seed	Amygdalin	290
Apricot kernel	Amygdalin	60
Bamboo stem (unripe)	Taxiphyllin	300
Bamboo sprout tops (unripe)	Taxiphyllin	800
Cassava tuber bark (less toxic clones)	Linamarin and Lotaustralin	69
Cassava inner tuber (less toxic clones)	Linamarin and Lotaustralin	7
Cassava tuber bark (very toxic clones)	Linamarin and Lotaustralin	84
Cassava inner tuber (very toxic clones)	Linamarin and Lotaustralin	33
Flax seedling tops	Linamarin, Linustatin and Neolinustatin	91
Black Lima bean, Puerto Rico (mature seed)	Linamarin	400
Peach kernel	Prunasin	160
Sorghum shoot tips	Dhurrin	240
Wild cherry leaves	Amygdalin	90–360

[a]Adapted from: Frehner, M., Scalet, M. and Conn, *Plant Physiology,* 1990, 94, 28–34.

Bitter apricot kernels have been marketed as a health food in the UK and elsewhere. They can contain high levels of the cyanogenic glycoside amygdalin. Analytical data indicates that the bitter apricot kernels currently on sale have a cyanide content of 1450 mg kg^{-1} (approximately 0.5 mg per kernel). While swallowing of apricot kernels whole may not release much cyanide, grinding or chewing them significantly increases its release.

Hazard Characterisation

Effects on Health

The toxicity of a cyanogenic plant depends largely on the amount of hydrogen cyanide that could be released on consumption of the plant. Adequate processing or preparation is required to ensure that detoxification of the food is complete before consumption. However, if the processing or preparation is insufficient to ensure detoxification, the potential hydrogen cyanide concentration released during consumption can be high. Upon consumption of the food, the enzyme β-glycosidase will be released and hydrolysis of the cyanogenic glycoside will commence, resulting in hydrogen cyanide formation. Certain gut microflora also produce β-glycosidases, which can contribute to the breakdown of cyanogenic glycosides to hydrogen cyanide.

Hydrogen cyanide is cytotoxic and blocks the activity of cytochrome oxidase—an enzyme critical for cellular respiration. When cytochrome oxidase is blocked, ATP production stops and cellular organelles cease to function. However, cyanide is readily detoxified in animals as all animal tissues contain the enzyme rhodanese—a thiosulfate sulfurtransferase enzyme that converts cyanide to thiocyanate, which is then excreted in urine. Acute poisoning only occurs when this detoxification mechanism is overwhelmed.

The symptoms of acute cyanide poisoning include rapid breathing, drop in blood pressure, raised pulse rate, dizziness, headache, stomach pains, vomiting, diarrhoea, confusion, twitching and convulsions. In extreme cases, death may occur. The minimum lethal dose of hydrogen cyanide taken orally is approximately 0.5–3.5 mg per kg of body weight, or 35–245 mg for a person weighing 75 kg.

The chronic effects of cyanide consumption are associated with regular long-term consumption of foods containing cyanogenic glycosides in individuals with poor nutrition. These effects are most notable in the tropics, where cassava, and to a lesser extent, sorghum, bamboo shoots and lima beans are staple components of human diets. Malnutrition, growth retardation, diabetes, congenital malformations, neurological disorders and myelopathy are all associated with cassava-eating populations subject to chronic cyanide intake.

There are a number of documented cases of poisoning caused by consumption of apricot kernels. One report concerned a 41-year-old female found comatose after eating approximately 30 bitter apricot kernels, who eventually recovered after treatment. There are also case reports of children being poisoned after consumption of wild apricot kernels and where the kernels were

made into sweets without proper processing. The UK Committee on Toxicity recommended in March 2006 that a tolerable daily intake (TDI) of 20 μg cyanide per kg of body weight per day be applied, which is the equivalent of 1–2 bitter apricot kernels per day.

Sources

There are over 2500 known species of plants that produce cyanogenic glycosides, usually in combination with a corresponding hydrolytic enzyme—a β-glycosidase. When the cell structure of the plant is disrupted in some way, for example by predation, the β-glycosidase is brought into contact with its substrate – the cyanogenic glycoside. This leads to the breakdown of the glycoside to sugar and a cyanohydrin, which rapidly decomposes to release hydrogen cyanide. The purpose of the reaction is to protect the plant from predation.

Numerous plants known to produce cyanogenic glycosides are cited in the EFSA "Compendium of Botanicals that have been reported to contain toxic, addictive, psychotropic, or other substances of concern".

Stability in Foods

Cyanogenic glycosides break down when the cells of the plant are damaged, for example during preparation and processing, and release hydrogen cyanide. Hydrogen cyanide itself is not heat stable and does not survive boiling and cooking processes. It can also be eliminated by fermentation.

Control Options

Processing

Adequate processing of cyanogenic glycoside-containing plants should be sufficient to significantly reduce or remove the toxic agents prior to consumption. Processing procedures, such as peeling and slicing, disrupt the cell structure of the plant so that β-glycosidases are released and the cyanogenic glycosides are hydrolysed. Hydrogen cyanide is thus released and can be removed by cooking processes such as baking, boiling or roasting. Fermentation is also used to remove hydrogen cyanide. These methods are particularly suitable for products such as cassava and bamboo shoots. There are two main types of cassava—bitter cassava and sweet cassava. The sweet variety contains a significantly lower concentration of cyanogenic glycosides than the bitter variety, and it is the sweet variety that is used commercially. Cassava is consumed largely as cassava flour, cassava chips and tapioca pearls, all of which are processed products with a long history of safe consumption.

Treatments for removing cyanogenic compounds from flaxseed include boiling in water, dry and wet autoclaving and acid treatment followed by autoclaving. Solvent extraction has also been used to remove cyanogenic glycosides from flaxseed and oil.

Legislation

A safe level of cyanide in cassava flour for human consumption has been set by the World Health Organization (WHO) at 10 ppm.

Low levels of cyanide are also present in almonds, sweet apricot kernels and in the stones of other fruit such as cherries, as well as in bitter apricot kernels. In the UK, the maximum level of cyanide that can be present as a result of using such materials as flavourings is regulated under the terms of the Flavourings in Food Regulations 1992 (as amended).

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2.1.2.3 Furocoumarins

Hazard Identification

What are Furocoumarins?

The furocoumarins are a group of naturally occurring chemicals that are found in a wide variety of plants, but which are present at their highest concentrations in members of the *Umbelliferae* family, particularly parsnips, celery and parsley. They are also present in lower concentrations in other foods such as citrus fruit, celeriac and figs, and in herbal preparations containing *Angelica archangelica* L. There are many different furocoumarins, but they all have similar molecular structures. Examples include psoralen, bergapten, xanthotoxin and isoimperatorin. The furocoumarins all have insecticidal and/or fungicidal activity, but they are also photoactivated carcinogens and are therefore significant from a food safety point of view.

Occurrence in Foods

The highest concentrations of furocoumarins are found in parsnips, celery and parsley (see Table 2.1.2).

Organically grown vegetables often have higher levels of furocoumarins. This may be because conventional cultivation involves the use of pesticides, and conventionally grown plants have less need to produce natural chemical defences in response to the threat of predation by insects. Damaged vegetables also contain significantly higher levels of furocoumarins than intact produce.

Furocoumarins have also been detected in some processed foods, particularly purees and soups, with the highest levels being found in soups containing celery. Other sources include citrus fruits, marmalade and sweet fennel. Herbal preparations containing *Angelica archangelica* L. are also known to be a source of furocoumarins.

Hazard Characterisation

Effects on Health

Furocoumarins are photoactivated carcinogens. This means that they absorb long-wave UV radiation upon exposure of the skin to sunlight and are activated by the light to form carcinogens. Prolonged exposure can result in cell damage, by binding pyrimidine bases and nucleic acids and thus inhibiting DNA

Table 2.1.2 Furocoumarins in commonly eaten foods.[a]

Plant	*Main furocoumarin*	*Concentration/mg kg^{-1}*
Celery	Bergapten	1.3–47
Parsnip	Bergapten	40–1740
Parsley	Isoimperatorin	11–112

[a]MAFF survey 1996.

synthesis. The oral LD_{50} for psoralen in rats has been reported to be 791 mg per kg of body weight.

They can also cause skin sensitisation to UV light, resulting in skin rashes after prolonged skin exposure to the sun. A fairly high intake is required to cause photosensitisation. The main symptom is peeling and blistering of the light-exposed parts of the skin of someone who has consumed a fairly large quantity of parsnips or celery, particularly damaged produce that has been organically produced. There have been two reported cases of phototoxic reactions after consumption of celery. Both involved extreme intakes of celery and strong UVA exposure.

A condition known as "celery dermatitis" has also been noted. The symptoms include blistering of the arms of farm workers handling celery when the celery is diseased with pink rot (*Sclerotinia sclerotiorum*) and produces xanthotoxin and trisoralen.

Health authorities in a number of countries, including Switzerland, the USA, the UK and Germany have made risk assessments of dietary furocoumarins. On the basis of these risk assessments, an average daily intake of 1.45 mg of furocoumarins has been estimated. The assessments have concluded overall that the risk from dietary furocoumarins is very small, or insignificant.

An assessment made by the Committee on Herbal Medicinal Products in 2007 concluded that, for herbal products containing *Angelica archangelica* L., daily exposure of 1.5 mg would provide no unacceptable risk for the consumer. However, for preparations providing more than 1.5 mg furocoumarins per day, a benefit–risk assessment would be required. In addition, groups such as children and pregnant women should be contraindicated for all preparations containing *Angelica archangelica* L., irrespective of dose. Warnings with respect to co-factors, particularly UV light exposure should be provided.

Sources

Furocoumarins are produced by many plants in response to stresses such as bruising or injury caused by predation. The plants respond to damage by up-regulating natural pesticide production to prevent insect attack or fungal infection.

Stability in Foods

Furocoumarins are quite heat stable and cooking does not reduce their concentration significantly.

Control Options

There are few effective controls for furocoumarins in celery and parsnips, although it has been recommended that new cultivated varieties be monitored for furocoumarin content before widespread planting. Avoiding damage to crops in the field and during harvesting may help to reduce furocoumarin levels.

Processing

Although furocoumarins are not inactivated by heating, they are water soluble. Therefore, if furocoumarin-containing vegetables are cooked in water, the levels in the vegetable can be appreciably reduced.

Product Use

Consumers with high dietary exposure to vegetables with potentially high furocoumarin levels may benefit by avoiding produce showing evidence of physical damage.

Legislation

The content of furocoumarins in vegetables is not generally regulated by legislation.

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2.1.2.4 Glycoalkaloids

Hazard Identification

What are Glycoalkaloids?

Many plants in the *Solanaceae* family contain glycoalkaloids, and they are considered to be natural toxins. They are active as pesticides and fungicides and are produced by the plants as a natural defence against animals, insects and fungi that might attack them.

The plant glycoalkaloids are toxic steroidal glycosides and the commonest types found in food plants are α-solanine and α-chaconine, with α-solanine ($C_{45}H_{73}NO_{15}$, CAS No. 20562-02-1) being the more toxic of the two.

Occurrence in Foods

Amongst the most widely cultivated food crops, aubergines, tomatoes and potatoes are in the *Solanaceae* family; however, the levels of glycoalkaloids in tomatoes and aubergines are generally quite low and are therefore not a concern. The glycoalkaloids of most relevance to food safety are those occurring in the potato, since even in commercially available tubers destined for human consumption a residual level of these compounds is always present.

The predominant toxic steroidal glycosides in potato are α-solanine and α-chaconine. They occur in potato tubers, peel, sprouts and blossoms and their concentration in tubers depends on a number of factors, such as cultivar, maturity, environmental factors and stress conditions.

In the UK, the total glycoalkaloid level in tubers destined for human consumption is generally in the range 25–150 mg kg^{-1} fresh weight, but considerably higher levels have been recorded for certain commercial varieties. As an example, the *Lenape* potato variety was withdrawn from commercial growing in Canada and the USA as it contained unacceptably high levels of glycoalkaloids. In Sweden, a conditional sales ban had to be imposed on potato tubers of the commercially established variety *Magnum Bonum* harvested in 1986, as they contained potentially toxic levels of glycoalkaloids.

Hazard Characterisation

Effects on Health

Most cases of suspected potato poisoning involve only mild gastrointestinal effects, which generally begin within 8–12 hours after ingestion and resolve within one or two days. However, reported symptoms have included nausea and vomiting, diarrhoea, stomach cramps and headache. More serious cases have experienced neurological problems, including hallucinations and paralysis, and fatalities have also been recorded.

Table 2.1.3 Documented Incidents of potato poisoning.[a]

Year	*Details*	*Effects*
1925	Seven family members ate greened potatoes.	Extreme exhaustion, restlessness, rapid breathing, loss of consciousness. Death of two family members.
1933	In Cyprus, 60 people consumed young potato shoots and leaves as a vegetable.	Headache, nausea, vomiting, diarrhoea, fever, throat irritations. One death.
1952–53	382 North Koreans affected following consumption of rotten potatoes.	Pain, nausea, vomiting, facial oedema, respiratory failure, cardiac arrest. 52 hospitalised and 22 deaths.
1979	78 London schoolboys consumed potatoes left over from a previous term.	Diarrhoea, vomiting, circulatory, neurological, dermatological problems. 17 hospitalised.
1986	11 people in Sweden consuming Magnum Bonum variety potatoes.	Nausea, vomiting, pain, headache.

[a]Partially taken from McMillan, M. and Thompson, J.C., *Quarterly Journal of Medicine*, 1979, 48, 227–43.

Although suspected potato poisoning is rare, a number of incidents have been documented, and a few of the more recent ones are tabulated in Table 2.1.3.

Although glycoalkaloids are suspected to be the cause of these symptoms, there is little data to confirm this. One study examined case reports of poisoning incidents and estimated that glycoalkaloid doses of 2–5 mg per kg of body weight would be enough to cause symptoms in humans and that 3–6 mg per kg of body weight could be fatal. However, a toxicological monograph produced by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1992 states that "Glycoalkaloids are not acutely toxic by the oral route in laboratory animals even at very high doses (up to 1 g per kg body weight) in some species". The Committee considered that the evidence implicating glycoalkaloids in potato poisoning cases was not convincing. JECFA concluded that levels of α-solanine and α-chaconine normally found in potatoes (20–100 mg kg^{-1}) were not of toxicological concern.

Nevertheless, JECFA and others have expressed concern about glycoalkaloids in skin-on potato products, such as crisps, that became widely available in the mid 1990s. Glycoalkaloid concentrations of up to 720 mg kg^{-1} were found in 'green-skinned' crisps, compared with a maximum of 150 mg kg^{-1} in normal crisps.

Apart from their toxicity, glycoalkaloids are also associated with a bitter taste and burning sensation in the throat.

Sources

Although glycoalkaloids in potatoes are produced naturally by the plant, certain factors can have a significant effect on the levels present.

Maturity

The highest concentrations of glycoalkaloids are usually associated with areas that are undergoing high metabolic activity, such as potato flowers, young leaves, sprouts, peels and the area around the potato 'eyes'. Small immature tubers are normally high in glycoalkaloids since they are still metabolically active.

Exposure to Light

Exposure to light has a significant effect on the concentration of both total and individual glycoalkaloids. Potatoes that become sunburned during growth and start to 'green', owing to lack of soil cover, tend to taste very bitter as a result of their high glycoalkaloid content.

In retail outlets, tubers may be displayed under fluorescent lighting and this can increase glycoalkaloid concentration. Studies have indicated that replacing fluorescent lights with mercury lighting for potatoes on display would significantly reduce glycoalkaloid content and improve food safety.

Storage Temperature

Storage at very low temperatures (0–5 °C) results in more bitter-tasting potatoes and thus more glycoalkaloids than storage at higher temperatures (up to 20 °C). On the whole, storage at lower temperatures will prolong potato quality, but at very low temperatures (0–5 °C), stress becomes a factor and glycoalkaloid accumulation starts to occur.

Injury/Damage

Any type of injury or damage to the tuber will result in the accumulation of glycoalkaloids. Disease, insect attack or rough handling, during or after harvest, will all initiate glycoalkaloid synthesis (as it is a defence response). Damaged potatoes from retail generally contain elevated levels of glycoalkaloids.

Stability in Foods

Glycoalkaloids are relatively stable in potatoes and levels are not affected by boiling, freeze-drying, or dehydration. Microwave cooking has only a limited effect, but cooking at temperatures at or above 170 °C is more effective at lowering levels.

Control Options

Cultivar Selection

The amounts of total and individual potato glycoalkaloids are genetically controlled. The most effective way of obtaining low levels is to select breed varieties that are initially very low in glycoalkaloids.

Processing

Peeling

In normal tubers, potato glycoalkaloids appear to be concentrated in a small 1.5-mm layer immediately under the skin, therefore, with normal tubers, peeling will remove between 60–95% of the glycoalkaloids present. However, if the tubers are very high in glycoalkaloids, peeling will remove only up to 35%, as diffusion into the deeper tissues occurs at higher concentrations. Unfortunately, peeling or slicing also elicits a stress response in the tubers and causes a slow rise in glycoalkaloid levels. If long delays occur before subsequent processing, glycoalkaloids can accumulate.

Cooking

The heat stability of glycoalkaloids means that only high-temperature processing, such as deep-frying, has any significant effect on levels in potatoes. Other processes give little or no reduction in the concentration of these compounds.

Physical/Chemical Treatments

Gamma-irradiation has been shown to control glycoalkaloid levels, particularly in damaged tubers. Treatment with certain chemicals, most of which function as sprout inhibitors, has also been shown to control glycoalkaloid accumulation.

Legislation

Although there is no specific legislation governing glycoalkaloid levels in potatoes, the generally accepted safe upper limit is considered to be 200 mg glycoalkaloids per kg of fresh potato. Plants of the Solanum family are included in the EFSA ''Compendium of Botanicals that have been reported to contain toxic, addictive, psychotropic or other substances of concern'', published in the EFSA Journal in 2009.

Sources of Further Information

Published

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European Food Safety Authority. Compendium of Botanicals that have been reported to contain toxic, addictive, psychotropic or other substances of concern. *EFSA Journal,* 2009, 7(9), 281.

Cantwell, M. Glycoalkaloids in Solanaceae. *Food Reviews International*, 1994, 10(4), 385–418.

On the Web

JECFA review (1992). http://www.inchem.org/documents/jecfa/jecmono/v30je19.htm

USA National Toxicology Program literature review (α-solanine and α-chaconine) 1998. http://ntp.niehs.nih.gov/ntpweb/index.cfm?objectid = 6F5E933B-F1F6-975E-7B1D19DE73F21505

2.1.2.5 Grayanotoxin

Hazard Identification

What is Grayanotoxin?

Grayanotoxins are natural plant toxins found in rhododendrons and other plants of the family *Ericaceae*. Specific grayanotoxins vary according to the plant species in which they are found. They can be found in honey made from the nectar produced by the flowers of these plants, and can cause a very rare poisonous reaction.

Grayanotoxin compounds are diterpenes—polyhydroxylated cyclic hydrocarbons that do not contain nitrogen. Alternative names for grayanotoxin include andromedotoxin, acetylandromedol, and rhodotoxin.

Occurrence in Foods

Honeys originating from Japan, the USA, British Colombia, Brazil, Turkey and Nepal are those most likely to be contaminated with grayanotoxin. Honey obtained locally from farmers who may have only a few hives is at increased risk, particularly in regions where plants of the *Ericaceae* family dominate the vegetation. The pooling of massive quantities of honey during commercial processing generally dilutes any toxic substances.

Hazard Characterisation

Effects on Health

Grayanotoxins elicit their effects by binding to sodium channels in cell membranes. All of the observed responses of skeletal and heart muscles, nerves, and the central nervous system are related to these membrane-binding effects.

Grayanotoxin intoxication is rarely fatal. Symptoms include dizziness, weakness, excessive perspiration, nausea, and vomiting shortly after the toxic honey is ingested. Other symptoms may include low blood pressure or shock, bradyarrhythmia (slowness of the heart beat associated with an irregularity in the heart rhythm) and other cardiac abnormalities. Despite the potential cardiac problems, the condition is rarely fatal and generally lasts less than a day.

Several cases of grayanotoxin poisoning have been documented, many associated with honey originating in Turkey. Between 1984 and 1986, 16 patients in Turkey had to be treated for honey intoxication. One case in Austria, which resulted in cardiac arrhythmia, was attributed to honey brought back from a holiday in Turkey. In this case, the patient needed a temporary cardiac pacemaker to deal with the decrease in heart rate. Grayanotoxin poisoning has also been reported in goats in the UK.

Sources

Rhododendrons are the main documented source of grayanotoxins, but not all rhododendrons produce them. *Rhododendron ponticum*, which grows extensively in the mountains of the Eastern Black Sea area of Turkey has been associated with honey poisoning since 401 BC (according to the writings of Pliny the Elder). Other species known to produce the toxins grow over large areas of the USA. In the Eastern part of the USA, grayanotoxin-contaminated honey may be derived from other members of the family *Ericaceae*.

Control Options

Most honey contaminated with grayanotoxin originates in areas of the world where the vegetation is dominated by *Ericaceae*, particularly areas of Turkey, Japan, Brazil, the United States, Nepal, and British Columbia. Extra care should be taken with honeys originating from these parts.

Processing

Chemical Analysis

The grayanotoxins can be isolated from the suspect product by the typical extraction procedures used for naturally occurring terpenes, and the toxins can be identified by thin layer chromatography.

Legislation

No specific legislation regarding grayanotoxin levels in honey exists. A number of plant species that produce grayanotoxin are cited in the EFSA ''Compendium of Botanicals that have been reported to contain toxic, addictive, psychotropic or other substances of concern''.

Sources of Further Information

Published

Gunduz, A., Turedi, S., Uzun, H. and Topbas, M. Mad Honey Poisoning. *American Journal of Emerging Medicine*, 2006, 24(5), 595–8.

Lampe, K.F. Rhododendrons, mountain laurel, and mad honey. *Journal of the American Medical Association*, 1988, 259(13), 2009.

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2.1.2.6 Lectins

Hazard Identification

What are Lectins?

Lectins are proteins that are widely distributed in nature and occur in many plants commonly consumed in the diets of humans and animals. They are toxic to humans and animals but their toxicity varies depending on their source. They were originally discovered in the 19th Century, when it was found that the extreme toxicity of castor beans could be attributed to a protein fraction capable of agglutinating erythrocytes (red blood cells). This protein fraction was given the name *ricin*, as it was derived from *Ricinus communis* (the castor oil plant). Since then, many other lectins similar to ricin have been discovered. For example, lectins are found in common edible legumes such as kidney beans, soya beans, lentils, peas and peanuts. Lectins are also commonly known as phytohaemagglutinins, owing to their ability to agglutinate red blood cells.

Lectins are characterised by their highly specific carbohydrate-binding activity, and it was this high degree of specificity that led Boyd and Shapleigh in the 1950 s to coin the term ''lectins'' from the Latin word *legere*, meaning to choose.

Most lectins are actually glycoproteins containing two or four subunits, each of which has a sugar-binding site. Lectins are generally identified by the plant species that they are derived from.

Occurrence in Foods

As can be seen from Table 2.1.4, leguminous vegetables are the most frequently encountered food sources of lectins, although other sources have been reported, such as dry cereals and wheat germ. The amounts and specificity of the lectins obtained from different sources vary widely, but the highest concentration is found in red kidney beans (*Phaseolus vulgaris*). The unit of toxin measure is the

Table 2.1.4 Properties of some common lectins.

Common name	*Botanical name*	*Molecular mass*	*Number of subunits*
Peanut	*Arachis hypogeae*	110 000	4
Kidney bean	*Phaseolus vulgaris*	126 000	4
Fava bean	*Vicia faba*	52 500	4
Soya bean	*Glycine max*	120 000	4
Lentil	*Lens esculenta*	46 000	4
Winged bean	*Psophocarpus tetragonolobus*	58 000	2
Garden pea	*Pisium sativum*	49 000	4
Horse gram	*Dolichos biflorus*	110 000	4
Lima bean	*Phaseolus lunatus*	60 000	2
Navy bean	*Phaseolus vulgaris*	128 000	4
Jack bean	*Canavalia ensiformis*	110 000	4

hemagglutinating unit (hau). Raw kidney beans contain from 20 000 to 70 000 hau, while fully cooked beans contain from 200 to 400 hau. White kidney beans, another variety of *Phaseolus vulgaris*, contain about one-third the amount of toxin as the red variety; broad beans (*Vicia faba*) contain 5 to 10% the amount that red kidney beans contain.

Despite the fact that most food-derived lectins are inactivated by heat processing, lectin activity has been detected in processed food items such as dry cereals and peanuts, dry-roasted beans and processed wheat germ.

Hazard Characterisation

Effects on Health

One of the most important structural features of lectins is the fact that they consist of two or four subunits, each having a sugar-binding site. This feature of multivalency enables the lectins to agglutinate red blood cells by binding to one cell *via* its surface proteins and attaching another cell to a different part of the protein molecule, effectively sticking the red blood cells together to form a clot, which can block blood vessels.

It has been shown that kidney bean lectins are able to bind specific receptor sites on the surface of the epithelial cells lining the intestine. This is accompanied by the appearance of lesions and disruption of the microvilli lining the digestive tract, which then leads to a severe impairment in the absorption of nutrients across the intestinal wall. Some lectins are highly toxic, for example, phasin from red kidney beans can lead to death at a concentration as low as 5 μg per kg of body weight.

Onset of symptoms usually starts within 1–3 hours of consumption of raw or undercooked lectins. Symptoms include acute gastroenteritis, sickness and abdominal pain, which may be severe enough to require hospitalisation. The symptoms generally clear within 3–4 hours and recovery is usually rapid and complete.

A number of incidents of human intoxication by lectins have been documented in the literature. In 1948, the population of West Berlin suffered a serious bout of gastroenteritis caused by the consumption of partially cooked beans that had been airlifted into the city during the Russian blockade. Illness has been reported in countries such as Tanzania, where a mixture of beans and maize is cooked as porridge for infants. The mixture often retains lectin activity owing to insufficient cooking, possibly caused by poor heat transfer to the beans through the viscous food mass. In 1976, an acute outbreak of sickness and diarrhoea occurred in a group of schoolboys in the UK and was attributed to the consumption of kidney beans that had been soaked in water but not cooked. An intake of 4–5 beans was sufficient to elicit the response, and two of the boys were hospitalised and required intravenous infusion. Following this incident, the Ministry of Health asked the public to report any similar experiences, which resulted in over 800 reports of illness.

Several UK outbreaks have been associated with slow-cooking devices, or casseroles, which had not reached a high enough internal temperature to destroy the glycoprotein lectin. It has been shown that heating beans to 80 °C may potentiate toxicity five-fold, so that these beans are more toxic than if eaten raw. In studies of casseroles cooked in slow cookers, internal temperatures often did not exceed 75 °C, and were probably insufficient to destroy all of the lectin activity, even though the beans were deemed acceptable in terms of texture and palatability.

Sources

Although many different lectins have now been identified in a wide range of plant species as detailed above (see Table 2.1.4), their role in plants is still uncertain. It seems likely that they do perform a physiological function connected with their ability to bind to carbohydrate-containing molecules. However, in some plants they are also thought to play a role in protecting the plant against attack by insects and fungi, and physical damage or fungal invasion may result in elevated lectin levels.

Stability in Foods

Lectins are proteins, and are denatured and inactivated by an adequate heat process. Boiling or autoclaving lectin-containing beans has been found to be effective, although preliminary soaking in water may be required. Dry heat is much less effective and lectin activity in some beans may remain after heating for several hours if they have not been soaked in water.

Control Options

Toxic lectins in edible legume species can be inactivated by adequate preparation and cooking procedures.

The following procedure is recommended by the UK Health Protection Agency and other authorities for the safe cooking of red kidney beans:

1. Soak in water for at least 5 hours
2. Pour away the soaking water
3. Boil briskly in fresh water, with occasional stirring, for at least 10 min

Food processors should be aware of these guidelines when using lectin-containing bean species as ingredients. Canning processes will inactivate lectins and canned beans can be used without further treatment.

Legislation

There is no specific legislation governing lectin levels in foods. A number of plant species that produce lectins are cited in the EFSA "Compendium of

Botanicals that have been reported to contain toxic, addictive, psychotropic or other substances of concern''.

Sources of Further Information

Published

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Nachbar, M. and Oppenheim, J. Lectins in the US diet: A survey of lectins in commonly consumed foods and a review of the literature. *American Journal of Clinical Nutrition*, 1980, 33, 2338.

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On the Web

Paper in Livestock Research for Rural Development (vol. 3, issue 3, December 1991). http://www.fao.org/ag/AGA/AGAP/FRG/lrrd/lrrd3/3/tropap.htm

Cornell University factsheet. http://www.ansci.cornell.edu/plants/toxicagents/lectins.html

2.1.2.7 Pyrrolizidine Alkaloids

Hazard Identification

What are Pyrrolizidine Alkaloids?

Pyrrolizidine alkaloids (PAs) are a large group of more than 350 naturally-occurring toxins produced as secondary metabolites by many plant species, possibly to protect the plant against grazing by herbivores. They are named for their inclusion of the pyrrolizidine nucleus, consisting of a pair of linked five-sided pyrrole rings containing four carbon and one nitrogen atom. Not all PAs are toxic, although many are. A number have been found to be hepatotoxic and some are probably carcinogenic.

PAs have been associated with disease in livestock caused by grazing on plants containing the alkaloids or by consuming contaminated feed. Humans may be exposed to PAs in the diet by consuming foods prepared from cereals contaminated with toxigenic weed species, or foods derived from animals given contaminated feed. Cases of human poisoning have also occurred following consumption of medicinal herbs containing PAs.

Occurrence in Foods

PAs can be detected in foods produced from animals that have fed on plants containing these natural toxins. Foods that have been found to contain PAs include grains, eggs, offal and milk. Honey produced by bees visiting PA-containing plants has also been found to contain significant amounts of toxins. Food products, such as flour and bread, produced from cereal crops accidently contaminated by pyrrolizidine-containing weeds have also been reported to contain high levels of PA toxins.

A number of medicinal herbs contain PAs, including borage, coltsfoot and comfrey, and cases of human poisoning have been associated with consumption of these herbs.

Hazard Characterisation

Effects on Health

Most PAs are rendered toxic by a process of metabolic activation in the bodies of animals and humans. The parent alkaloids are largely unreactive, but after they are ingested, they are progressively transformed into much more toxic and reactive dehydropyrrhole alkaloids. This process occurs principally in the liver, which is the main target organ for PA toxicity, and its extent is strongly influenced by the chemical structure of the parent PA. Some PAs are readily hydrolysed and are thus largely detoxified by esterases, while those with more highly branched side chains resist hydrolysis and undergo bioactivation to produce toxic pyrrhole compounds. These toxic

pyrroles are powerful alkylating agents and can cause significant tissue damage.

The toxicity of PAs varies significantly between different animal species, probably because of differences in the metabolic activation process. Cattle and horses are considered to be most sensitive to the toxins, sheep and goats less so, while human sensitivity falls somewhere between. Livestock poisonings caused by PAs have been reported worldwide, mainly in cattle and horses, but also in sheep. Animals tend to avoid plants containing high PA levels when grazing, unless pasture is over-grazed, but are less able to detect such plants in hay and other feed.

Very small doses of toxic PAs can cause enlargement of liver cells, impairment of liver metabolism and fatty degeneration. Longer term administration of smaller doses may cause liver cirrhosis. In both acute and long-term exposure, veno-occlusive liver disease can occur, involving obstruction of the small veins bringing blood from the liver back to the heart and causing acute upper-gastric pain, nausea and abdominal distension. Certain PAs also affect the nervous system or other organs, notably the lungs. A NOEL of 10 μg per kg body weight has been derived for PAs in humans from the available epidemiological data. The Australian Food Safety Authority has proposed a PTDI of 1 μg per kg body weight per day.

Studies in animals have shown that PAs can be both genotoxic and mutagenic, giving rise to concerns that individuals taking certain herbal preparations on a long-term basis might increase their chances of developing liver disease, including cancers.

Incidence and Outbreaks

A number of documented cases of poisoning caused by PAs have been reported. These are generally as a result of contamination of cereals with PA-containing plants, or from the consumption of herbal remedies.

Numerous incidents of hepatic veno-occlusive disease associated with PA poisoning have occurred worldwide. Outbreaks have been recorded in a number of countries, including South Africa, Uzbekistan and Afghanistan. The largest outbreak ever reported lasted from 1974 to 1976 in the Gulran district of Afghanistan, affecting an estimated 7800 people with about 1600 deaths. "Gulran Disease" was attributed to consumption of bread made from wheat contaminated with seeds of a weed, locally called *charmac*, and hepatic veno-occlusive disease was diagnosed by liver biopsy. The most recent large-scale outbreak reported was also in Afghanistan in 2008. Contaminated wheat flour used to make bread was the likely source, and hundreds of people were affected.

Many cases of illness have also been linked to regular intake of medicinal herbs containing PAs, notably comfrey, but also other herbs, such as *Senecio* species, *Gnaphalium* and *Heliotropium*.

Sources

Over 6000 plant species produce PAs and they are likely to be found in almost any environment. Most are members of three plant families: the *Asteracea* (*Compositae*) in plants of the *Senecioneae* sub-tribe and the *Eupatorieae* sub-tribe; in the *Boraginaceae*; and in the *Fabaceae* (*Leguminosae*), in the sub-tribe *Crotalariaceae* (mainly in the genus *Crotalaria* but also in *Chromolaena* and *Lotononis*). It has been estimated that about 3% of the world's flowering plants contain PAs.

The type and distribution of PAs differ with the plant variety, climatic conditions, season and location within the plant. For example, basic alkaloids appear to accumulate in seeds, whereas the respective *N*-oxides are distributed in the green parts of the plants.

Stability in Foods

The stability of PAs during food processing has been partially investigated. One study examined the stability of unsaturated PAs during the preparation of herbal tea and during the cooking of maize porridge. It concluded that the PAs in question were unaffected by the high temperatures involved. Bread-related outbreaks recorded in Afghanistan also suggest that PAs remain toxic during baking processes.

Control Options

In the case of PA-containing medicinal herbs, the main control option is increased public awareness of the dangers associated with taking these products. Government Agencies throughout the world have issued recommendations regarding their use. Comfrey, for example, has been restricted in most countries as a medicinal plant and its use is permitted only for topical applications.

Appropriate training programmes and implementation of GAP are recommended to reduce the exposure of livestock to PAs. This includes the use of herbicides where appropriate and the cleaning of grains and removal of weed seeds at the post-harvest stage. In addition, the EFSA has recommended monitoring feed materials for the presence of the more common PAs.

Legislation

Directive 2002/32/EC of the European Parliament and of the Council of 7th May 2002 on undesirable substances in animal feed lists several plant species or specific parts of these species containing PAs (see Table 2.1.5).

The United States Department of Agriculture has decreed that any animal showing signs of PA poisoning at slaughter must be condemned and not allowed to enter the food chain.

Table 2.1.5 Plant species or specific parts of these species containing pyrrolizidine alkaloids.

Undesirable substances (or plants)	*Product intended for animal feed*	*Maximum content/mg kg^{-1} relative to a feedstuff with a 12% moisture content*
Weed seeds and unground and uncrushed fruits containing alkaloids including:		3000
a) *Lolium temulentum*		1000
b) *Lolium remotum*		1000
Crotalaria species		100

Sources of Further Information

Published

Special issue: Pyrrolizidine alkaloids. *Food Additives and Contaminants*, 2011, 28(3), 259–372.

On the Web

Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) – Statement on Pyrrolizidine Alkaloids in Food (2008). http://cot.food.gov.uk/pdfs/cotstatementpa200806.pdf

Opinion of the Scientific Panel on Contaminants in the Food Chain related to pyrrolizidine alkaloids as undesirable substances in animal feed – European Food Safety Authority. http://www.efsa.europa.eu/en/efsajournal/doc/447.pdf

Food Standards Australia New Zealand Technical Report. http://catalogue.nla.gov.au/Record/626693

Pyrrolizidine alkaloids in foods and animal feeds – FAO Consumer Protection Fact Sheets No. 2: Pyrrolizidine Alkaloids. http://www.fao.org/ag/agn/agns/files/FAO_Fact_Sheet_Pyrrolizidine_Alkaloids.pdf

Dharmananda, S. Safety issues affecting herbs: pyrrolizidine alkaloids. http://www.itmonline.org/arts/pas.htm

2.1.3 FISH TOXINS

2.1.3.1 Azaspiracids

Hazard Characterisation

What are Azaspiracids?

The azaspiracids (AZAs) are a group of marine biotoxins, which cause a food-borne intoxication known as azaspiracid shellfish poisoning (AZP). AZP is associated with the consumption of contaminated shellfish harvested from waters affected by growth of certain types of toxic algae. The intoxication was first recognised in 1995 following an outbreak of illness in the Netherlands associated with the consumption of mussels imported from Ireland. AZP is a form of food poisoning with symptoms typical of gastroenteritis, broadly similar to diarrhoeic shellfish poisoning (DSP).

The AZA-group toxins are polyether compounds with an unusual spiral ring structure. Up to 11 AZA analogues have been identified and characterised, but some of these are thought to be shellfish metabolytes and are less toxic than AZA. Only AZA-1, AZA-2 and AZA-3 are considered to have public health significance and AZA-1 is thought to be the main cause of illness.

Occurrence in Foods

Recorded cases of AZP have been associated with consumption of mussels, but AZAs have also been found in oysters, clams, scallops, razor clams and cockles. There have also been reports of AZA contamination in crabs.

AZAs tend to accumulate in shellfish digestive glands initially, but unlike other shellfish toxins, they can be readily transported to other tissues, though not predictably. This means that the rate of natural detoxification (depuration) in contaminated shellfish can be very slow.

Hazard Characterisation

Effects on Health

The precise mechanism of AZA toxicity remains uncertain, but toxicological studies suggest that it affects the gastrointestinal tract, lymphoid tissues and the immune sytem. Evidence from AZP outbreaks suggests that a lowest observable adverse effect level (LOAEL) of AZA is 23 to 86 μg per person (mean value 51.7 μg). Mussels collected from Irish waters after outbreaks were found to contain total AZAs at levels up to 1.4 μg g^{-1} of meat.

Symptoms of AZP resemble those of DSP and include nausea, vomiting, severe diarrhoea and stomach cramps. Severity of symptoms appears to be linked to the quantity of toxin ingested.

Incidence and Outbreaks

AZP has only been associated with shellfish harvested from Irish waters to date. However, AZAs have also been isolated from shellfish harvested from the coastal waters of other Western EU countries (*e.g.* the UK and Norway), Morocco and Canada. This suggests that distribution of the toxins may be more widespread than once thought.

The first recorded outbreak of AZP affected eight people in the Netherlands who had consumed mussels imported from Ireland. Since 1996 other incidents have been reported in Ireland, notably in 1997 when contaminated mussels from Arranmore, in Donegal, caused human cases in Ireland and elsewhere in the EU. Further incidents were reported in 2001 and 2005, resulting in mussel fisheries being closed for prolonged periods.

Sources

Until recently, the source of AZAs was unknown, but was suspected to be a species of dinoflagellate. Early evidence suggested that *Protoperidinium crassipes* was most likely to be responsible, but as this species preys on other dinoflagellates, the finding was inconclusive. Then in 2009 a new dinoflagellate species was isolated from waters off the East coast of Scotland, which produced AZA-1 and AZA-2 in pure culture. This species has been named *Azadinium spinosum* and is now widely considered to be a primary producer of AZAs.

Incidents have not been linked to visible algal blooms and the cell density needed to produce hazardous AZA levels is not known.

Stability in Foods

There are conflicting reports on the heat stability of AZAs, but recent evidence suggests that they survive cooking processes, as do other polyether shellfish toxins. Heating homogenised contaminated mussel tissue at 90 °C for 10 min was reported to produce no change in AZA concentration.

Natural detoxification in shellfish does occur, but the rate of this process in mussels is slow, and toxicity has been reported to last for up to six months.

Control Options

The stability of AZAs and the prolonged duration of natural detoxification mean that neither depuration in clean water nor cooking processes are effective or economically viable methods of reducing the toxicity of affected shellfish to safe levels.

The only effective control available currently is the regular monitoring of shellfish samples for the presence of AZAs using a mouse bioassay or LC-MS analysis.

When toxic conditions are detected, bans on harvesting shellfish have to be imposed until toxicity can be shown to have returned to safe levels and contaminated shellfish should not be allowed to enter the human food chain.

Legislation

There are regulations relating specifically to AZA-group toxins in the EU where the European Commission (EC) has set a maximum level of 160 μg kg^{-1} in bivalve molluscs, echinoderms, tunicates and marine gastropods. The reference method for analysis is the mouse bioassay, although other alternative or complementary methods can be used.

The Irish authorities undertake weekly shellfish testing for several toxins, including AZAs.

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2.1.3.2 Brevetoxins

Hazard Identification

What are Brevetoxins?

The brevetoxins (BTXs) are a group of marine biotoxins, which cause a food-borne intoxication known as neurologic shellfish poisoning (NSP). NSP is associated with the consumption of contaminated shellfish harvested from waters affected by growth of certain types of toxic algae. It is also sometimes referred to as neurotoxic shellfish poisoning. NSP-like symptoms associated with 'red tides' off the Florida coast and in the Gulf of Mexico were first noted in the nineteenth century. NSP is an acute toxic syndrome having some similarities with paralytic shellfish poisoning (PSP), although PSP is usually more severe. NSP causes a wide range of symptoms, but is not reported to be fatal.

At least 10 BTX-group toxins have been isolated from algal blooms or cultures, the most common being BTX-2. They are stable, lipid-soluble polyether neurotoxins, consisting of 10 (type A) or 11 (type B) rings and having molecular masses of around 900. In addition to the 10 naturally occurring BTXs, several further analogues have been found in contaminated shellfish. These are thought to arise through biotransformation of BTX-1 and BTX-2, probably in the digestive glands of some shellfish species.

Occurrence in Foods

Most human cases of NSP are related to bivalve molluscs, including oysters, clams and mussels, all of which can accumulate BTXs during feeding when the water contains sufficient levels of toxin-producing algae. BTXs have also been reported in some seabirds and finfish, but most fish, birds and mammals are susceptible to the toxins and toxic algal blooms have caused extensive fish kills and the deaths of marine mammals and birds.

There is little published information on the rate or site of BTX accumulation in shellfish. Toxin levels in shellfish do reduce naturally after they stop feeding on toxic algae, but little is known about this process and retention times vary greatly between species. Furthermore, biotransformation of BTXs in some shellfish may produce analogues that are more toxic than the natural toxins.

Hazard Characterisation

Effects on Health

BTX-group toxins are neurotoxins that act by affecting the sodium channels in the membranes of nerve cells. This causes the cells to fire repeatedly, giving rise to various neurological symptoms. BTX is considered potentially toxic to humans at any detectable level in shellfish, but a residue toxicity of 20 mouse units (MU) per 100 g of shellfish flesh is commonly used for regulatory purposes.

The onset of symptoms of NSP occurs between 30 min and 3 hours after ingestion of toxic shellfish. The main symptoms of NSP include nausea, vomiting, diarrhoea, chills and sweating, hypotension, numbness, pins and needles, cramps and in some severe cases, paralysis and coma, but deaths have not been reported. Symptoms usually persist only for a few days.

BTXs can also cause skin and eye irritation in people swimming in waters affected by algal blooms and inhalation of toxic aerosols can cause respiratory problems.

Incidence and Outbreaks

For many years NSP was known only in Florida and the coasts around the Gulf of Mexico. However, in 1993 an outbreak of NSP-like illness was reported in New Zealand. Algal species known to produce BTXs have also been identified in the coastal waters of several Western EU countries, South Africa, Canada, the East and West coasts of the USA, Japan and Australia.

The first documented outbreak caused by shellfish harvested from waters north of Florida occurred in North Carolina in 1987. This outbreak affected 48 people and lasted for several months. In the 1993 outbreak in New Zealand, 186 cases of illness were recorded. This outbreak was identified as NSP, but it seems that PSP may also have been involved in some of the cases. BTX levels in contaminated shellfish were reported to have reached 592 MU per 100 g at the height of the outbreak.

Sources

BTXs are produced by the motile form of a dinoflagellate species now referred to as *Karenia brevis* (previously known as *Gymnodinium breve* or *Ptychodiscus brevis*). This is the species causing toxic red tides around the Florida coast, but it probably has a much wider geographical distribution. Toxins that correspond closely to BTXs have also been identified in four species of algae belonging to the class *Raphidophyceaea*. These species are *Chattonella antiqua* and *Chattonella marina*, *Fibrocapsa japonica* and *Heterosigma akashiwo* and they too are widely distributed.

The presence of low numbers of these algae is probably not a health hazard, but under certain conditions rapid growth may occur resulting in an algal bloom. When this happens the numbers of cells can be come high enough to colour the water reddish brown (a red tide). Cell densities of *K. brevis* of $>10^7$ cells per litre have been recorded during a red tide along the Southwest coast of Florida.

Any filter-feeding shellfish in water affected by a toxic bloom are likely to accumulate high levels of toxin quite quickly as they feed on and digest the algal cells. Thus shellfish harvested from such waters carry a high risk of toxicity.

Stability in Foods

BTXs are known to be relatively heat stable, and acid stable. They have been reported to survive both cooking and freezing processes. Even retorting processes cannot be relied upon to eliminate the toxin.

Natural detoxification (depuration) in shellfish does occur, but the rate of this process varies greatly between and even within species. Commercially grown shellfish are generally regarded as safe to eat after one or two months following the end of a toxic algal bloom.

Control Options

The stability of BTXs and the variability of natural detoxification mean that neither depuration in clean water nor cooking processes are effective or economically viable methods of reducing the toxicity of affected shellfish to safe levels. Depuration of mussels with ozonated water has been investigated and appears to enhance the elimination of BTXs.

The development of potentially toxic *K. brevis* blooms is highly unpredictable and the only effective control is the monitoring of the marine environment for evidence of a bloom, such as large fish kills and discoloured water. Toxicity is then confirmed using chemical analysis or mouse bioassay. Monitoring of water quality using microscopy to identify and count potentially toxic algae can be of value in preventing NSP outbreaks, but it is time consuming and requires highly skilled staff. New diagnostic tests using biomarkers for *K. brevis* have been investigated in the laboratory.

When potentially toxic conditions are detected, bans on harvesting shellfish have to be imposed until toxicity can be shown to have returned to safe levels and contaminated shellfish should not be allowed to enter the human food chain.

Legislation

There are regulations relating specifically to BTX-group toxins in shellfish in the USA and New Zealand.

In the USA a regulatory limit of 80 µg BTX-2 equivalents per 100 g of shellfish tissue (equivalent to 20 MU per 100 g) determined by the APHA mouse bioassay is applied. The health authorities in Florida monitor coastal waters for *K. brevis* and close shellfish fisheries when cell densities exceed 5000 cells per litre.

In New Zealand, a maximum acceptable level for BTX in shellfish of 20 MU per 100 g has also been adopted, again determined using the APHA mouse bioassay. Water from shellfish harvesting areas is monitored every week throughout the year.

Some South American and EU countries also carry out monitoring, but have not set regulatory limits.

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2.1.3.3 Ciguatoxins

Hazard Identification

What are Ciguatoxins?

The ciguatoxins (CTXs) are a group of marine biotoxins, which are the cause of a food-borne intoxication known as ciguatera fish poisoning (CFP). CFP is associated with consumption of coral reef fish from tropical and subtropical waters in the Pacific and Indian Oceans and the Caribbean sea. It was first recorded by Spanish explorers some 500 years ago. CFP is the commonest form of marine food poisoning worldwide and is considered to be a significant public health problem.

CTXs accumulate in certain fish species that feed on toxic algae, or prey on toxic herbivorous fish species. They are lipid-soluble polyether compounds made up of 13 or 14 rings fused into rigid ladder-like structures. Multiple forms of CTX with small structural differences have been described and there are important geographic differences. The Pacific ciguatoxin-1 (P-CTX-1) is the most potent and its structure is slightly different from that of the Caribbean ciguatoxin-1 (C-CTX-1). These differences are also reflected in the symptoms produced.

Occurrence in Foods

CTXs are found in broad range of fish that live in or around coral reefs in comparatively shallow tropical waters. Over 400 species have been reported to be involved in CFP outbreaks. The toxins tend to concentrate as they move up the food chain, so that large carnivorous fish are more likely to be toxic. Species such as barracuda, grouper, snapper, jack, moray eel, Spanish mackerel and some in-shore tuna carry the highest risk, but herbivorous and coral eating species such as parrot fish may also cause CFP outbreaks.

Recently, CTXs have been detected in coastal fish species harvested from Israeli waters in the Eastern Mediterranean. There is also evidence that recent CFP outbreaks in Madeira and the Canary Islands were caused by locally caught fish. These findings are causing concerns that the geographic range of CTX production may be extending into more temperate waters.

The highest concentrations of toxins in the fish are found in the viscera, particularly in the liver and kidneys, and levels can be up to 100 times higher than in other tissues. The fish themselves suffer no detectable symptoms even though the toxin is persistent and affected fish can remain toxic for long periods.

In former times, CFP was restricted to indigenous populations in areas where CTXs are endemic, but this has changed in recent years with the increase in global travel and the increasing importation of exotic foodfish species into developed countries.

Hazard Characterisation

Effects on Health

CTXs cause a wide variety of neurological, gastrointestinal and cardiovascular symptoms. They are extremely powerful toxins and an oral dose of 0.1 μg may be enough to cause illness. They act by increasing the sodium ion permeability of the plasma membranes in nerve and muscle cells, causing membrane depolarisation and thus disrupting cell function. Similarly, they affect intracellular calcium transport in gut epithelial cells.

Symptoms may appear within one hour in severe cases, but onset may be delayed for 24 or even 48 hours in milder cases. Gastrointestinal symptoms, including nausea, vomiting, diarrhoea and abdominal pain often occur first, followed by neurological symptoms, such as a tingling of the lips and extremities and severe localised skin irritation. However, there is geographic variation, with neurological symptoms being more common in the Pacific and gastrointestinal in the Caribbean.

Other recorded symptoms include hallucinations, depression and anxiety, fatigue and aching in the muscles and joints. Hypotension, respiratory problems and even paralysis can occur in severe cases, but death is uncommon, with a reported fatality rate of less than 1%. Gastrointestinal symptoms usually resolve within a few days, but where neurological symptoms occur they may last much longer, typically several weeks or months. Individuals can also become sensitised to CTXs so that they may react to fish that do not affect others.

The varied nature of the symptoms can result in CFP being misdiagnosed as multiple sclerosis or chronic fatigue disorder in developed countries.

Incidence and Outbreaks

It is estimated that between 10 000 and 50 000 cases of CFP occur each year. Most of these cases occur in tropical and sub-tropical coastal regions adjoining the Pacific and Indian Oceans and the Caribbean. However, more cases are being reported in temperate developed countries and it is thought that underreporting could be significant in the EU and North America because of misdiagnosis.

CFP outbreaks have been reported in France, Italy, Germany and the Netherlands. In the USA, 129 outbreaks affecting 508 people were recorded between 1983 and 1992. Most of these occurred in Hawaii and Florida, but outbreaks linked to imported fish were reported elsewhere. A number of outbreaks have occurred in Australia and an annual incidence of 30 per 100 000 has been estimated.

Sources

The principal known source of ciguatoxins is an alga, the marine dinoflagellate *Gambierdiscus toxicus*, which is associated with seaweeds, sediments and dead

coral. It is distributed around the tropics within the latitudes 32°N and 32°S and grows in shallow waters, but its presence and numbers are unpredictable. There is also evidence that other species of dinoflagellates may sometimes be involved.

Certain strains of *G. toxicus* produce toxins referred to as gambiertoxins—less oxidised and less toxic precursors of CTXs. When the algae are consumed by herbivorous fish, the gambiertoxins accumulate in the fish and a biotransformation begins to occur, in which they are converted to CTXs. Over time, the toxins become transferred to carnivorous fish and the biotransformation is completed. The highest levels of CTXs are found in the largest carnivorous fish. Different strains of *G. toxicus* are thought to produce different CTX precursors, which are then transformed into the various CTX types.

G. toxicus also produces another type of highly potent toxin called maitotoxins. These occur in the guts of herbivorous fish, but are not now thought to be involved in CFP.

Stability in Foods

CTXs are temperature-stable and are not destroyed by cooking or by freezing. Other processes, including salting and smoking, also have little or no effect. Affected fish can remain toxic for years, even when their diet ceases to contain toxin or precursors.

Control Options

CTXs are odourless and tasteless and do not alter the appearance of the fish. They can be detected using a number of techniques following extraction and purification techniques. The most widely used test method is a mouse bioassay, but biomolecular assay methods, such as cytotoxicity, receptor binding and immunoassay can also be applied. An ELISA-based method for CTX detection has recently been developed. The difficulty of detecting CTXs in fish, plus their stability, severely limits the control options available.

The only practical control is to avoid consumption of susceptible fish species from areas where CFP is endemic. Large predatory reef fish, such as barracuda, present a high risk and should be particularly avoided. Parts of the fish where the highest toxin levels accumulate, such as the head, gut, liver and roe should not be eaten. Health Canada advises travellers not to eat large reef fish weighing more than 3 kg.

Legislation

There are few specific regulations for CTXs toxins in fish.

In the EU, legislation covering fishery products states that ‘‘fishery products containing biotoxins such as ciguatera toxins’’ cannot be placed on the market, but no methods of analysis are given.

In the USA no action limits have so far been established. However, the Food and Drug Administration (FDA) has proposed guidance levels of <0.1 μg kg^{-1} C-CTX-1 equivalents and <0.01 μg kg^{-1} P-CTX-1 equivalents.

The most common legislative control in use around the world is the prohibition of the sale of high-risk fish taken from areas where CTXs are known to be present. Such bans have been used with success in Australia, Fiji, Hawaii and Florida.

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2.1.3.4 Cyclic Imines

Hazard Characterisation

What are Cyclic Imines?

The cyclic imines (CIs) are a group of emerging shellfish toxins, which include spirolides (SPXs), gymnodimines (GYMs), pinnatoxins (PnTXs), pteriatoxins (PtTXs), prorocentrolides and spiro-prorocentrimines. They have been discovered and characterised mainly during the last 20 years. None have yet been confirmed as a cause of food-borne illness in humans, but they are powerful, fast-acting neurotoxins in rodents and have recently been detected in shellfish harvested in several parts of the world.

CIs are produced by a number of different species of marine algae and accumulate in the tissues of shellfish feeding in waters containing significant numbers of toxin-producing algae. They are grouped together because of their very similar chemical structures and their acute toxicity in mice.

The CIs are described as macrocylic compounds with imine and spiro-linked ether moieties. The members of the group are all very similar in structure and the SPXs and PnTXs have been found to be approximately 70% homologous. The largest group of CIs are the SPXs, with 12 analogues having been characterised. Three GYM analogues, seven PnTX analogues and three PtTX analogues have been classified to date. The prorocentrolides and spiro-prorocentrimines have been less widely studied, but their chemical structures are similar.

Occurrence in Foods

The presence of CIs in shellfish was first identified because of their high acute toxicity in mice. Intra-peritoneal injections of shellfish extracts containing high levels of the toxins cause rapid death and can interfere with mouse bioassays for other marine toxins.

SPXs were first isolated from scallops and mussels harvested in Canada about 20 years ago and have since been found in shellfish harvested from the Mediterranean and the North Atlantic. GYMs were also first identified approximately 20 years ago, this time in oysters harvested in New Zealand. They have since been found in clams imported into the EU from Tunisia. PnTXs were first found in Japanese shellfish and have also been identified in China. Surveys in 2010 found PnTXs in blue mussels harvested from waters off the coast of Norway, but other EU countries have not yet carried out similar surveys and the extent of their presence in EU waters is uncertain. PtTXs were first isolated from shellfish in Japan in 2001. Prorocentrolides were first reported in extracts from marine algae in 1996.

Recent survey results suggest that CIs are more widespread in distribution than previously thought and these toxins are considered to be potential emerging food safety hazards.

Hazard Characterisation

Effects on Health

The limited toxicological data available for these toxins indicates that they have acute toxicity for mice when administered by intra-peritoneal injection, or by feeding, indicating that they can be absorbed from the gut. They act as neurotoxins and are thought to bind to acetylcholine receptors in the nervous system, causing muscular paralysis by blocking receptors at neuro–muscular junctions. High doses cause respiratory paralysis and eventual death in mice, but sub-lethal doses seem to cause no permanent damage and the mice quickly recover completely. LD_{50} values for SPXs in feed are of the order of 500 μg per kg of body weight (EFSA), but they are much more toxic if injected. Recent reports suggest that PnTXs may be the most toxic CIs when ingested.

There is no available data on the chronic or long-term toxicity of CIs in mice or other animals.

To date there are no confirmed reports of CI toxicity in humans and no reported outbreaks of food-borne illness. There have been reports of illness in people consuming shellfish thought to contain SPXs in Nova Scotia, but the symptoms were unlike those reported in mice and could not be linked directly to the toxins. Reports from New Zealand suggest that consumption of shellfish contaminated with GYMs and PnTXs did not result in any cases of illness, but the levels of toxin present in the shellfish are unknown.

Sources

The source of SPXs has been identified as the dinoflagellate *Alexandrium ostenfeldii*. The proportions of different SPX analogues produced by the alga have been reported to vary considerably with the environmental conditions prevailing in different locations.

GYMs are known to be produced by another dinoflagellate, *Karenia selliformis*, but the organism responsible for production of PnTXs has not yet been identified. It is currently suspected that two PnTX analogues, F and G, are produced by two different species of peridinoid dinoflagellates. These two analogues are currently thought to be progenitors for the other PnTX analogues and also for all PtTXs isolated from shellfish. These toxins are most likely to be produced by bio-transformations in shellfish, rather than directly by dinoflagellates.

Prorocentrolides and spiro-centrimines have been shown to be produced by dinoflagellates of the genus *Prorocentrum*.

Stability in Foods

There is very little information available on the stability of CIs in foods, but reports from New Zealand indicate that GYM persisted in oysters for several years after a contamination event.

There is no published information relating to the effect of processing on the stability and persistence of CIs in shellfish.

Control Options

The lack of information about the stability of CIs makes it difficult to estimate whether depuration in clean water or cooking processes would be effective or economically viable methods of reducing toxin levels in affected shellfish. However, the reported persistence of GYM in oysters suggests that depuration might be ineffective.

The only effective control available currently is the regular monitoring of shellfish samples for the presence of CIs using a mouse bioassay or LC-MS/MS analysis, but current methods have not been formally validated.

Legislation

There are no regulatory limits currently in force for CIs in shellfish anywhere in the world.

Sources of Further Information

Published

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2.1.3.5 Domoic Acid

Hazard Characterisation

What is Domoic Acid?

Domoic acid (DA) is a marine biotoxin, which causes a food-borne intoxication known as amnesic shellfish poisoning (ASP). ASP is associated with the consumption of contaminated shellfish harvested from waters affected by growth of certain types of toxic algae. It is also sometimes referred to simply as domoic acid poisoning because amnesia is not a symptom in every case. ASP was first identified in 1987 following a shellfish-related food poisoning incident in Canada. ASP is an acute form of human poisoning, which causes a wide range of symptoms and can sometimes be fatal.

DA ($C_{15}H_{21}NO_6$, CAS No. 14277-97-5) is a water-soluble cyclic amino acid and has been isolated from a number of marine macro- and micro-algae species. It is a powerful neurotoxin and belongs to the kainoid class of compounds.

Occurrence in Foods

Most human cases of ASP are related to bivalve molluscs, especially scallops and mussels, but DA has also been detected in oysters, cockles, razor clams and other species. DA has been found at levels high enough to cause human illness in Dungeness crabs, carnivorous gastropods, and anchovies. Mussels and other bivalves are filter feeders and accumulate toxins when the water contains sufficient levels of toxin-producing algae. It is thought that some small finfish, such as anchovies, may also feed directly on high densities of algae when other food sources are limited. There have been instances of other marine predators, notably pelicans and sealions, dying in large numbers after feeding on contaminated fish.

DA has been shown to accumulate in several bivalve species. Most of the toxin is concentrated in the viscera, especially in the digestive gland (hepatopancreas). Different species accumulate DA at different rates and variation has been observed in individuals of the same species growing in the same area. A toxin level of $>3000\ \mu g\ g^{-1}$ in the digestive gland of scallops has been reported, but negligible amounts were found in muscle tissue.

DA levels in shellfish do reduce naturally after they stop feeding on toxic algae, but retention times vary greatly between species. For example, mussels accumulate DA quite quickly, but it is also lost quickly from their tissues. Razor clams by contrast, lose DA from their tissues only slowly, and the toxin can remain in the edible muscle for a considerable time.

Hazard Characterisation

Effects on Health

DA is a potent neurotoxin, can affect both central and peripheral nervous systems in humans and is also an emetic. Its toxic effects are caused by

high-affinity binding and agonist action on glutamate receptor proteins in nerve cells. Repeated depolarisation of the cell leads to its eventual destruction and can cause damage in some parts of the brain, notably the hippocampus. In the first documented ASP outbreak in Canada, consumption of 60–110 mg DA (0.9–2.0 mg per kg of body weight) was sufficient to cause mild symptoms.

The onset of symptoms of ASP in the Canadian outbreak occurred between 15 min and 38 hours after ingestion of toxic shellfish. The main symptoms of ASP include nausea, vomiting, abdominal cramps headache, diarrhoea and memory loss. Memory loss is usually temporary and is more common in older people. The severity of symptoms depends on the amount of DA ingested, and a wide variety of more severe neurological symptoms can occur, including coma, disorientation, seizures, uncontrolled weeping or aggressive behaviour, eye problems and unstable blood pressure and pulse. Patients falling into a coma may not recover and may eventually die.

The effect of long-term exposure to small concentrations of DA is unknown.

Incidence and Outbreaks

Documented ASP outbreaks in humans are known only from Canada and the USA, but DA has been found in shellfish taken from EU waters. This has resulted in the closure of fisheries in several countries, including Scotland, Ireland and Spain. High levels of the toxin have also been isolated from shellfish harvested in New Zealand, but no outbreaks in humans are recorded. Algal species known to be capable of producing DA have been found over a much wider geographical area, including the Pacific Ocean.

The first documented outbreak in 1987 affected over 100 people in Prince Edward Island off the East Canadian coast. Three deaths were reported during the outbreak. The toxin was traced to blue mussels produced locally by aquaculture. Since then, dangerous levels of DA have been found in shellfish on a number of occasions. In 1991, 24 people in the USA state of Washington were taken ill suffering from gastrointestinal symptoms and memory loss. Although ASP was not confirmed, the outbreak coincided with high DA levels being identified in razor clams and a ban on harvesting the shellfish. There have been repeated incidents of DA being found in shellfish from USA waters, especially on the West coast, and a number of examples of fisheries being closed.

Sources

DA is unusual among shellfish toxins, in that it is not produced by species of dinoflagellates. It was first isolated from the red macroalga *Chondria armata* in the 1950 s, but the source of DA implicated in the first documented ASP outbreak was identified as a microalga, the diatom *Pseudo-nitzschia pungens* forma *multiseries* (now recognised as two separate species, *P. pungens* and *P. multiseries*). DA production has been reported in at least nine species of *Pseudo-nitzschia*: *P.australis*, *P. delicatissima*, *P. pseudodelicatissima*, *P. multiseries*, *P. pungens*, *P. seriata*, *P. multistriata*, *P. turgidula* and *P. fraudulenta*. Another species, *Nitzschia navis-varingica*, isolated from shrimp ponds in Vietnam, has

also been shown to produce DA. These species are widely distributed around the world's oceans, although certain species tend to be found more often in a specific region.

Production of DA by the different species is very variable and seems to be affected by environmental conditions, although the relationship with factors such as temperature and nutrient availability is unclear. Generally, DA is produced when rapid growth of *Pseudo-nitzschia* species occurs, forming an algal bloom. Toxin production has been observed during exponential and stationary growth phases. Reports suggest that cell densities of at least 3×10^5 cells per litre are required before feeding shellfish accumulate sufficient toxin to cause ASP.

Stability in Foods

DA is relatively heat stable and is not destroyed by practical cooking processes, or by frozen storage. In scallops, DA has been shown to spread from the digestive gland into other tissues during frozen storage and even a canning process was found to be ineffective in reducing DA levels, although migration from flesh to canning brine was observed. The meat of Dungeness crabs can also become contaminated during cooking if they are not eviscerated before processing.

Natural detoxification (depuration) in shellfish does occur, but the rate of this process varies greatly with the species, being rapid in mussels, but very slow in razor clams.

Control Options

The stability of DA and the variability of natural detoxification mean that neither depuration in clean water nor cooking processes are effective or economically viable methods of reducing the toxicity of affected shellfish to safe levels.

The only effective controls available currently are the monitoring of the marine environment and the testing of shellfish for DA when contamination is suspected. Regular inspection of the waters where shellfish are harvested, or produced by aquaculture, for the presence of toxic algae can be a useful source of data and indicate when a risk of toxicity is present. *Pseudo-nitzschia* diatoms are quite easy to identify under the microscope, but distinguishing between species is very difficult. As species vary in their ability to produce DA it is important to be able to identify individual species and molecular biology methods have been developed to do this.

When potentially toxic conditions are detected, bans on harvesting shellfish have to be imposed until toxicity can be shown to have returned to safe levels and contaminated shellfish should not be allowed to enter the human food chain.

Legislation

There are regulations relating specifically to ASP toxins in shellfish in a number of countries.

In the EU the EC has set a guideline limit for DA in the edible parts of molluscs of 20 mg kg^{-1}. An HPLC method is specified, but an ELISA-based method may be used for screening purposes. If levels above the guideline value are found, then the complete batch of shellfish must be destroyed. Monitoring of toxin-producing algae and DA in shellfish occurs in several EU countries.

In both Canada and the USA a guideline value of 20 mg DA per kg of mussel and/or bivalves is in force and an LC-based method must be used. In the USA, a guideline value for cooked crab (viscera and hepatopancreas) of 30 mg DA per kg is in place. Some monitoring for toxin producing algae and DA in shellfish is carried out in both countries.

Monitoring is also undertaken in Australia and New Zealand and New Zealand has set a regulatory limit of 20 mg DA per kg of shellfish meat, to be determined by LC.

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2.1.3.6 Gempylotoxin

Hazard Identification

What is Gempylotoxin?

Gempylotoxin is a naturally occurring toxin found in certain marine fish of the *Gempylidae* family, such as escolar and oilfish. It is the cause of a food-borne intoxication, gempylid fish poisoning, associated with the consumption of these species.

Unlike most other fish toxins, gempylotoxin is not derived from toxic algae, but occurs naturally within the flesh of the fish. It is a strong, purgative oil composed mainly of waxy esters of C32, C34, C36 and C38 fatty acids, with the main component being $C_{34}H_{66}O_2$. The oil is indigestible and can cause a mild, though unpleasant, form of gastrointestinal illness.

Occurrence in Foods

Gempylotoxin is only known to occur in the flesh of marine fish belonging to the Gempylidae family, particularly escolar and oilfish. Other species of fish have occasionally been implicated in cases of gempylid fish poisoning, but these reports have not been confirmed. Escolar and other gempylid species may sometimes be mis-labelled and sold as other, more valuable fish species such as butterfish, or ''white tuna'', leading to confusion about the type of fish associated with illness.

Hazard Characterisation

Effects on Health

Because the waxy esters in gempylotoxin cannot be digested in the human gut, they may have a laxative effect in some individuals. Symptoms usually appear between about 30 min to 36 hours after consumption of fish containing the toxin. The main symptom is an oily diarrhoea or rectal discharge (keriorrhea), but nausea and abdominal cramps have also been reported occasionally. Keriorrhea is not associated with dehydration and so is not life threatening. Symptoms generally moderate within 24 to 48 hours.

Incidence and Outbreaks

Sporadic cases of gempylid fish poisoning have occurred in many parts of the world, including North America, the EU, Japan, Hong Kong and Australasia. Since escolar in particular is an important food fish in many countries, it is likely that the condition is significantly under-reported.

Outbreaks have also been described. For example, in 2001, at least 17 people developed the symptoms of gempylid fish poisoning after attending a conference lunch in New South Wales. Analysis of fish served at the lunch

confirmed that it was escolar. A similar outbreak, affecting at least eight people, was reported in California in 2000.

Sources

The only confirmed sources of gempylotoxin are certain fish of the family Gempylidae, specifically Oilfish (*Ruvettus pretiosus*) and Escolar (*Lepidocybium flavobrunneum*). These species may sometimes be referred to by other names, such as rudderfish, Cocco, or castor oil fish. They are widely distributed in temperate and tropical waters worldwide.

These species do not metabolise wax esters found in their diet, but store them in muscle and other tissues. They therefore have very oily flesh with a lipid content of 20% or more. Analysis of oil from the muscle tissue of escolar showed that it contained approximately 90% wax.

Stability in Foods

Gempylotoxin is stable and is not destroyed during cooking. It is a natural component of the fish flesh and always present in the flesh of certain gempylid fish species.

Control Options

Processing

There is no practical method of eliminating gempylotoxin from fish during processing. However, cooking methods such as grilling where much of the oil in the flesh can be separated and discarded after cooking are reported to reduce the risk of illness.

Product Use

According to advice given by the Hong Kong Government Centre for Food Safety, consumers should either avoid eating these fish, or eat only very small portions initially to determine whether they are susceptible to the toxin. However, in certain countries, the import and sale of escolar and oilfish have been banned because they are considered toxic.

Legislation

EU

Existing legislation relating to the marketing of poisonous fish does not include reference to members of the Gempylidae. However, the Italian Government is reported to have issued a ban on the import and marketing of escolar and oilfish.

USA

The FDA has issued advice that escolar and oilfish should ''not be marketed in interstate commerce''.

Other Countries

The sale of escolar for human consumption has been banned in Japan because it is considered to be toxic.

The Government of Hong Kong has also issued guidelines on labelling the fish for consumers following reports of cases of illness associated with mislabelling. Other governments in the EU and elsewhere have issued guidance about the risks of consuming escolar and oilfish for importers, caterers and consumers.

Sources of Further Information

Published

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2.1.3.7 Okadaic Acid Toxins

Hazard Identification

What are Okadaic Acid Toxins?

The okadaic acid group of toxins (OA-toxins) are the cause of diarrhoeic shellfish poisoning (DSP), a food-borne intoxication associated with the consumption of contaminated shellfish harvested from waters affected by growth of certain types of toxic algae. DSP is a non-lethal form of food poisoning with symptoms typical of gastroenteritis, especially diarrhoea. It has been known for around 35 years and is most common in the EU and Japan, but OA-toxins are being increasingly reported in shellfish from previously unaffected areas.

The group of OA-toxins comprises OA itself, plus the dinophysis toxins, DTX1, DTX2 and DTX3, which are all analogues of OA. They are lipophilic, heat-stable polyether compounds.

Occurrence in Foods

Most cases of DSP are related to bivalve molluscs, especially mussels, but also scallops, oysters and clams. These species are filter feeders and accumulate OA-toxins when the water contains sufficient levels of toxin-producing algae. Toxicity is seasonal and tends to be highest during the summer months in the EU and Japan, although DSP cases in Scandinavia have been reported in February and in October.

Predatory fish and other marine animals that prey on toxic shellfish may also accumulate OA-toxins, especially in liver tissue, but the significance of this for human health is uncertain.

OA-toxins are fat soluble and so tend to accumulate in the fatty tissue of affected shellfish. The highest levels are normally found in the viscera and shellfish can accumulate enough toxin to cause illness within hours when large populations of toxic algae are present in the water. OA-toxins may also be metabolised in the digestive gland (hepatopancreas) of contaminated shellfish, producing related toxic by-products. Toxin levels as high as 10 mg OA per g of hepatopancreas have been reported in mussels grown in Japanese waters.

OA-toxin levels in shellfish do reduce naturally after they stop feeding on toxic algae, but there is little definite information on how this process occurs or on toxin retention times in different species. It is likely that some toxin is excreted in faeces before it can be assimilated.

Hazard Characterisation

Effects on Health

Both pectenotoxins and yessotoxins are often found in association with OA-toxins and were once thought to be involved in causing DSP. However, since

neither group gives rise to diarrhoea, OA-toxins are now considered to be the principal cause of the intoxication.

OA-toxins are powerful phosphatase inhibitors and have been reported to act by disrupting the barrier function of the intestinal epithelial cells, making them more permeable. This property is associated with inflammation of the gut in humans, leading to fluid loss from intestinal cells and to diarrhoea. A minimum dose of OA for toxic effects to occur is estimated to be 48 μg, whereas for its derivative DTX1 the minimum it is 38.4 μg.

Levels of OA-toxins are commonly expressed as toxic equivalents of OA (mg OA eq per kg) or as Mouse Units (MU per kg) relating to a standard mouse bioassay method.

The onset of symptoms of DSP may occur between 30 min and 12 hours after ingestion of toxic shellfish. The main symptoms of DSP include diarrhoea, nausea and vomiting, and abdominal pain. The severity of symptoms depends on the amount of OA-toxins ingested, but complete recovery typically occurs within three days. No fatalities caused by DSP have been reported to date and hospital treatment is not usually needed.

OA-toxins have also been shown to have other effects in animals and in cell cultures. For example OA and DTX1 are probable carcinogens, but the significance of this for human health is unknown.

Incidence and Outbreaks

DSP mainly affects the Western EU and Japan, but OA-toxin contaminated shellfish and toxin-producing algae have been found in more widespread locations, including Canada, Mexico, South America, India, Thailand, China and Australia, and detections of OA-toxins seem to be increasing.

There have been a number of major outbreaks of DSP in the EU. Mussels imported from Denmark caused 415 cases of illness in France in 1990. In 1984, 10 000 people in France were affected by DSP symptoms caused by domestically produced mussels and a further 2000 became ill the following year in a similar outbreak. 1984 also saw a major outbreak in Norway affecting at least 300 people. Over 5000 cases of DSP-related gastroenteritis were reported in Spain in 1981, and OA-toxins have repeatedly been found at high levels in shellfish from the Galician region, resulting in prolonged disruption to local fisheries from 1993 onward. DSP cases were not reported in the UK until 1997, when 49 people were made ill after eating mussels in a London restaurant. Since then, the frequency of DSP events in UK waters has increased and shellfish harvesting has been restricted in several areas on a regular basis.

In Japan, cases of DSP were first reported in 1976 and 1977 when more than 150 people were affected by vomiting and diarrhoea. A total of at least 1300 cases were reported between 1976 and 1981.

Elsewhere, outbreaks have been reported in Australia, Canada, Chile and the USA. Although the Northeast USA, especially New York and New Jersey, experienced large outbreaks of DSP-like illness between 1980 and 1985,

outbreaks of human illness have not been reported since then, although OA-toxins have been found occasionally in USA waters.

Sources

OA-toxins are produced by dinoflagellates of the genus *Dinophysis*. Seven species have been shown to produce the toxins. These are *D. acuminata* (the EU) *D. acuta*, *D. fortii* (Japan), *D. mitra*, *D. norvegica* (Scandinavia), *D. rotundata* and *D. tripos*. Three other species are also suspected of being able to produce toxins. Certain *Prorocentrum* species (*P. concavum*, *P. lima* and *P. redfieldi*) also produce OA-toxins.

If conditions are favourable, exponential growth of these species may occur resulting in an algal bloom. However, it is not necessary for visible blooms to occur for OA-toxins to be present at harmful levels. The production of toxins by different dinoflagellate species is highly variable and the same species may produce widely varying quantities of toxin in different locations. Some *Dinophysis* species can produce sufficient toxin in shellfish to cause illness in consumers at populations as low as 200 cells per litre. On other occasions much greater densities (>20 000 cells per litre) may be involved.

Stability in Foods

OA-toxins are all relatively heat stable and are not destroyed by practical cooking processes.

Natural detoxification in shellfish does occur, but the rate of this process varies greatly with the species, the season (low water temperature slows toxin loss) and with the site of toxin accumulation. It has been reported that the retention time of OA-toxins in mussels can vary from one week to six months.

Control Options

The stability of OA-toxins and the variability of natural detoxification mean that neither depuration in clean water nor cooking processes are effective or economically viable methods of reducing the toxicity of affected shellfish to safe levels.

The only effective controls available currently are the monitoring of the marine environment and the testing of shellfish flesh for OA-toxins. Regular inspection of the waters where shellfish are harvested, or produced by aquaculture, for the presence of toxic algae can be a useful source of data and indicate when a risk of toxicity is present. The routine testing of shellfish, especially mussels, for OA-toxins by chemical, immunological, or bioassay methods is the key prevention measure. However, the variability of toxin production by the algae and other factors must be taken into account when designing a suitable sampling plan.

When toxic conditions are detected, bans on harvesting shellfish have to be imposed until toxicity can be shown to have returned to safe levels and contaminated shellfish should not be allowed to enter the human food chain.

Legislation

There are regulations relating specifically to OA-toxins in shellfish in a number of countries.

In the EU the EC has set a maximum limit for combined OA, DTXs and pectenotoxins in molluscs, echinoderms, tunicates and marine gastropods of 160 μg OA eq per kg of edible tissues. The grouping of OA-toxins with pectenotoxins is mainly due to their co-occurrence and the previously suspected role of pectenotoxins in DSP. This is now recognised as inappropriate and is under review. The mouse bioassay method is the official reference method of analysis, in association with a chemical detection method if required. Monitoring programmes for toxic dinoflagellates are in place in most EU countries where shellfish are harvested.

Japan actively monitors both phytoplankton and shellfish and applies a tolerance level for OA-toxins of 5 MU per 100 g whole meat, when detected by the mouse bioassay method. This equates to approximately 0.2 μg per g.

In the USA, there is no current monitoring programme or limit for DSP toxins in shellfish, although monitoring is carried out in Canada.

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2.1.3.8 Palytoxins

Hazard Characterisation

What are Palytoxins?

The palytoxins (PlTXs) are a group of marine biotoxins first reported in about 1971 and isolated in Japan and Hawaii in the early 1980s. They are now found worldwide and may contaminate shellfish destined for human consumption. They have been reported to cause occasional food-borne intoxications in humans for at least 40 years.

PlTXs are produced by certain species of marine algae and have also been detected in species of soft corals.

Chemically, PlTX-group toxins are very large complex molecules consisting of long-chain polyhydroxylated compounds with lipophilic and hydrophilic components. To date, eight different PlTX analogues have been identified, but only two, PlTX and ostreocin-D, have been characterised. The molecular formula of PlTX is $C_{129}H_{233}N_3O_{54}$ (CAS No. 77734-91-9). The other members of the PlTX-group are ovatoxin-A, homopalytoxin, bishomopalytoxin, neopalytoxin, deopalytoxin and 42-hydropalytoxin.

Occurrence in Foods

Surveillance activity for PlTXs has been quite limited to date, but they have been shown to be present in mussels and sea urchins harvested from Mediterranean waters containing toxic algal blooms. PlTXs have also been found in crustaceans and several species of tropical marine fish.

Because the distribution of the marine algae that produce PlTXs has been expanding recently, the potential for food contamination is rising and these toxins are considered to be emerging food safety hazards.

Hazard Characterisation

Effects on Health

The toxins of the PlTX-group are considered to be some of the most potent non-protein natural toxins known. PlTX is acutely toxic to rats, mice and other mammals, showing an LD_{50} of 10–100 ng per kg body weight when administered intravenously, although LD_{50} values are much higher (767 μg per kg body weight) when the toxins are administered orally. PlTX and ostreocin-D are similarly potent, but the relative toxicity of other analogues is not known. There is little data on chronic toxicity, or the effects of long-term exposure.

The mode of action is by interference with the Na^+/K^+-ATPase ion-pump, causing an osmotic imbalance. The principal targets are muscle tissues, including heart and skeletal muscles, which contract. Death in mice administered with lethal doses of PlTX has been reported to be caused by vasoconstriction and heart failure.

Toxicity in humans is characterised by a range of poorly defined symptoms, typically including mayalgia and weakness, fever, vomiting and nausea. Recovery is reported to take several months and fatalities in severe cases have been recorded, though rarely.

It is thought likely that PlTXs are at least partly responsible for clupeotoxism, a toxic syndrome associated with the consumption of certain species of tropical clupeid fish, such as sardines. Clupeotoxism is characterised by neurological and gastrointestinal symptoms and is potentially fatal.

Incidence and Outbreaks

Many reports thought to refer to PlTXs are anecdotal and it is therefore difficult to estimate the incidence of illness caused by these toxins.

Clinically investigated individual cases and small outbreaks of illness caused by PlTXs have been reported in Japan and the Phillippines. Clupeotoxism outbreaks have been reported in Hawaii, Jamaica, the Phillippines and Madagascar.

Sources

PlTX was first detected in marine soft corals of the genus *Palythoa*, but of more concern from a food safety point of view is the production of PlTXs by marine dinoflagellates of the genus *Ostreopsis*. Examples of toxin-producing species of this genus are *Ostreopsis siamensis*, *O. mascarenensis* and *O. ovata*. These algae are the cause of growing concern as their geographical distribution is reported to have expanded in recent years. Blooms of *Ostreopsis* species have been observed in coastal waters of a number of temperate countries, including Greece, Italy, Spain and France, as well as in tropical regions. This expansion may increase the risk of contamination in commercial shellfish production areas.

Stability in Foods

There is currently little or no information available on the stability of PlTXs in foods and no published information relating to the effect of processing on their stability and persistence in shellfish.

Control Options

The lack of information about the stability of PlTXs makes it difficult to estimate whether depuration in clean water or cooking processes would be effective or economically viable methods of reducing toxin levels in affected shellfish.

The only effective control available currently is the regular monitoring of shellfish samples for the presence of PlTXs using a mouse bioassay, cell-based methods, HPLC or LC-MS/MS analysis, but standard methods have not yet been formally validated.

Legislation

There are no regulatory limits currently in force for PlTXs in shellfish anywhere in the world. A provisional limit of 250 μg per kg shellfish has been proposed by an EU working group on marine biotoxins.

Sources of Further Information

Published

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2.1.3.9 Pectenotoxins

Hazard Characterisation

What are Pectenotoxins?

Until recently pectenotoxin (PTX)-group toxins were grouped with okadaic acid (OA)-group toxins as a probable cause of diarrhoeic shellfish poisoning (DSP), since they always co-occur with OA-group toxins in shellfish. However, their role in DSP is unclear and they have been found to be acutely toxic to mice when present in shellfish extracts. For these reasons PTX-group toxins are now classified as a separate group of marine biotoxins. They were first isolated from scallops in Japan in 1985 and have since been frequently found in shellfish, but their potential for involvement in food-borne intoxications is uncertain.

PTXs are produced by certain species of marine algae that have been found in temperate waters worldwide.

Chemically, the PTXs are lipophilic macrolide compounds containing multiple polyether-lactone rings. To date, at least 15 PTX analogues have been identified and characterised in algae and shellfish. PTX-2 is commonly found in certain marine algae and is thought to be the main precursor of PTX-group toxins, which are produced by biotransformation in the guts of various species of shellfish. However, PTX-1 has also been detected in algae.

Occurrence in Foods

PTX-group toxins have been detected in a number of bivalve molluscan shellfish, including oysters, mussels and scallops. They have been found in shellfish in a number of EU countries, including Germany, Italy, Norway, Spain and the UK, as well as in Australia, Japan and New Zealand.

Surveillance activity for PTXs in shellfish has been very limited to date. The highest reported level of PTX-2 in shellfish from EU waters is 418 µg per kg shellfish meat, in a sample tested in the UK in 2006–2007.

Hazard Characterisation

Effects on Health

Until recently, PTXs were considered to be associated with DSP because they were often found with OA-group toxins and early studies suggested that PTX-2 caused diarrhoea in mice. However, further investigation showed that the PTX-2 used in these tests was contaminated with OA-group toxins and more recent studies have failed to reproduce the results, even at high doses. It is now generally accepted that PTXs do not cause diarrhoea and they are therefore now classified as a separate group of toxins.

PTXs are acutely toxic in mice when administered by intraperitoneal injection, causing severe damage to the liver at higher doses. Adverse effects on the livers of mice have been demonstrated at doses above 100 µg per kg body

weight. The mechanism of action is thought to be through the depolymerisation of actin-based structures in the cystoskeleton, leading to disruption of the cell structure and eventual cell death.

PTXs appear to be much less toxic to mice when taken orally even at higher levels. Absorption is reported to be low and toxicity is limited to the gastrointestinal tract. There is little or no published data on the chronic or long-term toxicity of PTXs in animals.

There are no confirmed reports of PTX intoxications in humans. Isolated reports of gastrointestinal symptoms caused by PTX-2 in shellfish have since been attributed to the co-occurrence of OA-group toxins. But in view of their acute toxicity in mice, they are considered to be potentially toxic to humans. Since PTXs have been found to accumulate in shellfish intended for human consumption in several parts of the world they should be regarded as an emerging food safety risk.

Sources

PTXs are produced exclusively by species of marine dinoflagellates from the genus *Dinophysis*, specifically *Dinophysis fortii*, *D. acuta*, *D. acuminata*, *D. caudata* and *D. norvegica*. These species may be found worldwide. PTX-2 is by far the commonest analogue found in *Dinophysis* species and other analogues are thought to originate in the guts of bivalve molluscs where they are produced by biotransformation.

Stability in Foods

There is currently little published information available on the stability of PTXs in foods or the effect of processing on their stability and persistence in shellfish. However, there is evidence to suggest that they are heat stable and it seems reasonable to assume that cooking could increase the concentration in shellfish flesh if water is lost.

Control Options

The lack of information about the stability of PTXs makes it difficult to estimate whether depuration in clean water would be an effective method of reducing toxin levels in affected shellfish. However, it is unlikely that conventional cooking processes would reduce PTXs to safe levels.

The only effective control available currently is the regular monitoring of shellfish samples for the presence of PTXs using a mouse bioassay, or an adequately validated alternative, such as LC-MS/MS analysis.

Legislation

The EC has a set a maximum level for PTXs in live bivalve molluscs of 160 μg okadaic acid equivalents per kg. The grouping together of OA-group toxins and PTXs in the regulation is based on their co-occurrence and previously

suspected involvement in DSP. This is no longer considered appropriate from a toxicological point of view and may be subject to amendment in the near future.

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Published

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2.1.3.10 Saxitoxins

Hazard Characterisation

What are Saxitoxins?

The saxitoxins (STXs) are a group of largely marine alkaloid biotoxins, which can cause a food-borne intoxication known as paralytic shellfish poisoning (PSP). PSP is associated with the consumption of contaminated marine shellfish harvested from waters affected by a sudden and rapid growth of certain types of toxic algae. PSP was recorded in Canada over 100 years ago and reports were restricted to temperate waters until the 1970 s. Since then there has been an apparent increase in outbreaks and a geographical spread into more tropical Southern waters. PSP can cause a variety of neurological symptoms and severe cases can prove fatal within hours.

Chemically, the STX-group toxins are all closely related tetrahydropurines. To date, at least 57 analogues of STX have been identified. The analogues vary in their toxicity, but STX, NeoSTX, GTX1 and dc-STX are considered to be the most toxic.

Occurrence in Foods

Most cases of PSP are related to bivalve molluscs, especially mussels and clams, but also oysters and scallops. In total, at least 50 shellfish species have been associated with cases of PSP. All these species are filter feeders and accumulate toxins when the water contains significant levels of toxin-producing algae. When the algae are digested, STXs are released into the animal's digestive tissue. PSP cases in Japan have also been associated with consumption of certain reef-dwelling crab species.

Different shellfish species vary greatly in the way that they accumulate STXs and in the retention time of the toxins within the body. Some species seem to be able to detect toxins in the water and stop feeding, but others do not have this ability. Some detoxification within the body also occurs as the toxins are broken down, but the rate varies enormously between species. Generally, the viscera accumulate the highest levels of toxins, but detoxification tends to proceed more rapidly in these tissues. The variation is illustrated by a comparison between mussels and oysters. Mussels accumulate toxins much more quickly and at higher concentrations than oysters, but they also detoxify much more quickly. For these reasons, potential levels of STXs in affected shellfish are almost impossible to predict.

Hazard Characterisation

Effects on Health

STXs are potent neurotoxins, and operate by selectively blocking the voltage-gated sodium channel—a large protein that extends across the plasma membrane of nerve and muscle cells. This slows or stops the cells ability to generate

an action potential and so affects cell function. Reports of the level needed to cause symptoms vary greatly. The Australia New Zealand Food Authority has reported that 120 to 180 μg of STX is sufficient to produce symptoms in humans, 400 to 1060 μg may prove fatal and levels above 2000 μg are likely to cause death. However, in some reported cases, 300 μg of STX proved fatal, while intakes as high as 320 μg have apparently not caused symptoms. It is likely that varying sensitivity between individuals may be partly responsible for these observations.

Levels of STXs are commonly expressed as toxic equivalents of STX (mg STX eq per kg) or as Mouse Units (MU per 100 g) relating to a standard AOAC mouse bioassay method.

The first symptom of toxicity in mild cases is usually numbness, or tingling around the mouth, which normally appears within 30 min. This then spreads to the head and neck. Within a few hours, other symptoms, including 'pins and needles' in the hands and feet, headaches, nausea, vomiting and diarrhoea usually occur and vision may be affected temporarily. Muscular weakness is also common and symptoms can last for several days.

Symptoms of more severe toxicity include numbness or tingling and weakness in arms and legs, incoherent speech and dizziness, motor coordination is affected and the patient may have difficulty breathing. In very severe cases, muscle and respiratory paralysis can develop, leading to death within 2 to 24 hours of ingestion of toxin. Mortality rate is variable (0–14%), but if the patient can be kept alive for at least 24 hours, the chances of recovery are good.

Incidence and Outbreaks

The geographical distribution of PSP appears to have been expanding since the 1970 s. Before then, PSP contamination events were restricted to temperate waters off the coasts of the EU, North America and Japan. More recently, STXs have been reported in shellfish all over the Southern hemisphere, including South Africa, Central and South America, Australia, China, India, Malaysia and Thailand. It is not clear whether this is due to increasing awareness of toxic algae and improved diagnosis of PSP, or whether other factors are involved. There are estimated to be 1600 cases of PSP each year worldwide, with approximately 300 of these proving fatal.

STX contamination in shellfish has been recorded repeatedly in Western EU waters, especially off the coasts of Scotland, Spain, Portugal and Norway. Harvesting of scallops, mussels and other shellfish is regularly prohibited during the summer months when contamination occurs.

In the UK, an outbreak of PSP in 1968 affected many people in Northeast England, with 78 requiring hospital treatment, but no deaths. The outbreak was linked to mussels containing between 600 and 6000 μg STX eq per kg. Since then monitoring of the fishing grounds has largely prevented similar outbreaks.

In 1976, mussels exported from Spain caused PSP outbreaks in several other EU countries, including France, Germany and Italy. At least 120 people were affected, but there were no deaths.

PSP outbreaks have been known in Canada for over 200 years. Between 1880 and 1995, some 106 documented outbreaks occurred affecting 538 people and killing 32. Outbreaks have also occurred repeatedly on the east coast of the USA and in Alaska. In 1980 an outbreak in the Northeast USA affected 51 people who had eaten locally caught mussels and oysters containing 3000 to 40 000 μg STX eq per kg.

Sources

STXs are produced mainly by dinoflagellates of the genus *Alexandrium* (previously called *Gonyaulax* species). Several species are involved, notably *A. catenella*, *A. cohorticula*, *A. fraterculus*, *A. fundyense*, *A. minutum* and *A. tamarensis*. Certain other dinoflagellate species, such as *Pyrodinium bahamense* and *Gymnodinium catenatum* also produce STXs. Many of these species exist as free-swimming forms and as resting cysts that are also toxic.

The presence of low numbers of these algae is not a health hazard, but if conditions are right—increasing temperature, high nutrient levels and sunlight—exponential growth may occur, resulting in an algal bloom. When this happens the numbers of cells can be come high enough to colour the water reddish brown (a red tide). During a bloom the cells are at their most toxic during the late exponential phase. Any filter-feeding shellfish in water affected by a toxic bloom are likely to accumulate high levels of toxin quite quickly as they feed on and digest the algal cells. Thus shellfish harvested from such waters carry a high risk of toxicity.

Production of STXs has also been detected in fresh and brackish water, where they are produced by cyanobacteria belonging to several genera, including *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis* and *Lyngbya*. Toxigenic cyanobacterial blooms can contaminate fresh water supplies for drinking and recreation. STX-group toxins have been reported in freshwater resources in a number of countries, including Australia, the USA and China. Accumulation of STXs in farmed freshwater fish, notably Tilapia, has recently been reported.

Stability in Foods

STXs are relatively heat stable, especially at acid pH, but easily oxidised under alkaline pH conditions. Conventional cooking processes reduce toxin levels, mainly by leaching of toxin into the cooking liquor, but do not eliminate the risk of toxicity. Canning and retorting processes at temperatures of 110 °C to 115 °C have been reported to reduce STX levels in shellfish by 70–90%, partly by leaching and partly by thermal destruction, but cannot be relied upon to eliminate toxin completely. Their effectiveness depends on the initial toxin concentration and only very severe processes (120 °C for 60 min) have been shown to give complete detoxification.

Natural detoxification in shellfish does occur, but the rate of this process varies greatly between species and some may remain toxic for months or even years in the case of clams.

Control Options

The relative stability of STXs and the variability of natural detoxification mean that neither depuration in clean water nor cooking processes are effective or economically viable methods of reducing the toxicity of affected shellfish to safe levels.

Research into better methods is ongoing, but the only effective control available currently is the monitoring of waters where bivalve molluscs are harvested or produced by aquaculture. This can be done by regular inspection of water for the presence of dinoflagellates and their cysts, using biological, or immunological detection and identification methods and, more recently, molecular biology techniques. Regular inspection and testing of shellfish flesh for the presence of toxins using bioassay or chemical methods is also important.

When toxic conditions are detected, bans on harvesting shellfish have to be imposed until toxicity can be shown to have returned to safe levels and contaminated shellfish should not be allowed to enter the human food chain.

Legislation

There are regulations relating specifically to STXs in shellfish in a number of countries.

In the EU there is a limit for bivalve molluscs of 80 μg STX eq per 100 g of meat. The mouse bioassay method is the official reference method of analysis, in association with a chemical detection method if required. Monitoring programmes to check for toxic dinoflagellates are in place in most EU countries where shellfish are harvested.

In the USA, Canada and Australia the limit for bivalves is also 80 μg STX eq per 100 g of meat and the mouse bioassay method is used. However, in the USA and Canada, some shellfish with higher levels of PSP toxin can be harvested if they are to be canned.

Both China and Japan set a limit of 400 MU per 100 g in bivalves and specify the mouse bioassay as the reference method.

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2.1.3.11 Tetrodotoxin

Hazard Characterisation

What is Tetrodotoxin?

Tetrodotoxin (TTX), also known as anhydrotetrodotoxin 4-epitetrodotoxin, or tetrodonic acid, is a marine biotoxin associated with certain fish species, notably pufferfish. Consumption of these fish can cause very severe food-borne intoxication, often referred to as pufferfish poisoning, or fugu poisoning. Unlike other marine biotoxins, it is not produced by the growth of toxic algae. Pufferfish poisoning has been known for many years, especially in Japan where the fish are a delicacy. Probable cases were documented by Captain James Cook as long ago as the eighteenth century. The term tetrodotoxin was first applied to the toxin nearly 100 years ago and the TTX molecule itself was first characterised in 1964.

TTX is a potent non-proteinaceous neurotoxin belonging to a group referred to as guanidinium toxins, which also includes the paralytic shellfish poisoning (PSP) toxin, saxitoxin (STX). It consists of a positively charged guanidinium group and a pyrimidine ring with five additional fused rings. A number of derivatives of TTX have also been identified.

Occurrence in Foods

TTX is mainly associated with fish of the order Tetraodontidae (pufferfish, balloon fish, fugu, globe fish, blowfish, toad fish) from the Pacific and Indian Oceans. These fish are a traditional food in Japan, where they are sold as ''fugu'' in specialised restaurants employing specially trained and licensed chefs who are able to remove the most toxic parts of the fish to reduce the poisoning risk. The highest levels of TTX are found in the viscera, particularly the liver and ovaries, and skin of the fish, but the muscle tissue does not usually contain dangerous levels of toxin.

TTX has also been found in a wide range of other animals, such as the blue-ringed octopus, goby, triggerfish, parrotfish, angelfish, xanthid crabs, certain marine molluscs and worms and some terrestrial amphibians, such as the Californian newt.

The trumpet shell (*Charonia sauliae*) has also been reported to contain a TTX derivative and has been implicated in some cases of food-borne intoxication.

Hazard Characterisation

Effects on Health

TTX is a very potent neurotoxin, and operates in a similar way to the STX group of biotoxins by selectively blocking the voltage-gated sodium channel—a large protein that extends across the plasma membrane of nerve and muscle

cells. This slows or stops the cells ability to generate an action potential and so affects cell function. A minimum dose of 0.2 mg has been estimated to be sufficient to cause symptoms and an LD_{50} in man of 2 mg has been reported.

Initial symptoms appear between 20–180 min of ingestion and are similar to those of PSP caused by STX. A slight numbness of the lips and tongue is then followed by increasing paraethesia (tingling, 'pins and needles') in the face, hands and feet. Those affected may also suffer dizziness, headaches, nausea and diarrhoea.

These symptoms may then develop into increasing paralysis and respiratory problems. Victims may be completely paralysed and unable to move or speak, yet remain conscious. Death usually occurs within 4–6 hours but may be as rapid as 20 min in some cases. Those who have not died within 24 hours generally recover completely. Mortality rates of almost 50% have been reported, but this is strongly influenced by the quantity of TTX ingested.

Incidence and Outbreaks

TTX poisoning is most frequently reported in Japan. Between 1987 and 1996, almost 300 cases involving 500 individuals were recorded, with a mortality rate averaging approximately 7%. Most of these cases are thought to be associated with home preparation of fugu. Other Pacific countries, including the USA, have reported sporadic cases. Outbreaks elsewhere are rare, although three people died in Italy in 1977 after consuming wrongly labelled imported frozen pufferfish from Taiwan.

Cases of TTX poisoning associated with the consumption of small gastropods have been reported in China and Taiwan. During the period 1977–2001, more than 300 people were affected in China and 16 deaths were recorded.

Sources

No algal source of TTX has ever been identified, and it was thought until quite recently that the toxin was produced endogenously by pufferfish as a metabolic by-product. However, there is now considerable evidence suggesting that this is not the case. The toxicity of pufferfish is very variable and when they are grown in culture they do not become toxic unless fed material containing TTX. Furthermore, the discovery that many other unrelated animals also contain TTX suggests an exogenous source.

It is now generally accepted that TTX is produced by certain marine bacteria—notably members of the *Vibrionaceae*, some *Pseudomonas*, *Shewanella*, *Photobacterium phosphoreum* and *Alteromonas* species. It is thought that the toxin passes up the food chain through plankton, small gastropods and flatworms and is eventually accumulated in the tissues of pufferfish species, possibly as a defence against predators. Pufferfish appear to be immune to the toxic effects of TTX, but other fish species do not accumulate it, even when fed low-dose toxic material. Some other marine animals, especially the blue-ringed octopus, are reported to accumulate the toxin in special glands and may use it as venom to subdue their prey.

Stability in Foods

TTX is reported to be relatively heat stable and is not affected by normal cooking procedures. Furthermore, it does not appear to be significantly reduced during prolonged frozen storage.

Control Options

The stability and toxicity of TTX means that the only effective control for prevention of poisoning is to avoid consuming those fish species that are known to contain the toxin. In Japan, where pufferfish are traditionally eaten, strict licensing and training of fugu chefs is required to protect the consumer. These individuals are skilled in the removal of toxin-containing tissue from the fish, but the possibility of human error remains.

TTX can be monitored in pufferfish using the same mouse bioassay developed for quantifying PSP toxin and an HPLC method has also been developed. These methods may be useful in cases where pre-prepared frozen tissues from unknown, or wrongly identified species of fish are intended for consumption.

Legislation

Neither the USA, nor the EU normally permit the importation of pufferfish products for human consumption, although exceptions may be granted under special circumstances.

In Japan, there is a strict licensing system covering the marketing and preparation of pufferfish for human consumption.

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2.1.3.12 Yessotoxins

Hazard Characterisation

What are Yessotoxins?

Yessotoxin (YTX) was first isolated from scallops in Japan in 1986 and until recently yessotoxins were thought to be associated with diarrhoeic shellfish poisoning (DSP). However, they are now classified as a separate group of marine biotoxins. YTXs are cytotoxic and may be present in seafood intended for human consumption, but their potential for involvement in food-borne intoxications is uncertain.

YTXs are produced by certain species of marine algae that have been found in temperate waters worldwide.

Chemically, the YTXs are polyether compounds with a characteristically ladder-shaped molecule formed by 11 adjacent ether rings, with an unsaturated side-chain and two sulphate esters. The molecular formula of YTX is $C_{55}H_{82}O_{21}S_2Na_2$ (CAS No. 112514-54-2). To date, at least 100 YTX analogues have been identified in algae and in shellfish and about 40 of these have been characterised. The most toxicologically studied of the analogues is homoyessotoxin, but 45-hydroxy-homoyessotoxin and desulpho-yessotoxin have also been investigated.

Occurrence in Foods

Since the first report of the isolation of YTX in Japan, YTXs have been found in several different species of filter-feeding molluscan shellfish, including mussels, scallops, oysters and clams, in Norway, New Zealand, Chile, Russia, Spain and Italy.

Surveillance activity for YTXs in shellfish has been limited to date, but levels as high as 9620 μg per of kg shellfish meat have been reported, notably from the Northern waters of the Italian Adriatic, where YTX has been found regularly since 2000.

Hazard Characterisation

Effects on Health

Until recently, YTXs were considered to be associated with DSP because they were often found with DSP toxins and give a positive result in a mouse bioassay DSP toxin test. However, they differ from other DSP toxins, such as okadaic acid, in that they do not cause diarrhoea or inhibit phosphatases. They are therefore now classified as a separate group of toxins.

YTXs are acutely toxic in mice when administered by intraperitoneal injection, causing symptoms similar to those observed in cases of paralytic shellfish poisoning (PSP). They have been reported to target the cardiac muscle, liver and pancreas and may also cause nerve damage in the brain. LD_{50} values vary greatly, from about 80 μg per kg to 750 μg per kg, for reasons that are not

clear. The mechanism of action of YTXs is also uncertain, although four different processes have been implicated and investigated.

YTXs appear to be much less toxic to mice when taken orally even at high levels. A single dose of YTX of 50 mg per kg of body weight produced no clinical symptoms in a recent study (EFSA). However, there are reports of damage to the heart muscle detectable by microscopic examination at lower doses (>5 mg per kg of body weight). There is little or no published data on the chronic or long-term toxicity of YTXs in animals.

There are no reports of YTXs intoxications in humans, but because of their acute toxicity in mice they are considered to be potentially toxic to humans. Since they have been found to accumulate in shellfish intended for human consumption in several parts of the world, they should be regarded as an emerging food safety risk.

Sources

The primary source of YTXs is the marine dinoflagellate *Protoceratium reticulatum*. YTXs have been detected in this species in temperate waters all over the world, including Japan, Canada, New Zealand, The Mediterranean, Norway and the UK. Other dinoflagellate species have also been found to produce YTXs, notably *Lingulodinium polyedrum* and *Gonyaulax spinifera*. *P. reticulatum* is reported to be able to produce a wide range of YTX analogues, but some of the known analogues are much more common in shellfish and and may be produced by biotransformation in the gut.

Stability in Foods

There is currently little published information available on the stability of YTXs in foods or the effect of processing on their stability and persistence in shellfish. However, there is evidence to suggest that they are heat stable and it seems reasonable to assume that cooking could increase the concentration in shellfish flesh if water is lost.

Control Options

The lack of information about the stability of YTXs makes it difficult to estimate whether depuration in clean water would be an effective method of reducing toxin levels in affected shellfish. However, it is unlikely that conventional cooking processes would reduce YTXs to safe levels.

The only effective control available currently is the regular monitoring of shellfish samples for the presence of YTXs using a mouse bioassay, or an adequately validated alternative, such as LC-MS/MS analysis.

Legislation

The EC has a set a maximum level for YTXs in edible tissues of 1 mg YTX eq per kg in the EU.

Sources of Further Information

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2.1.4 BIOGENIC AMINES

2.1.4.1 Biogenic Amines (Excluding Histamine)

Hazard Identification

What are Biogenic Amines?

Biogenic amines are low-molecular-weight organic bases produced in a variety of foods by the decarboxylation of specific free amino acids. This may occur naturally as a result of the action of endogenous decarboxylase enzymes in the food, or more importantly as a by-product of bacterial growth and the production of exogenous decarboxylases. The presence of significant amounts of biogenic amines, especially in meat and fish products, is often an indicator of bacterial spoilage.

Histamine is the best known and most studied biogenic amine in foods, but this is considered in detail in the chapter on scombrotoxic poisoning. Other important biogenic amines and their precursor amino acids are given in Table 2.1.6.

In terms of chemical structure, cadaverine and putrescine are aliphatic diamines, tyramine and β-phenylethylamine are aromatic amines and tryptamine is a heterocyclic amine.

In addition to these compounds, certain other biogenic polyamines, such as spermine and spermidine, are present at significant levels in some foods, especially fish and vegetables. However, these are thought to be produced by endogenous decarboxylation pathways rather than as a result of microbial decomposition.

The presence of significant quantities of biogenic amines in foods can have adverse effects on health and is generally undesirable.

Occurrence in Foods

Biogenic amines are known to occur in a wide variety of food products, but they are of particular significance in foods that contain a high level of free amino acids and high numbers of decarboxylase-producing bacteria. These include fish products, cheese, meat products (especially fermented meats), wine, beer and fermented vegetable products, such as sauerkraut. Certain biogenic

Table 2.1.6 Important biogenic amines and their precursor amino acids.

Biogenic amine	*Precursor*
Tyramine	Tyrosine
Cadaverine	Lysine
Putrescine	Ornithine
Tryptamine	Tryptophan
β-Phenylethylamine	Phenylalanine

amines are also found naturally in a range of fruit juices and fresh fruit and vegetables, including cocoa beans, mushrooms and lettuce.

Different amines tend to predominate in different foods, depending on the amino acids present, the nature of the bacterial population and the nature of the processing and storage environments. Putrescine and cadaverine levels tend to increase in the tissues of fish after capture, especially under temperature abuse conditions, and high levels indicate spoilage. In ripened cheese, tyramine, putrescine and cadaverine predominate, while tyramine is found in higher concentrations (up to 150 mg per 100 g) than other amines in fermented meats.

Hazard Characterisation

Effects on Health

Although the role of histamine in scombrotoxic poisoning is well established, the food safety significance of other biogenic amines is much more uncertain.

In acute toxicity testing using rats, most biogenic amines are found to have quite low oral toxicity. Tyramine, cadaverine and putrescine all have acute oral toxicities of at least 2000 mg per kg of body weight. Spermine and spermidine were reported to be slightly more toxic, with acute oral toxicities of 600 mg per kg of body weight. When administered intravenously, all these amines, except tyramine, caused a drop in blood pressure. However, the levels of most biogenic amines that are toxic in humans have not been reliably determined and a wide range of figures has been suggested. Furthermore, there is evidence that individuals vary considerably in their sensitivity.

Tyramine has been associated with hypertension and headaches in sensitive individuals, especially those who suffer from migraine headaches. Tyramine also interacts with a class of drugs called monoamine oxidase inhibitors (MAOI). These drugs are antidepressants and, although largely superseded by more modern drugs, they are still prescribed for a minority of patients. MAOI inhibit monoamine oxidase enzymes in the gut that would normally inactivate tyramine in foods. This allows more tyramine to enter the circulatory system and increases the risk of dangerous rises in blood pressure. Patients taking MAOI are advised to avoid tyramine-rich foods, such as cheese.

There is evidence that some biogenic amines, particularly putrescine and cadaverine, may be indirectly involved in histamine poisoning. There have been reports of cases of scombrotoxic poisoning being caused by fish containing unusually low levels of histamine, but with high amounts of other amines. It is thought that other amines may increase histamine uptake by inhibiting intestinal enzymes, such as diamine oxidase, that would normally metabolise histamine. It has been suggested that cadaverine and putrescine may also facilitate histamine transport through the wall of the intestine, but the mechanism involved is unknown.

In foods containing nitrite, such as cured meat products, putrescine and cadaverine may react with nitrate and produce carcinogenic compounds.

Incidence and Outbreaks

The uncertainty surrounding the public health significance of dietary exposure to biogenic amines, other than histamine, means that there is virtually no published information on the incidence of toxic events or outbreaks.

Sources

Although biogenic enzymes such as spermine and spermidine are produced endogenously in foods as a result of cellular metabolism, it is the exogenous decarboxylation of free amino acids by bacteria that is of most significance for food safety.

Bacterial sources of biogenic amines vary with the food commodity concerned and with the environmental conditions of processing and storage. For example, putrescine and cadaverine are produced in fish tissue by a wide range of bacterial species, many of which are also involved in histamine production. Post-harvest contaminants, such as members of the Enterobacteriaceae, are particularly active amine producers, especially when temperature control is poor. *Proteus* spp., *Klebsiella* spp., *Morganella morganii* and *Hafnia alvei* are all capable of producing high levels of biogenic amines in fish. *Pseudomonas* species too have been reported to generate high levels of putrescine and cadaverine in fish stored at temperatures between 0 and 15 °C.

Tyramine in cheese is produced mainly by non-starter bacteria during the ripening process. Various *Lactobacillus* species, enterococci and propionibacteria have been reported to produce biogenic amines during cheese ripening. In fermented meats, lactobacilli have been found to produce tyramine, while members of the Enterobacteriaceae produced cadaverine and *Pseudomonas* species produced putrescine. Again, non-starter contaminating bacteria are thought to be mainly responsible.

In wines, lactobacilli that perform the malolactic fermentation have also been found to produce tyramine and putrescine and contaminating lactobacilli are also thought to produce biogenic amines in some beers.

Stability in Foods

Like histamine, other biogenic amines are relatively heat stable and are not destroyed by cooking or even during canning processes. However, unlike histamine, cadaverine and putrescine in particular are detectable by their unpleasant and pungent odours at high levels, especially in fish and meat.

Bacterial decarboxylase enzymes are heat labile and are destroyed by cooking, so that further biogenic amine production does not occur unless foods are re-contaminated.

Control Options

In non-fermented foods, biogenic amines are produced mainly by contaminating spoilage bacteria. Therefore many of the controls routinely applied to prevent microbial spoilage and extend shelf-life, such as modified atmosphere packaging, irradiation and high pressure processing, are also helpful in preventing their production at high levels. But the key controls are good hygienic practice and effective temperature control. These are particularly important for minimising the contamination of foods by spoilage bacteria and inhibiting their growth.

For fermented foods where a starter culture is used, it is recommended that a starter culture strain that has been shown not to produce biogenic amines is chosen. The initial microbiological quality of the raw materials also has a significant influence on amine production during manufacture and storage. Good hygiene is important in preventing contamination by species of non-starter species that may produce large amounts of amines, particularly for products with lengthy ripening periods. A heat treatment in processing helps to reduce non-starter bacterial populations and raw-milk cheeses and fermented meat products made without pasteurisation are more likely to develop high amine concentrations during ripening.

Legislation

Most of the legislation relating to biogenic amines applies specifically to histamine and is dealt with separately. However, the EC has suggested a maximum legal limit for total biogenic amines of 30 mg per 100 g in fish and fish products for future consideration.

Sources of Further Information

Published

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2.1.4.2 Scombrotoxin (Histamine)

Hazard Identification

What is Scombrotoxin?

Scombrotoxin is a food-borne toxin most often associated with the consumption of fish, particularly species belonging to the *Scombridae* and *Scomberesocidae* families (scombroid fish), such as mackerel and tuna. It can cause a mild, though sometimes distressing, form of food-borne intoxication (scombroid or scombrotoxic food poisoning) when ingested in sufficient quantities.

Scombrotoxic poisoning is also known as histamine poisoning, since histamine is considered to be the principal toxic component of scombrotoxin, although other compounds may be involved. Histamine ($C_5H_9N_3$, CAS No. 51-45-6) is a biogenic amine and can be produced during processing and/or storage in fish and certain other foods, usually by the action of spoilage bacteria.

Occurrence in Foods

Scombrotoxin is most often associated with scombroid fish, especially tuna, skipjack, bonito and mackerel, but other non-scombroid fish, such as sardines, herring, pilchards, marlin and mahi-mahi have been involved in outbreaks of illness. There are also reports that scombrotoxin could occur in salmon species. Generally, fast swimming and migratory finfish species with red-coloured meat are more likely to develop high histamine levels that whitefish species.

The toxin is not limited to fresh and frozen fish. It may be present in canned and cured fish products at high enough concentrations to cause illness.

The concentration of histamine can vary considerably between different sampling sites in a single fish, or between individual cans in a single lot. Levels of >3000 ppm have been recorded in fish products implicated in outbreaks of scombrotoxic poisoning.

Histamine can also be produced at levels toxic to humans by bacterial action in other foods, notably Swiss cheese.

Hazard Characterisation

Effects on Health

Scombrotoxic (histamine) poisoning is a chemical intoxication, in which symptoms typically develop rapidly (from 10 min to 2 hours) after ingestion of food containing toxic histamine levels.

The range of symptoms experienced is quite wide, but may include an oral burning or tingling sensation, skin rash and localised inflammation, hypotension, headaches and flushing. In some cases vomiting and diarrhoea may develop and elderly or sick individuals may require hospital treatment. The symptoms usually resolve themselves within 24 hours.

The evidence for histamine as the active toxin in scombrotoxic poisoning is strong, but the condition is very difficult to replicate in humans using pure histamine and a clear dose–response effect is lacking. Scombroid poisoning is therefore not now considered to be simple histamine poisoning. A number of possible toxicity mechanisms have been proposed, but the true cause remains uncertain. One possibility is that other biogenic amines in spoiled fish, such as putrescine and cadaverine, may act as potentiators for histamine toxicity.

Because of the uncertainty over the mechanism of toxicity, the threshold toxic level for histamine remains unclear. Individuals also vary in the severity of their response to histamine in fish. Analysis of outbreaks suggests that levels of histamine above 200 ppm are potentially toxic. Although histamine occurs naturally in the human body, exposure to large doses can rapidly produce the symptoms of toxicity.

Incidence and Outbreaks

The symptoms of histamine poisoning resemble an allergic reaction and there is potential for misdiagnosis. Furthermore, since symptoms are usually mild, it is likely that the illness is considerably under-reported. Nevertheless, it is thought that histamine poisoning is one of the commonest forms of fish-related toxicity.

The highest numbers of cases are reported in the USA, Japan and the UK, but this may be a reflection of reporting systems rather than incidence. Between 1992 and 2009, England and Wales reported 71 outbreaks affecting 336 people. Outbreaks were more common in summer than in winter. In the USA, between 1968 and 1980, 103 outbreaks involving 827 people were reported, and in Japan over the same period, 42 outbreaks affecting 4122 people. A more recent report (2008) stated that scombroid poisoning accounted for 38% of all seafood-related poisoning outbreaks in the USA.

Large outbreaks also occur; in 1973, at least 200 USA consumers became ill after eating domestic canned tuna.

In the first six months of 2005 an unusual increase in incidence was reported in England and Wales, with 16 outbreaks affecting 38 people. This was thought to be associated with poor temperature control and hygiene in certain catering premises. A similar trend was reported in 2010.

Sources

Histamine in fish and other foods is produced by the decarboxylation of the amino acid histidine and fish species that have high levels of free histidine in their tissues are most likely to develop toxic histamine levels. This is usually the result of the action of the enzyme histidine decarboxylase, which is found in a number of bacterial species that may occur on fish.

Species such as *Vibrio*, *Pseudomonas* and *Photobacterium* are found in the marine environment and occur naturally on fish. Others, especially the Enterobacteriaceae, are contaminants that are introduced post harvest. It is this second group that is considered most important in the development of histamine. Species such as *Morganella morganii*, *Raoultella planticola* and *Hafnia*

alvei are able to produce high levels of histamine very rapidly at mesophilic temperatures (20–30 °C). For this reason, histamine is more often produced during spoilage in this temperature range, although high levels can also develop at temperatures as low as 0–5 °C over time. Recently, significant histamine production has been found in psychrotolerant species, such as *Morganella psychrotolerans* and *Photobacterium phosphoreum*.

In tropical waters the indigenous microflora may be more important histamine-producing organisms, particularly when fishing methods such as long-lining are used, where the fish may die before landing. Under these conditions, it is possible for histamine to be formed before the fish is landed and chilled.

There is evidence that histidine decarboxylase remains active at chill temperatures, even though the bacteria themselves are not active. Therefore once the enzyme has been formed at higher temperatures, it may continue to produce histamine even when the fish is properly chilled.

It is also possible for histamine to form after cooking or canning if the fish subsequently becomes contaminated with histidine decarboxylase-producing bacteria. This can happen when canned fish is handled under conditions of poor hygiene.

Stability in Foods

Histamine is extremely stable once formed and is not affected by cooking. It can survive canning and retorting processes and is not reduced during freezing or frozen storage. Furthermore, high histamine levels may not be accompanied by other signs of spoilage and may be undetectable other than by chemical analysis.

The enzyme histidine decarboxylase is inactivated by cooking and further histamine will not then be produced unless re-contamination occurs.

Control Options

Temperature Control

Chilling

The key measure for the control of histamine production in fish is rapid chilling as soon as possible after death, particularly where the fish has been exposed to warm water. This will inhibit the formation of bacterial histidine decarboxylase. Once the enzyme is present, control options are very limited.

Accepted guidelines (FDA 2011) recommend that fish exposed to air or water temperatures of 28.3 °C or less should be placed in ice, chilled seawater or brine at $\leq$4.4 °C as soon as possible, but not more than nine hours after the time of death. If the fish have been exposed to air or water temperatures above 28.3 °C they should be chilled to $\leq$4.4 °C as soon as possible, but not more than six hours from the time of death. Fish gutted and gilled before chilling should be chilled to $\leq$4.4 °C as soon as possible, but not more than 12 hours from the time of death. Very large fish such as tuna that are eviscerated before chilling also should have the body cavity packed with ice.

Further chilling to a temperature as close to the freezing point as possible is desirable to prevent less rapid formation of histidine decarboxylase at lower temperatures. Even rapid chilling to $\leq 4.4\,^{\circ}C$ may only give a safe shelf-life of 5–7 days.

Once frozen, the fish can be stored safely for extended periods and further histidine decarboxylase will not be formed. However, enzyme produced before freezing will not be destroyed and will continue to produce histamine after thawing.

Cooking

Cooking will destroy both histamine-producing bacteria and bacterial decarboxylases, but not histamine itself. Cooked fish therefore can be stored safely for longer periods and canned fish can be kept almost indefinitely.

It is important to note that once cooked or canned fish becomes recontaminated with histamine producing bacteria, temperature control again becomes critical to prevent a hazard. For example, canned tuna that is not consumed immediately after opening should be stored at $<5\,^{\circ}C$ as soon as possible.

Good Hygienic Practice

Good hygienic practice on-board fishing vessels, especially during landing and processing, is important to minimise contamination with non-indigenous histamine-producing bacterial species.

Careful handling of fish to avoid damage to muscle tissue is also important in preventing contamination. For example, puncture wounds in fish can introduce contaminating bacteria into deep tissue where large concentrations of histidine are available. Histamine production may then happen much more quickly.

Good hygiene at processing and preparation stages further along the supply chain, such as cutting and packing or in catering operations, is also important to prevent contamination of fresh fish, or recontamination of frozen and cooked fish.

Chemical Testing

Histamine is only detectable by analysis and the sensory characteristics of affected fish may appear satisfactory. Testing by chemical methods such as HPLC, or by ELISA and other immunological techniques can provide some assurance that toxic levels of histamine are not present, but the variability in histamine levels in a single fish mean that very large numbers of samples must be taken. For this reason, chemical testing cannot be relied upon to demonstrate adequate control of the hazard, but can be useful as a HACCP verification tool. Rapid 'dipstick' type methods have been developed recently and may be valuable screening tests for fish processors.

Legislation

EU legislation states that fish species belonging to families known to contain large amounts of histidine (*e.g.* Scombridae, Clupeidae *etc.*) in their tissues

should be tested for the presence of histamine. Nine samples should be tested from each lot and the mean value should be $\leq$100 ppm. The lot is considered unsatisfactory if more than two samples give results of between 100 and 200 ppm, or if any sample gives a result of $\geq$200 ppm. A mean level of 200 ppm and a maximum limit of 400 ppm are permitted for fish that have undergone enzyme maturation in brine.

In the USA the FDA has issued guidelines for tuna and related fish establishing a "defect action level" of 50 ppm in any sample. This is said to be indicative of spoilage and may mean that toxic levels are present in other samples. A separate toxicity level of 500 ppm is also given.

The international Codex standard for fish also includes histamine levels as indicators of decomposition and hygiene and handling. A maximum average level of not more than 100 ppm is considered satisfactory in relation to decomposition, while an upper limit of 200 ppm in any one sample is applied for hygiene and handling.

Australia and New Zealand also apply a maximum limit of 200 ppm for histamine in fish or fish products.

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CHAPTER 2.2

Non-biological Chemical Contaminants

2.2.1 CONTAMINANTS PRODUCED DURING PROCESSING

2.2.1.1 Acrylamide

Hazard Identification

What is Acrylamide?

Acrylamide (CH_2=CH–$CONH_2$, CAS No. 79-06-1) is a synthetic vinyl compound produced by the chemical industry mainly as a building block for polymers, particularly polyacrylamide. Polyacrylamide is widely used in industrial applications, such as in the treatment of wastewater, in textile and paper processing and in mining and mineral production. Acrylamide is also present in cigarette smoke.

The wide use of polyacrylamide in industry means that human exposure to acrylamide is likely and a number of toxicological studies have been carried out. The results of these studies suggest that acrylamide may have adverse effects on human health under some circumstances. In 2002 it was discovered that acrylamide could be generated in some food products during processing and should therefore be investigated as a potential food safety hazard.

Occurrence in Foods

The possibility of acrylamide contamination of foods did not become widely known until April 2002, when a report from the Swedish National Food Administration was published. This report revealed that acrylamide

The Food Safety Hazard Guidebook, 2nd Edition
Richard Lawley, Laurie Curtis and Judy Davis

Published by the Royal Society of Chemistry, www.rsc.org

could be produced in significant concentrations in certain carbohydrate-rich foods processed at relatively high temperatures, such as fried potato and baked cereal products. The work on which this report was based was done following an earlier study into the adverse health effects of polyacrylamide used by construction workers in the building of a tunnel. The discovery that control subjects showed evidence of unexplained exposure to acrylamide gave rise to the idea that food could be a source of the chemical.

Since 2002 a very wide range of foods around the world have been surveyed for the presence of acrylamide and the contaminant has been found to occur widely in many different food categories. Fried potato products, notably French fries and crisps, and baked cereal products, such as biscuits, bread, toasted breakfast cereals and pastries are the main foods affected, but roasted and ground coffee has also been found to be an important source. Animal-based foods and plant foods that are eaten raw, or cooked at lower temperatures, tend not to contain significant levels of acrylamide.

Acrylamide is not confined to commercially processed foods. It can also be found in home-baked or fried foods at relatively high levels. It seems certain that acrylamide has been present, but gone undetected, in cooked foods for centuries. It has been found in such diverse products as olives, prune juice and chocolate confectionery and many countries have published survey data covering a wide range of foods.

The amount of acrylamide found in foods varies widely, both with the food category and with the process applied. Some approximate examples of recorded levels in different food groups are given in Table 2.2.1.

Acrylamide levels in a number of food groups have been monitored in the EU since 2003. The most recent data, covering the period from 2007–2009, indicates that levels have fallen in some foods, but remained static, or even increased, in others. The reasons for this are unclear and further monitoring may be needed before clear trends emerge. Nevertheless overall exposure to acrylamide in the diets of EU consumers is reported to have fallen by approximately 30% since 2003.

Table 2.2.1 Approximate observed ranges of acrylamide concentration by food group.[a]

Food group	*Acrylamide/ppb*
Breakfast cereals	20–250
Bread	10–130
Roast and ground coffee	100–400
Crackers	50–600
Potato crisps and snacks	100–2500
Chocolate products	10–100

[a]Taken from the FAO/WHO Acrylamide Infonet Analytical Database

Hazard Characterisation

Effects on Health

Acrylamide is a neurotoxin at high levels of exposure and may cause a range of symptoms such as numbness in the hands and feet. It has also been shown to be genotoxic in animal studies. However, it is considered unlikely that the levels found in foods could result in sufficient exposure to cause neurological damage or reproductive toxicity.

Of more concern to the food industry is the finding that acrylamide is also carcinogenic in animal studies. The International Agency on Research on Cancer (IARC) classifies it as ''probably carcinogenic to humans (IARC Group 2A)''. Results of epidemiological studies searching for evidence of a link between acrylamide in the diet and the development of certain common cancers in humans have so far been inconclusive. A number of long-term carcinogenicity and toxicological studies are currently in progress and these should help to reduce the level of uncertainty.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) reviewed all the available toxicity and likely intake data for acrylamide in 2005 and carried out a risk assessment for the effect on human health. They found that the average person ingests enough acrylamide in the diet each day to equate to 1/300th of the dose required to cause a 10% increase in the risk of breast cancer in rats, with high consumers ingesting as much as 1/75th of that dose. The Committee considered this to be a low safety margin in comparison with other carcinogens in the diet. They concluded that, although there was considerable uncertainty in estimating the risk to human health, exposure to acrylamide in the diet might indeed be a concern.

The JECFA review acknowledged that acrylamide is an inadvertent contaminant introduced during cooking and unlikely ever to be eliminated from foods. Nevertheless, the Committee recommended that the food industry should work towards lowering acrylamide levels in critical food groups, such as potato crisps and chips, coffee, bakery products and biscuits and that guidance should be developed to help consumers reduce the levels produced in home-cooked foods.

JECFA carried out a re-evaluation of acrylamide in 2010 using new exposure and toxicity data. They noted that, despite attempts to reduce levels of acrylamide in some foods, overall dietary exposure for most people had remained the same. The committee concluded that the additional data confirmed that acrylamide in the diet is a ''human health concern''.

Sources

The original Swedish report into acrylamide in food in 2002 indicated that the contaminant is produced as a result of heating certain foods, especially those containing high levels of carbohydrate, at temperatures above 120 °C. It is therefore a contaminant generated during processing. Since then considerable

research has been carried out into the mechanism by which acrylamide is generated during frying, baking or roasting.

The major mechanism for the formation of acrylamide during cooking is now acknowledged to be the reaction of the free amino acid asparagine with reducing sugars, such as glucose or fructose, during the Maillard browning reactions that occur during cooking at high temperatures. Other mechanisms have since been suggested, including formation *via* acrolein, produced during the degradation of lipids from frying oil. However, most attention has been focused on Maillard browning as the main source of acrylamide. The key factors that affect the quantity of acrylamide produced appear to be the amount of free asparagine and sugars present in the food and the cooking time and temperature.

Stability in Foods

The large amount of data collected from food surveys suggests that acrylamide is relatively stable in food, but this has not been widely studied to date. Nevertheless, acrylamide levels have been found not to decrease significantly in crisps or baked cereal products during shelf life, while levels in roast and ground coffee are reported to decrease significantly.

Control Options

A considerable amount of research has been initiated since 2002 to investigate possible strategies for minimising the formation of acrylamide during the cooking of food products. Much of this work has been published and many of the most useful and practical techniques have been brought together by FoodDrinkEurope (formerly the Confederation of the Food and Drink Industries of the EU, or CIAA) in an "Acrylamide Toolbox" available online (link provided below). The Codex Alimentarius Commission has published a "Code of Practice for the Reduction of Acrylamide in Foods" and this too is available online.

Product Formulation

One obvious strategy for the control of acrylamide formation is to minimise the amount of free asparagine and reducing sugars in food prior to cooking. The development of low-asparagine varieties of potato is one approach that is receiving attention.

The modification of product recipes also shows some promise. For example, replacing ammonium bicarbonate with other raising agents in baked products can reduce acrylamide formation significantly, as can a reduction in pH. However, care must be taken to ensure that unacceptable textural and flavour changes do not result from such modifications.

Processing

The main factors that can be modified to minimise acrylamide formation are cooking time and temperature. The 'thermal input' to a cooking process has been shown to be directly linked to the amount of acrylamide produced. As a general rule, higher thermal input results in higher levels, with the exception of coffee production, where acrylamide levels decrease with longer roasting times and 'darker' roasts.

Frying, baking and roasting at lower temperatures and for shorter times reduce the amount of browning of the product and also reduce the amount of acrylamide produced. For example, consumers have been advised to cook French fries only until golden, rather than brown, and some crisp manufacturers have altered frying times and temperatures to reduce acrylamide production. While this may be successful, it must be recognised that the browning of baked and fried foods is an essential component in their sensory acceptability. Also, frying at lower temperatures may allow foods to take up higher levels of fat, which may be undesirable from a nutritional point of view. Reducing acrylamide by changing processing times and temperatures results in a compromise between product quality and safety.

The food industry has already made significant progress in reducing acrylamide in processed foods and it is likely that improved strategies and techniques will be developed in the near future. However, it has been reported that an overall reduction of acrylamide in food of 40% may be the best currently achievable.

Legislation

Acrylamide is not yet covered specifically by legislation in the EU or North America and no permitted limits have been set. At present, national and international food safety and public health authorities request that the food industry continue to work to minimise the levels of acrylamide in critical food groups.

In the EU, member states have been requested to extend monitoring of acrylamide levels in food until 2012, when the European Commission (EC) will assess the situation.

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United States Food and Drug Administration acrylamide pages. http://www.fda.gov/Food/FoodSafety/FoodContaminantsAdulteration/ChemicalContaminants/Acrylamide/default.htm

2.2.1.2 Advanced Glycation End-Products

Hazard Identification

What are Advanced Glycation End-Products?

Advanced glycation end-products (AGEs), also referred to as glycotoxins, are a heterogeneous group of chemical compounds, which occur naturally in animal tissues and are also produced during the cooking and processing of certain foods. They are highly oxidative and are known to be involved in the pathology of a number of diseases, including diabetes, cardiovascular disease and Alzheimer's disease. Recent research has produced evidence that exposure to AGEs in the diet may contribute to elevated levels in the body, with potentially significant adverse effects on health. Some researchers now consider AGEs to be an emerging food safety hazard.

Although a number of different AGEs have been identified, three types have been studied in some detail and have been used in studies as markers for AGEs. These are N^6-(carboxymethyl)lysine (also known as N^{ε}-carboxymethyl-lysine or CML), pentosidine and derivatives of methylglyoxal (MG).

Occurrence in Foods

AGEs occur naturally in a wide range of foods derived from animals, but higher concentrations are generated during high temperature 'dry' cooking processes, such as frying, roasting and grilling. High levels of both CML and MG have been found in cooked red meats, poultry, fish and eggs, but non-heat-processed animal foods, such as mature cheeses, have also been found to contain high concentrations. High-fat spreads, including butter, margarine and mayonnaise may also contain large amounts, as do some vegetable oils. It has been proposed that this is a result of AGE generation during processing. Generally, foods derived from vegetables and cereals, and those cooked at lower temperatures by boiling or poaching contain much lower levels of AGEs.

It has been reported that the average intake of AGEs in the diets of healthy adults in the city of New York was $14\,700 \pm 680$ AGE kU per day. It is estimated that a diet containing large amounts of grilled, or roasted meat, fat and highly processed foods could result in intakes of 20 000 AGE kU per day or more.

Hazard Characterisation

Effects on Health

AGEs are the products of normal metabolic processes in humans and occur naturally in the body. For example, MG is formed by a number of processes, including glycolysis and lipid peroxidation. Levels found in the blood tend to increase with age and have been reported to be 35% higher in people over the age of 65 than in those aged 45 or less. It is thought that this is because kidney

function in older people is reduced and less able to remove AGEs from the body.

AGEs are able to bind to cell surface receptors leading to oxidative stress and inflammation and may also react with proteins to alter their structure. If sufficiently high concentrations accumulate in blood and tissue, this can lead to pathological changes. AGEs have been linked to a number of chronic diseases, including diabetes and insulin resistance, cardiovascular disease, hypertension, kidney disease and Alzheimer's disease.

Until comparatively recently, it was thought that AGEs in food would be poorly absorbed in the gut, but this assumption is now proven to be false. Feeding studies with mice have shown that an AGE-rich diet can contribute to elevated AGE levels in blood and tissue and may be associated with chronic disease. Human experiments have also reported that AGEs in foods can be absorbed. High levels have also been reported in healthy younger people, leading to suggestions that a diet high in AGEs may cause these compounds to accumulate in the tissues more quickly than they can be removed and contribute to the early onset of chronic disease.

Sources

AGEs in food are largely the by-products of Maillard browning reactions during heating at high temperatures and are produced by non-enzymic reactions between reducing sugars and the free amino groups of proteins and fats. However, the presence of high levels in foods such as mature and high-fat cheeses, suggests that they may also be produced more slowly by other mechanisms.

A number of factors influence the amount of AGEs produced during cooking, notably the temperature and moisture content. High temperatures and low moisture generally increase AGE formation. High fat content can influence the generation of AGEs, but high levels can also be produced in lean meats cooked by dry heating methods. The use of cooking methods like boiling, steaming and poaching produce significantly lower AGE concentrations and acidic marinades used prior to cooking are also reported to reduce AGE generation in grilled and roasted meats.

Stability in Foods

There is little published information relating to the stability of AGEs in foods, but it is likely that these compounds vary in their stability. For example, CML is a chemically stable compound, whereas MG derivatives are highly reactive. The ability of AGEs to accumulate in the tissues suggests that they are not easily broken down or excreted.

Control Options

The control of AGEs in foods focuses on limiting their production during processing and on limiting intake by dietary modification.

Processing

Although the mechanisms for AGE production in foods are still uncertain, it is known that processing conditions affect the levels present. A number of recommendations for measures to reduce production in cooked foods have been made as follows:

- Cook meat and fish at temperatures of less than 200 °C and avoid prolonged cooking times
- Use indirect cooking methods, such as stewing, poaching and steaming, rather than grill, fry or barbecue
- Apply acidic marinades incorporating lemon juice or vinegar to meat before cooking

Product Use

It is possible for consumers to limit their exposure to dietary AGEs by reducing consumption of grilled and roasted meat products, high-fat and highly processed foods and increasing the amount of vegetables, fruits and cereals in the diet. This is in line with generally agreed recommendations for a healthy diet.

Legislation

As far as the authors are aware, no regulations or guidelines relating to limiting exposure to AGEs in the diet have been yet been published in the EU, North America or elswhere.

Sources of Further Information

Published

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2.2.1.3 Benzene

Hazard Identification

What is Benzene?

Benzene (C_6H_6, CAS No. 71-43-2) is an aromatic hydrocarbon compound used extensively in the chemical industry as an intermediate in the manufacture of polymers and other products. It is also a common atmospheric contaminant and is present in motor vehicle exhaust emissions and cigarette smoke.

In 1990, it was discovered by the USA soft drinks industry that benzene could be produced at low levels in certain soft drinks containing a benzoate preservative and ascorbic acid. Since benzene is a known human carcinogen, its presence in food and beverages is clearly undesirable.

Occurrence in Foods

Detectable levels of benzene have been found in a number of soft drinks that contain either a sodium or potassium benzoate preservative and ascorbic acid, and 'diet' type products containing no added sugar are reported to be particularly likely to contain benzene at detectable levels. Surveys carried out in the USA, the UK and Canada have all confirmed that a small proportion of these products may contain low levels of benzene. For example, in a survey of 86 samples analysed by the FDA between April 2006 and March 2007, only five products were found to contain benzene at concentrations above 5 μg kg^{-1}. The levels found were in a range from approximately 10–90 μg kg^{-1}. A survey of 150 UK-produced soft drinks by the Food Standards Agency (FSA) published in 2006 showed that four products contained benzene at levels above 10 μg kg^{-1}, and the highest level recorded was 28 μg kg^{-1}. However, it has been reported that higher levels may develop in these products during prolonged storage, especially if they are exposed to daylight.

Benzene may also be formed in some mango and cranberry drinks in the absence of added preservatives, because these fruits contain natural benzoates.

Hazard Characterisation

Effects on Health

Although benzene can cause acute toxicity, especially when inhaled at high levels, it is its carcinogenicity that is of concern in foods and beverages. Benzene is a proven carcinogen and has been shown to cause cancers in industrial workers exposed to high airborne levels. Much less is known about its effects when ingested at low levels over long periods, but current risk assessments suggest that the contribution of soft drinks to benzene exposure levels is negligible, as is any additional risk to human health. Nevertheless, the soft drinks industry has been requested to take action to eliminate benzene from its

products and product recalls have been initiated in the UK following the discovery of benzene contamination.

Sources

It has been established that the source of benzene in soft drinks is the decarboxylation of benzoic acid when ascorbic acid and trace amounts of a suitable metal catalyst (copper or iron) are present. Elevated temperature and light are both reported to stimulate this reaction, whereas it is inhibited by sugars and by EDTA salts. This may be why benzene is most likely to be found in diet drinks containing low sugar levels. Benzene levels may continue to rise during storage if the product is kept in the light and the storage temperature is high.

Stability in Foods

There is little published information available on the stability of benzene in soft drinks during storage.

Control Options

The preferred approach for controlling the production of benzene in soft drinks is to reformulate the product. Once a specific soft drink formulation has been shown to be capable of generating benzene during storage, alternatives to benzoate preservatives, such as potassium sorbate, should be evaluated. Benzene generation may be effectively prevented by the removal of benzoates from the product. However, it should be noted that the majority of soft drinks containing benzoates and ascorbic acid have not been shown to produce benzene and may not need to be reformulated in this way.

Legislation

Current USA and EU legislation does not set maximum limits for benzene in soft drinks. However, the FDA has adopted the Environmental Protection Agency (EPA) maximum contaminant level (MCL) for drinking water of 5 ppb as a quality standard for bottled water. This MCL has been used to evaluate the significance of benzene contamination in the soft drinks tested in surveys.

The FSA has used the World Health Organization (WHO) guideline level for benzene in water of 10 μg kg^{-1} as a point of reference for its own survey results.

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2.2.1.4 Chloropropanols

Hazard Identification

What are Chloropropanols?

The chloropropanols are a group of related chemical contaminants that may be produced in certain foods during processing. They first became a concern to the food industry in the late 1970s when small concentrations were found to be generated during the manufacture of acid-hydrolysed vegetable protein (acid-HVP) used as a savoury ingredient in soups, sauces, especially soy sauce, snacks, stock cubes and ready meals. Chloropropanols are potentially carcinogenic and their presence in food, even at low levels, is therefore undesirable.

Several different chloropropanols have been identified in food. The most common and the best studied is 3-monochloropropane-1,2-diol (3-MCPD), but other food-borne chloropropanols include 2-monochloro-1,3-propandiol (2-MCPD), 1,3-dichloro-2-propanol (1,3-DCP) and 2,3-dichloro-2-propanol (2,3-DCP). Chloropropanols are probably produced by a number of different mechanisms during food processing, but these are not yet fully understood.

Significant levels of fatty acid esters of chloropropanols have recently been found in refined oils. These compounds are also likely to be produced during processing and may be of concern from a public health point of view. Chloropropanol esters detected in oils, and in foods containing such oils, include 3-MCPD esters and 2-MCPD esters. Related glycidyl esters (fatty acid esters of glycidol) have also been found in foods and may be a further safety concern.

Occurrence in Foods

The highest levels of chloropropanols (mainly 3-MCPD and 1,3-DCP) have been found in acid-HVP and in soy sauce and related products. A UK survey of 3-MCPD in acid-HVP in 1980 showed levels of up to 100 mg kg^{-1} and surveys of soy sauce products in the EU and North America in 1999–2000 showed levels varying from undetectable (<0.01 mg kg^{-1}) to a highest concentration of 330 mg kg^{-1} in a sample tested in Canada. High levels were shown to be produced during the manufacturing process of acid-HVP, which is a major ingredient of soy sauce. Changes in acid-HVP manufacturing methods have produced a dramatic reduction in levels of 3-MCPD in products on the market in the UK since 1990, and typical levels in 1998 were in the range 0.01–0.02 mg kg^{-1}.

Since chloropropanols were first identified in acid-HVP and soy sauce, they have also been found in a variety of other food products that do not contain acid-HVP as an ingredient. For example, 3-MCPD has been found in bread, biscuits and other baked products, coffee, roasted barley malt, certain cured and fermented meat products, cheeses, salted fish and smoked foods. Levels of 3-MCPD are generally low in these foods. For example, a concentration of 0.5 mg kg^{-1} is not unusual in malt used as a food ingredient, and maximum

concentrations of 3-MCPD found in surveys of bakery products, meat, fish and cheese range from 0.01–0.1 mg kg^{-1}.

Chloropropanol esters and glycidyl esters have been detected in refined edible oils and fats, notably palm oil, margarine and spreads, but not in unrefined oils. They have also been found in foods containing such oils, including infant formula. Chloropropanol esters may be present in oils and fats in much higher concentrations than 3-MCPD and other chloropropanols.

Hazard Characterisation

Effects on Health

Although chloropropanols can cause acute toxicity at high concentrations, it is extremely unlikely that this could occur through consumption of contaminated food, and it is the effect of low doses over a long time that is of most concern from a food safety point of view. Both 3-MCPD and 1,3-DCP have been shown to be carcinogenic in animal studies and are therefore potential human carcinogens.

3-MCPD was formerly considered to be genotoxic, but recent studies suggest that there is little solid evidence for this. The JECFA has recently reviewed the toxicity of 3-MCPD and concluded that a threshold-based approach for deriving a TDI could be used. A provisional maximum tolerable daily intake (PMTDI) of 2 μg per kg of bodyweight has thus been set to replace the previous recommendation that levels in foods should be reduced as far as technically possible. For 1,3-DCP, JECFA was unable to rule out the possibility of genotoxicity and so no PMTDI has been set.

At present there is insufficient toxicological evidence to assess the public health significance of chloropropanol esters and glycidyl esters in foods. Nevertheless, concerns have been raised over their presence in infant formula. One reason for this concern is the possibility that these compounds could be hydrolysed by lipases in the gut, releasing significant quantities of 3-MCPD and other related compounds, including glycidol, a probable carcinogen. There is a need for further toxicological studies to fully understand risks of dietary exposure to these compounds.

Sources

It is thought that chloropropanols and chloroesters are usually produced during processing, especially at high temperatures, but the mechanism is not known in all cases.

The mechanism for chloropropanol production in acid-HVP is known to be a reaction between hydrochloric acid and lipids that occurs more rapidly at the high temperatures used in processing. 3-MCPD and other chloropropanols then contaminate other foods, for which acid-HVP is a key flavour ingredient.

In bread and other baked products, chloropropanols are thought to be formed by a reaction during the baking process between the chloride in added salt and glycerol from flour and yeast. In other foods, the mechanisms of

chloropropanol production are less clear. One proposed mechanism for 3-MCPD production in meat, fish and cheese at relatively low temperatures suggests that hydrolytic enzymes (lipases) may be involved, but this has yet to be confirmed.

Food-borne chloropropanols may be derived from migration from food contact materials, such as sausage casings and teabags, and they can also be produced during domestic cooking of such foods as grilled cheese and meats.

Chloropropanol esters and glycidyl esters are thought to be produced in the refining of edible fats and oils, particularly during the final deodourisation process, when temperatures above 250 °C may be reached.

Stability in Foods

Chloropropanols are relatively non-volatile and may be quite persistent in foods once formed. However degradation does occur during storage, and 3-MCPD has been shown to be lost more rapidly from foods processed at higher pH values and at higher temperatures.

Control Options

The control of chloropropanols in foods focuses on limiting their production during processing.

Processing

The production of chloropropanols during the manufacture of acid-HVP is well understood and control strategies have been successful in reducing the level of contamination significantly. This has been achieved by a number of changes to the manufacturing process.

- Replacing acid hydrolysis with an enzymatic process
- Reducing lipid concentrations in the raw materials
- Effective control of the acid hydrolysis process
- Use of an over-neutralisation treatment with NaOH to remove chlorohydrins after acid hydrolysis

The mechanism of formation of chloropropanols in other foods is less well known and it is therefore more difficult to design effective control strategies. However, in many cases common salt is a source of chloride ions and a precursor for chloropropanol production. Therefore reducing salt levels without compromising sensory properties or microbiological stability may be an effective control, especially in bread and other bakery products. Reducing processing temperatures and avoiding excessive browning of these products may also be useful controls.

For meat, fish and cheese, there is little information on how chloropropanols are formed at lower temperatures. However, salt concentration is again likely to be a factor and the inactivation of lipases may also be helpful. Fortunately,

levels of 3-MCPD and other contaminants are usually very low in these foods.

The production of chloropropanol esters and glycidyl esters in oils and fats is a relatively recent discovery and control measures have yet to be developed and tested.

Legislation

In the EU, permitted levels of 3-MCPD in hydrolysed vegetable protein and soy sauce are prescribed by a the EC regulation No. 1881/2006, which sets a maximum level of 20 μg kg^{-1}. This is based on the PMTDI for 3-MCPD of 2 μg per kg of bodyweight. For other chloropropanols, manufacturers are requested to reduce levels as far as is technically possible. Chloropropanol esters and glycidyl esters are not included in this legislation, but the German Federal Institute for Risk Assessment has recommended that levels of these compounds in infant foods should be reduced.

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2.2.1.5 Ethyl Carbamate

Hazard Identification

What is Ethyl Carbamate?

Ethyl carbamate ($C_3H_7NO_2$, CAS No. 51-79-6), also referred to as urethane, is the ethyl ester of carbamic acid. It is naturally present in many fermented foods and alcoholic beverages and is produced by a number of different mechanisms during processing and storage.

Since the 1940s ethyl carbamate has been considered to be a potential carcinogen, but it was first identified as a food safety hazard when comparatively high levels were detected in alcoholic beverages tested by the public health authorities in Canada.

Occurrence in Foods

Ethyl carbamate has been detected in many fermented beverages and foods, including wine, beer and spirits, bread, yoghurt and soy sauce. Survey findings reported by EU member states to the EFSA in 2007 contained few results for ethyl carbamate in food products, but did show that 41% of samples were below the limit of detection. By contrast, more than 33 000 test results were received for alcoholic beverages. These showed that median levels of ethyl carbamate ranged from up to 5 $\mu g\, l^{-1}$ for beer and wine to 21 $\mu g\, l^{-1}$ for spirits. However, by far the highest concentrations (260 $\mu g\, l^{-1}$) were found in stone fruit brandies. It has been estimated that dietary exposure to ethyl carbamate from food only would be 17 ng per kg of bodyweight per day, whereas this would rise to 65 ng per kg of bodyweight per day for consumers drinking a variety of alcoholic beverages. However, consumers drinking large amounts of fruit brandy regularly could be exposed to levels as high as 558 ng per kg of body weight per day.

Hazard Characterisation

Effects on Health

Ethyl carbamate has quite a low acute oral toxicity in animals and the LD_{50} in rodents is reported to be approximately 2000 mg per kg of bodyweight. However, it is known to be genotoxic and has been shown to be a multisite carcinogen in animals. It is generally considered to be a potential human carcinogen and in 2007 the IARC classified ethyl carbamate as "probably carcinogenic to humans (Group 2A)". It has been shown to cause tumours in a number of organs, including the lungs and liver, in rodents and in non-human primates when given as a single large dose, or administered at much lower levels over a long period.

Little data exists on toxicity or carcinogenicity in humans, but evidence suggests that some of the metabolic pathways involved in carcinogenicity in

rodents are also present in humans. For this reason, human carcinogenicity is considered likely and the presence of ethyl carbamate in food and beverages is undesirable. The potential exposure of consumers whose diets contain fermented foods and alcoholic beverages is considered by JECFA to be a matter of concern.

Sources

Ethyl carbamate can be formed from a number of precursors found in foods and beverages. These include hydrocyanic acid, urea, citrulline, cyanogenic glycosides and other *N*-carbamyl compounds. Ethyl carbamate in wine is reported to be mainly derived from urea produced from the degradation of arginine by yeasts during fermentation. Urea then reacts with ethanol to form ethyl carbamate. The high levels of ethyl carbamate present in stone fruit brandies are derived mainly from cyanogenic glycosides (*e.g.* amygdalin) present in the stones. These compounds are degraded by β-glucosidase and other enzymes, producing cyanide, which is then oxidised to cyanate. The cyanate then reacts with ethanol, producing ethyl carbamate.

A number of factors have been shown to influence ethyl carbamate production in wine, including yeast variety, concentration of arginine present in grapes, fortification of wine, temperature and storage conditions.

Stability in Foods

Ethyl carbamate is produced in fermented foods and beverages both during the fermentation process and in the course of long-term storage, providing suitable precursors are present. There is little published information relating to its stability in these products, but the presence of high levels in distilled spirits and evidence from survey results suggests that it is not readily broken down.

Control Options

The control of ethyl carbamate in foods and beverages focuses mainly on limiting the quantities of precursor compounds present in raw materials.

Processing

Since the identification of ethyl carbamate as a food safety hazard in 1985, a number of measures have been devised to reduce levels, especially in alcoholic beverages, by achieving a reduction in the concentrations of precursor compounds. For example, the whisky industry has achieved significant reductions by switching to barley varieties containing low levels of arginine and other precursors and by modifying processing conditions, such as reducing contact with copper surfaces. The wine industry has also developed effective controls to reduce ethyl carbamate levels by careful yeast selection and reducing the arginine content of grapes. Excluding light from bottled spirits to inhibit

oxidation of precursor compounds to cyanate may also be an effective means of reducing ethyl carbamate formation.

Legislation

There are no current harmonised regulations or guidelines in the EU relating to maximum permitted levels of ethyl carbamate in foods and beverages, although certain member states have introduced national recommendations for maximum permitted levels. For example, France has a recommended limit of 1000 μg l^{-1} for fruit brandy.

The Canadian government introduced legislation in 1986 setting limits of 30 μg l^{-1} for wine and 150 μg l^{-1} for distilled spirits, rising to 400 μg l^{-1} for fruit brandies. The FDA has published voluntary limits of 15 μg l^{-1} for wine and 60 μg l^{-1} for fortified wines.

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2.2.1.6 Furan

Hazard Identification

What is Furan?

Furan (C_4H_4O, CAS No. 110-00-9) is a volatile heterocyclic organic chemical often found as an intermediate in industrial processes for producing synthetic polymer materials. It is a very different compound from the diverse group of chemicals sometimes referred to collectively as *furans*, which includes various antimicrobials (nitrofurans) and dioxin-like toxins.

Concern over furan in foods dates back only to 2004, when an FDA survey of heat-processed foods in the USA revealed that low levels of furan could be found in an unexpectedly large proportion of products processed in closed containers, such as cans and jars. Furan is a possible human carcinogen, and therefore even low levels in foods are undesirable.

Occurrence in Foods

Furan has been recognised as a food flavour volatile for a considerable time, and quite high levels (up to 4000 μg kg^{-1}) were reported in canned meat as long ago as 1979. It was not known to occur widely in heat-processed foods until the 2004 FDA survey. This found furan at concentrations of up to 125 μg kg^{-1} in a variety of heat-processed foods, including baby foods, canned beans, soups, sauces and pasta meals. Since then, monitoring of a wide range of foods for furan by food safety authorities and food manufacturers has been ongoing in the USA and in the EU. Detectable levels of furan have now been found in savoury snacks, coffee, canned fruits and juices, preserves, canned vegetables, soy sauce, ready-to-use gravies and breakfast cereals. Many of the contaminated products are packed in sealed containers, such as cans and jars, but furan can also be found in potato crisps, crackers and crispbreads.

Most of the positive samples recorded levels of furan of less than 100 μg kg^{-1}, but much higher concentrations (up to 6900 μg kg^{-1}) have been reported in some ground, roasted coffee samples. Furanic compounds are known to be normal components of flavour volatiles in coffee and are probably formed during the roasting process. However, levels in ready-to-drink 'brewed' coffee are usually much lower as a result of dilution and losses during brewing. Recent monitoring results published by the EFSA have shown that the highest levels of furan recorded in non-coffee samples were found in infant food (224 μg kg^{-1}) and soups (225 μg kg^{-1}). Levels exceeding 100 μg kg^{-1} were also found in cereal products, canned fish and meat products soups and gravy. Both the FDA and the EFSA have appealed for the submission of more data on furan levels in foods so that valid risk assessments can be carried out.

Hazard Characterisation

Effects on Health

Furan is cytotoxic and the liver is the target organ for acute toxic effects. However, it is the effect of prolonged dietary exposure to furan and its possible carcinogenic potential that is of concern for food safety. Furan has been shown to be carcinogenic in rats and mice and is probably genotoxic. For this reason, it has been classified by the IARC as "possibly carcinogenic to humans (IARC Group 2B)". The EFSA Scientific Panel on Contaminants in the Food Chain concluded in 2004 that the difference between human exposure to furan and doses causing carcinogenic effects in animals was "relatively small". However, this conclusion was based on limited data, and the extent of the health risk presented by furan in food will not be properly established until more toxicity and exposure data are available for evaluation. Nevertheless, it is generally agreed that in view of the likely genotoxicity of furan, levels in food should be minimised as far as is practicable.

Sources

It is thought probable that furan is a by-product of the high temperatures involved in the heat processing of foods, but there is still some uncertainty over exactly how it is produced. In view of the wide variety of heat processed foods that may contain furan, it is considered likely that there are a number of different mechanisms, probably involving naturally occurring precursors, such as ascorbic acid, furoic acid, furfural and unsaturated oils.

Proposed pathways for furan formation include the thermal degradation of reducing sugars alone, or in combination with amino acids, thermal degradation of some amino acids, and thermal oxidation of ascorbic acid, polyunsaturated fatty acids and carotenoids. The highest levels have been reported to be formed from ascorbic acid as a precursor and at temperatures above 120 °C. Certain metals, notably copper, may also act as catalysts for furan formation in some cases.

The widespread presence of furan residues in canned foods, and products in sealed jars and other containers, is likely to be a consequence of the volatile compound being trapped in the container.

Stability in Foods

There is little data as yet on the stability of furan in food. It is a highly volatile compound and was thought likely to be driven off quickly if foods were cooked or reheated in open vessels. However, it has been reported that gentle heating does not reduce levels of furan significantly and that only vigorous and prolonged boiling will result in substantial losses from contaminated foods.

Control Options

Too little is currently known about the formation, occurrence and potential risk of furan in foods for any valid control options to have been developed to date. Suggested mitigation measures have focused on changes to thermal processes and reducing the amounts of precursor compounds present in foods before processing.

The FDA has published an Action Plan for furan in food. The goals of this plan are to develop reliable analytical methods, gather more data on dietary exposure to furan, learn more about the human toxicology of furan and produce sufficient data to undertake a full risk assessment. In the EU, the EFSA is engaged in a similar programme of data collection and risk assessment.

Legislation

As yet there is no legislation limiting levels of furan in foods. Any future regulation will be based on the results of ongoing risk analysis activities.

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Published

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2.2.1.7 Heterocyclic Amines

Hazard Identification

What are Heterocyclic Amines?

Heterocyclic amines (HCAs) are a large and diverse group of chemical compounds comprising at least one heterocyclic ring (a ring containing atoms of more than one element) and one or more amino groups. Those HCAs that are important from a food safety point of view share a common imidazole-ring structure with an exocyclic amino group. They are potential carcinogens produced when meat, poultry and fish are cooked at high temperatures. These compounds were first isolated from cooked meat and characterised in the late 1970s and early 1980s by research groups in Japan and the USA.

At least 15 different types of HCA have been isolated from from cooked foods and characterised. Those most commonly found in food include imidazoquinoxalines, imidazoquinolines and imidazopyridines. Five of these compounds are reported to be particularly important contributors to the overall level of HCAs in cooked foods

2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)
2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx)
2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (DiMeIQx)
2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ)
2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ)

Occurrence in Foods

The main source of HCAs in the diet is generally considered to be cooked meat products, including beef, pork, lamb, poultry and fish, although low levels of HCAs have also been found in processed food flavourings, gravy, wine and beer. The concentrations present in cooked meats range from <1 ng g^{-1} to approximately 500 ng g^{-1}, but are typically below 100 ng g^{-1}. By far the highest levels are found in meat cooked at high temperatures, by grilling, broiling, barbecueing and frying, and the dietary contribution from other foods is considered to be of little significance. Dietary exposure to the compounds has been estimated at <1–17 ng per kg of bodyweight per day, depending mainly on the amount of cooked meat in the diet.

Hazard Characterisation

Effects on Health

All of the HCAs found in cooked meats are reported to be powerful mutagens for bacteria and for mammalian cells. They have also been shown to be carcinogenic when fed to rodents, causing the development of tumours in several

organs, including the colon, breast and prostate. Although carcinogenicity in humans has not been confirmed, animal test data suggests that it is likely. The IARC has classified one HCA compound, IQ, as "probably carcinogenic to humans (IARC Group 2A)" and three more, MeIQ, MeIQx and PhIP, as "possibly carcinogenic to humans (IARC Group 2B)".

Some cancer specialists believe that high levels of HCAs in the Western diet may be an important contributor to the relatively high rates of bowel cancer seen in regions where large quantities of fried and grilled meat are consumed. However, such an association remains unproven and it is suspected that other factors may also be involved.

Sources

HCAs at significant concentrations are reportedly produced only in meat and fish during cooking, especially at the higher temperatures used in grilling and frying ($>200\,^{\circ}C$) and where a heat source is applied directly to the meat. They are thought to be produced as a by-product of Maillard browning reactions from amino acids, hexose sugars and creatine derived from muscle tissue, but the exact mechanism by which this occurs is still uncertain.

A number of factors influence the amount of HCAs produced during cooking, including the cooking temperature and time, pH, and the types and concentrations of precursor compounds present. Higher temperatures and longer cooking times generally produce higher HCA levels. Higher levels are also produced by cooking methods involving direct heat transfer, such as grilling and frying. Marinading before cooking has been reported to reduce HCA formation, but the effect varies with the marinade used.

Stability in Foods

There is little published information relating to the stability of HCAs in cooked meats. However, their detection in processed food flavourings, such as bouillons and gravy suggests that they are likely to persist for some time.

Control Options

The control of HCAs in foods focuses on limiting their production during processing.

Processing

Although the mechanisms for HCA production in foods are still uncertain, it is known that processing conditions affect the levels present. A number of recommendations for measures to reduce HCA production in cooked meats have been made as follows:

- Cook meat and fish at temperatures of less than $200\,^{\circ}C$ and avoid prolonged cooking times

- Use indirect cooking methods, such as stewing, poaching and steaming, rather than grill, fry or barbecue
- Turn meat regularly during cooking
- Partially pre-cook meat in a microwave oven and drain before conventional cooking
- Do not use liquids derived from high-temperature meat cooking to make sauces and gravy

Legislation

As far as the authors are aware, no regulations or guidelines relating to limiting exposure to HCAs in the diet have been yet been published in the EU, North America or elswhere.

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Published

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2.2.1.8 Polycyclic Aromatic Hydrocarbons (PAH) Hazard Identification

What are Polycyclic Aromatic Hydrocarbons?

The polycyclic aromatic hydrocarbons (PAHs) are a large group of stable, lipophilic organic chemical contaminants containing two or more fused aromatic rings. They can be produced during the partial combustion or pyrolysis of organic material and are common by-products of a number of industrial processes, including the processing and preparation of food. The presence of PAHs in burnt and partially carbonised food was first reported over 40 years ago. PAHs are potentially carcinogenic and their presence in food, even at low levels, is therefore undesirable.

Hundreds of PAHs have been identified as by-products of incomplete combustion. However, by far the most studied PAH is benzo[*a*]pyrene (BaP, $C_{20}H_{12}$, CAS No. 50-32-8). BaP is often used as a marker compound for all PAHs in food, and also in environmental studies. Although the profile of PAH contamination in different foods varies, BaP has been considered to be a valid marker compound for the most harmful group of higher molecular weight PAH compounds. Other toxicologically important PAHs detected in foods include chrysene, benz[*a*]anthracene and benzo[*a*]fluoranthene.

Occurrence in Foods

PAHs are common environmental contaminants in water, air and soil, and so may contaminate many foods by this route. Vegetables are especially vulnerable to environmental PAH contamination, particularly when grown in areas where industrial pollution levels are high. Seafood, such as some shellfish and crustaceans, may also accumulate PAHs from the water in which they are grown, but significant levels do not usually accumulate in the meat, milk, or eggs of food animals, because PAHs are rapidly metabolised in these species.

However, the main source of PAHs in the diet is generally considered to be food processing and preparation, especially foods processed at high temperatures. High levels (up to 130 $\mu g\ kg^{-1}$ against a background level of $<1\ \mu g\ kg^{-1}$) of individual PAHs have been reported in grilled and barbecued meats. Smoked foods are also often contaminated, with levels of up to 200 $\mu g\ kg^{-1}$ being reported in both smoked meat and fish. However, reported levels of PAHs in smoked foods vary widely, and are probably dependent on the nature of the smoking process, with traditional methods generally producing higher levels than newer processes. Smoke flavourings may also be contaminated with PAHs.

Vegetable oils, including olive pomace oils, are an important source of dietary PAHs, which are usually present as a consequence of direct seed-drying methods where the product comes in contact with combustion gases. Reported levels in oils vary widely. Both roasted coffee beans and dried tea leaves may

also contain high PAH levels—up to 1400 μg kg^{-1} in one report—but high levels have not been found in coffee or tea drinks as-consumed. Dried fruits and nuts have also been reported to contain high levels of PAHs on occasion.

Food is thought to be the main source of PAH exposure in non-smokers, especially cereals and seafood. Dietary intake of PAHs across six EU countries was estimated to be in the range 0.05–0.29 μg of BaP per day. Similar estimates have been produced in the USA. Surveillance of PAH levels in foods in the EU has shown that, although BaP could be detected in about half of the samples tested, a further 30% of samples tested negative for BaP but contained other potentially toxic PAHs, notably chrysene. This has lead to doubts over the validity of using BaP as the sole marker for PAHs in food (see below).

Hazard Characterisation

Effects on Health

Little is known about the potential for acute toxicity of PAH, but it is extremely unlikely that this could occur through consumption of contaminated food, and it is the effect of low doses over a long time that is of most concern from a food safety point of view. A number of PAHs, including BaP, have been shown to be both carcinogenic and genotoxic in experimental animals and are therefore potential human carcinogens. For example, BaP has been shown to cause tumours in the gastrointestinal tract, liver, lungs and mammary glands of rodents.

Individual PAHs have also been found to produce other, non-carcinogenic effects in animals, including liver toxicity, reproductive and developmental toxicity and suppression of the immune system.

Because some PAHs are likely to be both genotoxic and carcinogenic, the EU Scientific Committee on Food recommended that no TDI be set for PAHs. Instead the Committee recommended that levels in food should be as low as is reasonably achievable. However, it also noted that maximum dietary intakes are 5–6 times lower than the levels causing tumours in animals.

Sources

The main sources for PAHs in foods are air, soil, or water-borne environmental contamination and food processing involving high temperatures. However, humans are also exposed to PAHs in the air—from industrial and traffic pollution and from tobacco smoke.

The mechanism for PAH production during smoking, drying and cooking processes are not fully understood, but it is likely that more than one mechanism is involved. For example, when fat from cooking meat drips onto a heat source, it undergoes pyrolysis and PAHs may be produced and deposited on the food itself. Meat heated to temperatures above 200 °C may also undergo pyrolysis, producing PAHs on the surface. PAH production in grilled meats has been shown to be dependent on fat content and the time and temperatures used

during cooking. In dried products, PAH contamination is most likely to come from exposure to partially burnt combustion gases in direct flame dryers.

Stability in Foods

PAHs are generally very stable compounds, although photodegradation does occur. They are highly lipophilic and are particularly stable in oils and fats. They also readily adhere to particles in the soil and in foods.

Control Options

The control of PAHs in foods focuses on limiting their production during processing.

Processing

Although the mechanisms for PAH production in foods are still uncertain, it is known that processing conditions can have a dramatic effect on the levels present. It has therefore been possible to produce a number of recommendations for effective measures to reduce PAH production in a number of food types. For example:

- Select leaner meat and fish for grilling and barbecuing
- Do not allow fat to come in contact with the heat source during cooking (*e.g.* by using vertical barbecues and grills)
- Reduce cooking temperatures and do not brown food excessively
- Replace traditional direct smoking processes with indirect smoking, or use smoke flavouring
- Avoid direct contact of oil seeds and cereals with combustion gases during drying
- Wash, or peel, fruit and vegetables that have a waxy coating

Product Use

Similar advice on safer barbecuing and grilling of meat and fish in the domestic environment may help consumers to reduce levels of PAHs in their diet.

Legislation

In the EU, EC Regulation No. 1881/2006 (EC Regulation No. 420/2011), sets permitted levels of BaP (as a marker for PAHs) in a number of food products, including oils and fats, infant foods, and smoked meat and fish products. The maximum levels permitted in these products range from 1.0 μg per kg wet weight in baby foods to 10.0 μg per kg in bivalve molluscs.

In 2008, the EFSA CONTAM Panel concluded that BaP was not a suitable indicator for PAH contamination in food and suggested using the sum total of four common PAHs as an alternative. These were BaP, chrysene,

benz[*a*]anthracene and benzo[*a*]fluoranthene (PAH4). These findings are now under review.

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2.2.2 CONTAMINANTS FROM FOOD CONTACT MATERIALS

2.2.2.1 Bisphenol A

Hazard Identification

What is Bisphenol A?

Bisphenol A (BPA) is a phenolic compound ($C_{15}H_{16}O_2$, CAS No. 80-05-7), also referred to as 2,2-*bis*(4-hydroxyphenyl)propane. It was first synthesised over a hundred years ago and is an important industrial chemical used in manufacturing processes. BPA is a major component of rigid polycarbonate plastics and epoxy-resin coatings.

Polycarbonate is commonly used in the food industry for water and soft-drink bottles, and epoxy resins are used as protective linings for metal food cans, wine storage vats and other liquid containers, and as coatings on metal lids used for glass bottles and jars. In addition, polycarbonate plastic containers and tableware are widely used by consumers and the material is also used to manufacture infant feeding bottles.

Although materials containing BPA have been used in packaging and storage vessels for food and beverages for over 50 years, some scientific studies have shown that under certain conditions BPA can migrate into food products. This is of concern because BPA is known to cause adverse health effects in animals at high levels. Canada has recently placed bisphenol A on its list of toxic substances and the EPA has identified BPA as a chemical of concern.

Occurrence in Foods

Detection of BPA has been reported in various canned food and drink products including canned fruit, vegetables, coffee, tea, infant formula concentrate and sake.

A survey of 62 canned food and drink products by the FSA published in 2001 found detectable levels of BPA in fruits and vegetable products, stout, fish, soups, dairy products, meat products and pasta in tomato sauce. However, the Independent Committee on Toxicology of Chemicals in Food, Consumer Products and the Environment (COT) concluded that the levels of BPA found during the FSA survey were unlikely to be a concern for human health.

More recently Canadian surveys published between 2009–2010 detected low levels of BPA in: bottled and canned beer and soft drinks; canned tuna, soups, vegetables, and pasta products; baby foods in glass jars with metal lids; and canned liquid infant formula products.

Estimates of dietary exposure to BPA vary widely and can be based on different methods of calculation.

Using migration figures from food contact materials, levels of BPA found in foods and the amount of food consumed, a recent conservative estimate published in 2006 by the EFSA's Scientific Panel of Food Additives, Flavourings, Processing Aids and Materials in Contact with Food, gives values ranging from 0.2 μg per kg of bodyweight per day for a breast-fed three-month-old infant to 13 μg per kg of bodyweight per day for a six-month-old infant fed formula from a polycarbonate bottle and consuming commercial foods and beverages. This highest value falls to 1.5 μg per kg of bodyweight per day for an adult consuming commercial foods and beverages.

Following studies that identified possible developmental concerns following exposure to BPA, the FDA published a draft assessment of bisphenol A for use in food-contact applications in 2008. In this document BPA exposure estimates for formula-fed infants up to 12 months of age were: highest at 1–2 months at 2.25 and 2.42 μg per kg of bodyweight per day for males and females, respectively; and lowest at 12 months at 0.15 and 0.16 μg per kg of bodyweight per day for males and females, respectively.

Hazard Characterisation

Effects on Health

Based on studies in mice and rats, it is widely accepted that exposure to BPA (from the environment as well as from food) at high levels is potentially detrimental to human health. It is an endocrine disruptor and may have an effect on fertility. It has weak oestrogenic activity and has been shown to reduce sperm count and sperm activity. Studies indicate that it could affect development, and there are concerns that BPA can affect the brain, behaviour, and prostate gland in foetuses, infants, and young children. Some research suggests that BPA may be carcinogenic, possibly leading to the precursors of breast cancer. Some reports indicate that it has liver toxicity and may even be linked to obesity by triggering fat-cell activity.

The effect of low-level exposure to BPA on human health is far less clear. Some researchers believe that there is evidence in the literature demonstrating that animals exposed to very low doses of BPA suffer adverse affects. However, expert panels asked to review the data generally consider that there is not enough evidence from animal studies to suggest that low levels of BPA adversely affect humans.

It is generally agreed that the overall no-observed-effect level (NOEL) for BPA is 5 mg per kg of bodyweight. Government agencies add in an uncertainly factor to calculations to arrive at what is known as either a tolerable daily intake (TDI), or the maximum acceptable or oral reference dose (RfD). Currently the EFSA's TDI (established November 2006), and the Japanese TDI, as well as the EPA's RfD (established in 1993) for BPA is 0.05 mg per kg of bodyweight. This value is considerably greater than the highest estimates of dietary intake.

Sources

BPA can be present in foods as a result of migration from the epoxy-resin coatings used to line metallic food cans and on metal closures for glass jars and bottles. The other main source is the polycarbonate plastic bottles and containers used to package a wide range of products, such as water, soft drinks and milk. BPA in food may also originate from epoxy coatings and polycarbonate plastic used in tanks and containers in the processing environment.

Another potential source of BPA in food is polycarbonate tableware used to store foods in the domestic environment. BPA may migrate from tableware to foodstuffs, either from residual BPA in the material, or because various extreme conditions, including repeated cleaning, exposure to heat and contact with acid foods, result in the polycarbonate breaking down to produce BPA, which subsequently migrates into the food.

BPA is also found in a wide variety of non-food sources, such as drinking water storage tanks and water pipes, electrical equipment and various household appliances.

Stability in Foods

BPA appears to be readily biodegradable and after a short period of adaptation (3–8 days), levels in natural water environments rapidly decrease (100% removal in 2–17 days). Levels of BPA are also reduced rapidly during wastewater treatment.

Studies in fish indicate that BPA has low potential for bioaccumulation.

BPA is very heat stable. It has a melting point of 155–157 °C and polycarbonate plastics can be used up to temperatures of around 145 °C.

Control Options

It is generally agreed that the levels of ingested BPA should be as low as possible because of the uncertainties that exist about its potential adverse effects on human health. Health Canada has recommended that a general principle of ALARA (as low as reasonably achievable) be applied to limiting exposure of newborns and infants to BPA.

Processing

The food industry is being encouraged to implement techniques and procedures to reduce the migration of BPA into foodstuffs and to source can and container coatings that contain lower levels of BPA, or are BPA-free. In the USA, the FDA is working with manufacturers to develop safe alternatives to BPA for the linings of infant formula cans, and ensuring that these reach the market as soon as possible.

It is important to note that for canned food products, alternatives should not permit bacterial or metallic contamination of the contents, and should not give rise to other safety concerns. The use of alternatives may also reduce the final

shelf-life of a canned product, if the resistance of the alternative is lower than that of an epoxy-resin-based lining.

Product Use

Alternatives to BPA-containing plastics can be used for feeding infants and for storing and serving food. The FDA and Health Canada are actively supporting reasonable efforts to reduce levels of BPA in the food supply, especially for infants and young children.

The FDA is encouraging industry to stop producing new BPA-containing baby bottles and infant feeding cups destined for the USA market, and elsewhere some countries have introduced legislation banning the use of BPA in the manufacture of infant feeding bottles.

Legislation

There are no restrictions, at present, on the amount of BPA that can be present in most final plastic products, but the tendency of BPA to migrate from food contact materials has been acknowledged in EU food law. In 2002, EU legislation was introduced setting a Specific Migration Limit (SML) of 3 mg BPA per kg of food. This was amended in 2004 to set a SML(T) of 0.6 mg BPA per kg of food. The migration limit in Japan allows a maximum of 2.5 ppm. There is no SML in the USA at present.

Some countries have recently considered banning, or have banned, the use of BPA in plastics used for baby feeding bottles. Following national bans in Denmark and France, the EU banned the manufacture, marketing or importation of BPA-containing polycarbonate infant feeding bottles in 2011 and the Canadian Government is reported to be considering similar restrictions.

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2.2.2.2 Phthalates

Hazard Identification

What are Phthalates?

The phthalates (also known as phthalic acid diesters) are a group of related organic chemicals commonly used in the plastics industry as plasticisers. Plasticisers are routinely added to other materials, particularly polyvinyl chloride (PVC) and other polymers such as rubber and styrene, to make them more pliable and elastic.

The five phthalates most commonly used by industry are di-(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), di-isononyl phthalate (DINP), di-isodecyl phthalate (DIDP) and benzyl butyl phthalate (BBP).

Since the early 1980s there have been concerns about the effect that phthalates have on human health. Phthalates are able to leach from the materials to which they have been added and there is known to be widespread environmental exposure to the chemicals. Food products can become contaminated with phthalates from a wide variety of sources, but there has been particular concern over migration from food packaging. Phthalates can be present in some food packaging materials, including printing inks used on flexible food packaging, adhesives used for both paper, board and plastics, regenerated cellulose film (cellophane), aluminium foil-paper laminate and closure seals in bottles. It should be noted that many PVC "cling film" food wraps are no longer made with phthalates, but are now manufactured using other plasticisers.

Occurrence in Foods

Food can become contaminated with phthalates during processing, handling, transportation, and by migration from packaging, as well as from food-storage containers used in the home. Phthalates are fat soluble and have been found in many high-fat foods, such as dairy products, meat and poultry, eggs, fish, fats and oils. High levels of phthalates have been found in some olive oil samples.

Phthalates have also been found in a variety of other foods, such as infant formula, ready-to-use baby foods, bakery products, gravy granules, confectionery, pasta and cereal products, flour, sugar, vegetable burger mix and vegetables. They also occur in drinking water and in breast milk. In a UK survey of phthalates in foods from animal sources collected in 1993, DEHP was the most abundant individual phthalate found in each sample. A later UK survey of samples collected in 2007 demonstrated the presence of phthalates in a wide variety of foods such as dairy products, fish, poultry, meat products, cereals, fruit products and beverages. As found in the earlier survey, the highest dietary exposure to phthalates was associated with DEHP.

On occasion phthalates have been deliberately, and illegally, added to foods. In 2011 the Taiwanese government alerted food safety agencies to the misuse of

DEHP in various food products, which had been exported to other countries including the USA, Canada, the UK and Japan. The phthalate had been illegally added as a low-cost emulsifier to foods such as juices, tea-based beverages, sport and energy drinks, cakes and jams, and was reported to have been present at levels of 2.5 to 34 mg kg^{-1}.

A total diet study conducted in the UK on samples collected during 1993 estimated that the total phthalate intake for an average or high-level consumer is 0.013 and 0.027 mg per kg of bodyweight per day, respectively. Later Danish studies have suggested that this figure may be an underestimate of phthalate dietary intake, because the UK figures were based on foods from animal sources and did not take into account the high contribution that vegetables can make to phthalate intake. A further UK total diet study of food samples collected in 2007 calculated that total phthalate intake was highest for toddlers in the 1.5 to 2.5 year-old age group at 0.020 mg per kg of bodyweight per day, with total intake subsequently decreasing with age.

Hazard Characterisation

Effects on Health

Most of the data on the health effects of phthalates comes from experiments exposing rats and mice to high levels of the chemicals for prolonged periods. Long-term health effects of phthalates may include changes in sperm production, adverse effects on fertility and birth defects. They have also been reported to cause kidney and liver damage. Phthalates may be potential carcinogens and also endocrine disruptors, and as such could affect reproductive development. Very recent research has suggested that phthalates may also be linked to neurological and behavioural disorders such as attention deficit hyperactivity disorder (ADHD) and autism.

Individuals exposed to very high levels of DEHP for relatively short periods may experience mild gastrointestinal disturbances, vertigo and nausea.

There is no group TDI figure for phthalates, but TDIs have been set for some individual phthalates. For the five most commonly used phthalates, the EFSA's Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Foods (AFC) set TDI figures in 2005. The TDI for DEHP is 0.05 mg per kg of bodyweight; for BBP it is 0.5 mg per kg of bodyweight; for DBP it is 0.01 mg per kg of bodyweight; and for both DIDP and DINP it is 0.15 mg per kg of bodyweight.

The EPA has set an RfD of 0.02 mg per kg of bodyweight per day for DEHP, 0.20 mg per kg of bodyweight per day for BBP and 0.10 mg per kg of bodyweight per day for DBP. In a Concise International Chemical assessment Document in 2003 the WHO proposed a TDI for di-ethyl phthalate (DEP) of 0.5 mg per kg of bodyweight.

It is generally considered that the levels of individual phthalates currently found in foods are not a significant concern for human health.

Sources

Phthalates may be naturally produced by some animals and plants, and are also released into the environment during the manufacturing, use and disposal of products that contain them. As a result, consumers are exposed to these chemicals from a wide variety of environmental sources including air, drinking water and their physical surroundings. Phthalates are found in many plastics, cosmetics, medical devices, paints, lubricants, flooring materials, cleaning products, adhesives, inks, clothing, pesticides and toys. As a result, materials containing phthalates can be found everywhere in the domestic environment, but they are also used by the food industry and can be found in packaging materials, and also in manufacturing equipment such as conveyor belts and plastic hoses and tubing.

A number of food packaging materials can contain phthalates, including PVC and other plastics, printing inks used on flexible food packaging, adhesives used for paper and board, regenerated cellulose film (cellophane), aluminium foil–paper laminates and closure seals in bottles. Phthalates are known to migrate from packaging into foods, especially high-fat products and oils, and the rate of migration into food from packaging rises with increasing temperature.

Food surveys have determined that, although packaging contributes to the presence of phthalates in food products, it is not the only source of the chemicals. A UK survey published in 1995 found that measured levels of DEHP and DBP in some products were higher than would be expected if all the DEHP and DBP in the packaging had migrated into the foods concerned. In addition, the level of the phthalate at the core of products was equal to, or higher than, the level at the surface where the product was in contact with the packaging. These results may indicate that environmental sources contribute, at least in some part, to the presence of phthalates in foods.

Stability in Foods

Although phthalates are widespread in the environment, levels tend to be low because phthalates do not generally persist for extended periods when exposed to photochemical and biological breakdown.

DEHP in its gas form is broken down in the atmosphere by other chemicals in 1–2 days and solid particles are removed by various natural mechanisms in about 2–3 weeks. The chemical is broken down in surface soils by microorganisms into harmless components, but the rate of degradation is temperature dependent and is slower at cooler temperatures. However, DEHP persists for much longer in deep soil or at the bottom of lakes and rivers because anaerobic degradation is considerably slower than aerobic breakdown. The contaminant is found in plants and fish, but bioaccumulation is limited and animals higher up in the food chain can break the chemical down so that tissue levels tend to be low.

DBP persists in air for about 1.5 days, and in water environments for 2–20 days. As with DEHP, aerobic degradation is more efficient than anaerobic breakdown.

Control Options

Food campaign groups have raised consumer awareness of the possible health effects associated with soft PVC plastics and other materials containing phthalates. In the EU, there have been bans on the use of phthalates in some toys and cosmetics. Some measures designed to reduce the levels of phthalates in the environment and in foods have been introduced to address these concerns.

Processing

Reducing the levels of phthalates in the food processing environment and in food packaging can have a direct effect on the level of phthalates in food products. Where possible, soft PVC equipment parts containing phthalates, such as hoses, can be replaced with non-plastic parts, with other soft materials that do not contain plasticiser, or with plastics containing non-toxic plasticisers.

Manufacturers have developed glues and inks that do not contain phthalates to reduce levels in food packaging. PVC-free plastic food wrap materials have also been introduced as replacements for older "cling film" type food wraps. Products vulnerable to phthalate contamination, especially fatty foods, can be packaged in materials that do not contain phthalates.

Product Use

Advice for consumers on the safe use of plastic containers and food wraps in the home has been issued by a number of food safety authorities, including the UK FSA.

Legislation

The EU has legislation that limits the use of phthalates in food plastics, and where use is permitted, it limits the migration of these chemicals into foods by setting specific migration limits (SML). EU directive 2007/19/EC was adopted on 30th March 2007, and the manufacture, or import, of products that do not comply with this legislation were prohibited from 1st June 2008.

USA regulations treat phthalates that migrate into foodstuffs from food contact materials as indirect additives. In the USA, indirect food additives are defined as additives "that become part of the food in trace amounts due to its packaging, storage or other handling." The onus is on the food packaging manufacturers to prove to the FDA that food contact materials are safe.

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2.2.2.3 Semicarbazide

Hazard Identification

What is Semicarbazide?

Semicarbazide (SEM) is a chemical contaminant, which has been found in a number of food products—probably originating from several different sources. It is of concern from a food safety point of view because it has been shown to be a weak carcinogen in laboratory animals.

SEM ($H_2N–NH–CO–NH_2$, CAS No. 57-56-7) is a member of a group of chemicals known as the hydrazines. It was first detected in foods in 2003, when it was identified as a contaminant in foods packed in glass jars and bottles with sealed lids.

Occurrence in Foods

A number of EU studies were conducted during 2003 and 2004 to determine levels of SEM in foods. Baby foods in sealed glass jars contained the highest reported levels of SEM, ranging from not detectable to 140 μg kg^{-1}. Levels were similar in all EU countries reporting data, and the average level of SEM found in 385 samples of baby foods was 13 μg kg^{-1}. The average SEM level found in 121 samples of other food types (fruit, fish, vegetables, jams, pickles and sauces) included in these studies was 1.0 μg kg^{-1}.

Using figures derived from the studies of foods packaged in glass and jars and bottles, the EFSA estimated daily intakes of SEM. ''Reasonable worst case estimates'' of daily intakes of SEM for infants fed on products packed in glass jars and bottles ranged from 0.35 to 1.4 μg per kg of bodyweight per day. For adults, the estimates of SEM exposure from this source were much lower at 0.02 μg per kg of bodyweight per day, but these figures do not account for exposure to SEM from other dietary sources.

Canadian tests have found levels of SEM of up to 28 μg kg^{-1} in bread, with most SEM being found in the crust. Frozen breaded chicken or fish products can contain SEM in the breadcrumb coating, possibly at levels up to 5 μg per kg of product. SEM has also been detected in egg-white powder and in some types of carrageenan (particularly processed Euchema seaweed, E407a). SEM appears to occur naturally in some foods, but may also originate from currently unidentified sources. For example, wild crayfish caught in Finland during autumn 2004 were found to contain SEM at levels of up to 18 μg kg^{-1}.

A 2010 Australian survey of foods packaged in glass bottles or cans, including wine, canned beans, coffee, preserves, olive oil and olives, honey and pasta sauce failed to detect SEM in any of the 65 samples tested.

No international safety or migration limits have been established for SEM.

Hazard Characterisation

Effects on Health

Many of the hydrazine group of chemicals are known to cause cancer in laboratory animals. However, SEM is one of the least carcinogenic hydrazines. In 2005 the EFSA's Scientific Panel of Food Additives, Flavourings, Processing Aids and Materials in Contact with Food concluded that evidence indicates that SEM is a weak non-genotoxic carcinogen. Data on the potential developmental and reproductive toxicity of SEM is limited.

Based on recent studies, and the fact that efforts are being made in the EU to reduce the levels of SEM from its main food-related source, products in glass jars and bottles, EU experts consider that the risk, if any, to human health from SEM is very small, not only for adults, but also for infants.

Sources

There are thought to be several sources of SEM in foods, but by far the most significant is considered to be migration into foods from sealing gaskets fitted to the lids of glass jars and bottles. SEM from this source arises as a by-product of the breakdown of azodicarbonamide used as a 'blowing agent' in the formulation of PVC gaskets found on the inside of metal lids. Blowing agents change the texture of the gaskets and help to produce a better airtight seal. Azodicarbonamide has been employed to help seal metal 'twist' caps on glass jars used for a wide range of products including baby foods, fruit juices, conserves, pickles, mustard, mayonnaise and ketchups. Levels of SEM in the gaskets themselves have been found to vary from 1–7 mg per kg of gasket material.

In some countries, although not in the EU, azodicarbonamide is also approved as a food additive. It is used as a dough improver, and as a bleaching agent in cereal flour. SEM has been found in products made using flour to which azodicarbonamide has been added.

SEM is a metabolite of the veterinary drug nitrofurazone, and is used as a marker for the use of this drug in foods of animal origin. Nitrofurazone is not permitted for use in food-producing animals in the EU and so SEM from this source should not be detected in foods. However, this may be a source of dietary exposure in other countries where nitrofuran drugs are not illegal.

SEM is also formed during some manufacturing processes used to produce egg-white powder and some types of carrageenan, particularly processed Euchema seaweed. SEM is thought to be produced as a by-product of these processes as a result of a reaction between hypochlorite bleach and organic substances.

SEM may also occur naturally in the environment, and recent research has found that it is naturally present in freshwater prawns and other crustaceans, including crabs and langoustines, primarily in their shells. It is also thought that there may be still some unidentified sources of the contaminant in foods.

Stability in Foods

There is no available data on the persistence, or bioaccumulation of SEM in the environment. A study has shown that concentrations of SEM in pig muscle and liver did not drop significantly during storage for eight months at −20 °C, and that working standard solutions prepared in methanol stored at 4 °C for 10 months were generally stable.

The melting point of SEM is around 173–177 °C. A study concluded that SEM is largely resistant to conventional cooking techniques such as frying, microwaving, grilling and roasting.

Control Options

Processing

The WHO has said that ''the presence of SEM in baby foods is considered particularly undesirable''. Therefore as a precaution, efforts should be made to reduce levels, or eliminate SEM from foods, particularly baby foods, and these efforts should focus on avoiding processes that produce the chemical.

In order to eliminate SEM from the gaskets used for metal twist caps, food manufacturers have been encouraged to develop alternative materials so that azodicarbonamide is no longer used in food packaging. Care should be taken to choose alternative types of sealing for bottles and jars that do not compromise the microbiological safety of the contents.

In the EU, the use of azodicarbonamide in food contact materials was prohibited from August 2005, and once existing stocks of products have been used, the dietary intake of SEM derived from gaskets should have been eliminated.

The use of flour containing azodicarbonamide as an additive should be avoided to prevent the formation of SEM in baked foods, and in products with crumb coatings.

Legislation

Azodicarbonamide is not permitted as a flour treatment agent in the EU. At the time of writing it is permitted in some countries (*e.g.* the USA, Canada and Brazil), and can be used at levels up to 45 mg kg^{-1} flour.

The use of azodicarbonamide as a blowing agent has been prohibited in the EU since 2nd August 2005. Products filled before this date could continue to be placed on the market provided that the date of filling, or a mark indicating when it was filled, appeared on the product. At the time of writing the use of azodicarbonamide for food contact materials is still permitted in some other countries, including the USA.

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2.2.3 ENVIRONMENTAL CONTAMINANTS

2.2.3.1 Dioxins and PCBs

Hazard Identification

What are Dioxins and PCBs?

The term dioxins refers to a group of compounds with similar chemical and physical properties and structures. Dioxins are colourless, odourless organic compounds containing carbon, hydrogen, oxygen and chlorine. There are many different dioxins, of which 17 are known to be toxic to humans. The most toxic known dioxin is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD), and significant concentrations of this compound can be measured in parts per trillion (PPT).

Dioxins are ubiquitous environmental contaminants, having been found in soil, surface water, sediment, plants, and animal tissue worldwide. They are highly persistent in the environment with half-lives ranging from months to years. They have low water solubility and low volatility, meaning that they remain in soil and sediments that serve as environmental reservoirs from which the dioxins may be released over many years.

PCBs, or polychlorinated biphenyls, are chlorinated aromatic hydrocarbons produced by the direct chlorination of biphenyls. There are about 209 related PCBs, known as congeners of PCBs, of which 20 reportedly have toxicological effects. Some of the PCBs have toxicological properties similar to those of dioxins and are therefore often referred to as ''dioxin-like PCBs''.

Like dioxins, PCBs are widespread environmental contaminants and are very persistent in soil and sediments. It has been suggested that highly contaminated bottom sediments in sewers and receiving streams may represent a reservoir for the continued release of PCBs into the environment.

Occurrence in Foods

Dioxins and PCBs enter the food chain through a variety of routes. Grazing animals and growing vegetables may be exposed directly, or indirectly, to these contaminants in the soil. Leafy vegetables, pasture and roughage can also become contaminated through airborne transport of dioxins and PCBs. Dioxins in surface waters and sediments are accumulated by aquatic organisms and bioaccumulated through the food chain. The concentration of dioxins in fish may be hundreds to thousands of times higher than the concentrations found in surrounding water and sediments.

Because dioxins are not very soluble in water, they tend to accumulate in the fatty tissues of animals and fish. Theoretically, the longer the lifespan of the animal, the longer the time it has to accumulate dioxins and PCBs. Foods that are high in animal fat, such as milk, meat, fish, eggs and related products are the main source of dioxins and PCBs and contribute about 80% of the overall

human exposure, although almost all foods will contain these contaminants at some (generally very low) level owing to their ubiquitous nature.

The main contributors to the average daily human intake of dioxins and PCBs have been found to be milk and dairy products, contributing between 16 and 39%; meat and meat products, contributing between 6 and 32%; and fish and fish products, contributing between 11 and 63%. Other foods, mainly vegetables and cereals, contributed 6–26% in the countries for which data was available (Codex Alimentarius Commission, 2001).

Human milk can contain elevated levels of dioxins, some of which can pass to the infant during lactation. However, the intake of babies from their mothers is limited to a relatively short period of their lives.

It is estimated that the average dietary intake of dioxins and dioxin-like PCBs has fallen amongst adults in the UK from 1.8 pg World Health Organization toxic equivalents (WHO-TEQ) per kg of bodyweight per day* in 1997 to 0.9 pg WHO-TEQ per kg bodyweight per day in 2001. Similar decreases have been reported in other countries. In November 2001, the Independent Committee on Toxicity recommended a TDI of 2 pg WHO-TEQ per kg of bodyweight per day.

Hazard Characterisation

Effects on Health

Humans accumulate dioxins in fatty tissue mostly by eating dioxin-contaminated foods. The toxicity of dioxins is related to the amount accumulated in the body during the lifetime. Dioxins and PCBs have a broad range of toxic and biochemical effects, and some are classified as human carcinogens. In animal testing, dioxins have been implicated in causing damage to the immune and reproductive systems, developmental effects and neurobehavioural effects.

Despite the variety of adverse effects observed in animals exposed to dioxins, documented adverse health effects in humans have generally been limited to highly exposed populations in industrial environments, or following accidental chemical contamination.

The most commonly observed adverse health effect in humans following acute over-exposure to dioxins and PCBs is the skin disease chloracne, a particularly severe and prolonged acne-like skin disorder. The accidental contamination of edible rice bran oil with PCBs in Japan in 1968 led to a poisoning epidemic amongst those who consumed the oil. The poisoning caused chloracne, liver disturbances, abdominal pain, headaches, skin discolouration, and the birth of abnormally small babies to mothers who had consumed the oil.

More recent examples of dioxin contamination include an incident in Belgium in 1999, when PCB-contaminated feeds were fed to farm animals; in this case the contamination was discovered as a result of the direct biological

*The TEQ is a weighted toxicity value designed to take into account the variable toxicity of different dioxins and dioxin-like PCBs in comparison with the most toxic dioxins, and give a comparable overall measure of dioxin and PCB levels.

effects of dioxins observed in poultry. Other incidents include contamination of guar gum from India, discovered in 2007. The contamination involved pentachlorophenol and dioxins and led to the EC's imposition of special conditions governing guar gum originating in India. Another incident in 2008 involved contamination of pork meat in Ireland. The source of the contamination was found to be animal feed, to which had been added breadcrumbs produced from bakery waste by a process involving direct contact with combustible gases from an inappropriate fuel source. A potentially damaging incident involving dioxin-contaminated fatty acids produced using biodiesel and destined for animal feed use also occurred in Germany in 2010. Rapid measures by the German Authorities managed to prevent the incident from becoming a widespread problem.

Sources

Dioxins are often man-made contaminants and are formed as unwanted by-products of industrial chemical processes, such as the manufacture of paints, steel, pesticides and other synthetic chemicals, wood pulp and paper bleaching, and also in emissions from vehicle exhausts and incineration. Dioxins are also produced naturally during volcanic eruptions and forest fires. Most industrial releases of dioxins are strictly controlled under pollution prevention and control regulations. Currently, the major environmental source of dioxins is incineration.

PCBs have been used in manufacturing industry since the early 1930s, mainly as cooling and insulating fluids in electrical equipment. The manufacture and general use of PCBs was banned in the 1970s because of environmental and health concerns. However, some PCBs remain in use, sealed inside older electrical equipment, although the use of this equipment must be phased out, and the PCBs removed and destroyed.

Stability in Foods

Dioxins and PCBs are highly stable with reportedly long half-lives. In animals, they accumulate in fat and in the liver and are only very slowly metabolised by oxidation or reductive dechlorination and conjugation. They are therefore likely to persist in animal tissues, especially fatty tissue, for long periods. They are not generally affected significantly by food processing such as heat treatments, or fermentation.

Control Options

There is very little scope for the removal of dioxins and PCBs from foods once they have entered the food chain. It is generally agreed that the best means for preventing dioxins and PCBs from entering the food chain is to control their release into the environment.

The overall goal of EU policy is to reduce the contamination levels of dioxins and PCBs in the environment, and in food and feed. The EU has prohibited the use of most PCBs from 1978 and for certain applications from 1986. A deadline of 2010 was set for removing all PCB-containing equipment from service.

Dioxins, on the other hand, cannot be banned owing to their formation as unwanted by-products of many industrial processes. The amounts of dioxins and PCBs ingested in food are similar in the EU and the USA. Intakes are falling and have reduced by 85% since 1982, demonstrating some international success in controlling environmental contamination by these compounds.

Product Use

While studies suggest that there is no cause for alarm from potential health issues concerning dioxins in the diet, choosing leaner cuts of meat, removing the skin from chicken or trimming the fat off meat may help to minimise any potential exposure of consumers to dioxins in food. Similarly, drinking reduced- or low-fat milk may also help to reduce exposure slightly, as may the washing of fruit and vegetables to remove any airborne dioxin-contaminated dust particles that might have been deposited on produce in fields.

Legislation

EU

EU regulations on contaminant levels in foods have recently been introduced (March 2007), which require tougher safety controls in food manufacturing plants. The regulations aim to ensure a harmonised approach to the enforcement of permitted contaminant levels across the EU.

EC Regulation No. 1881/2006 sets maximum levels for certain contaminants, including dioxins and dioxin-like PCBs in foods. The limits for dioxins and PCBs as set out in this Regulation are as outlined in Table 2.2.2.

Methods of Sampling for Dioxins

EC Regulation No. 1883/2006 lays down the methods for sampling and analysis for the official control of levels of dioxins and dioxin-like PCBs in certain foodstuffs.

Other Regulatory Measures

Commission Recommendation 2006/88/EC of 6th February 2006 provides recommendations concerning the reduction of the presence of dioxins, furans and PCBs in animal feed and foodstuffs.

Commission Recommendation 2006/794/EC of 16th November 2006 relates to the monitoring of background levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in foodstuffs.

USA

There are no tolerances or other administrative levels for dioxins in food or feed in the USA and the FDA considers all detectable levels to be of concern. Action

Table 2.2.2 Limits for dioxins and PCBs set out in EC Regulation No. 1881/2006.

Foodstuff	*Maximum levels (sum of dioxins)*	*Maximum levels (sum of dioxins and dioxin-like PCBs)*
Meat and meat products (excluding edible offal) of the following animals:		
Bovine animals and sheep	3.0 pg per g of fat	4.5 pg per g of fat
Poultry	2.0 pg per g of fat	4.0 pg per g of fat
Pigs	1.0 pg per g of fat	1.5 pg per g of fat
Liver of terrestrial animals above and derived products thereof	6.0 pg per g of fat	12.0 pg per g of fat
Muscle meat of fish and fishery products and products thereof, excluding eel. The maximum level applies to crustaceans, excluding the brown meat of crab and excluding head and thorax meat of lobster and similar large crustaceans (*Nephropidae* and *Palinuridae*)	4.0 pg per g wet weight	8.0 pg per g wet weight
Muscle meat of eel (Anguilla anguilla) and products thereof	4.0 pg per g wet weight	12.0 pg per g wet weight
Raw milk and dairy products including butterfat	3.0 pg per g of fat	6.0 pg per g of fat
Hen eggs and egg products	3.0 pg per g of fat	6.0 pg per g of fat
Bovine and sheep fat	3.0 pg per g of fat	4.5 pg per g of fat
Poultry fat	2.0 pg per g of fat	4.0 pg per g of fat
Pig fat	1.0 pg per g of fat	1.5 pg per g of fat
Vegetable oils and fats	0.75 pg per g of fat	1.5 pg per g of fat
Marine oils (fish body oil, fish liver oil and oils of other marine organisms intended for human consumption)	2.0 pg per g of fat	10.0 pg per g of fat.

levels have been set for PCBs in red meat and fish. Temporary tolerances have also been set for animal feeds and paper packaging. These are published in the Federal Register.

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2.2.3.2 Heavy Metals

Hazard Identification

What are Heavy Metals?

The term *heavy metal* refers to any relatively high-density metallic element that is toxic or poisonous even at low concentrations. Heavy metals are natural components of the Earth's crust and cannot be destroyed. Although there are many elements that are classified as heavy metals, the ones of most concern, with respect to their biotoxic effects and presence in food, are arsenic, cadmium, lead, and mercury, and it is primarily these that are dealt with here. These elements have no known biological importance in human biochemistry and physiology, and consumption, even at very low concentrations, can cause toxic effects, because they tend to accumulate in the human body over time.

Because of their potential toxicity, regulatory bodies throughout the world have set a limit on the acceptable amounts of these contaminants in certain foods. In the EU, limits have been set on the amounts of the heavy metal tin in foods as well as on cadmium, lead and mercury. For this reason, tin has also been included in this section.

Occurrence in Foods

The EU Directorate-General, Health and Consumer Protection carried out a major study to assess the dietary intake of arsenic, cadmium, lead and mercury of the population of the EU Member States in March 2004 (the reference to the full report is given below). The report collected data on the occurrence, consumption and intake calculations for the populations of Belgium, Denmark, Finland, France, Germany, Ireland, Italy, the Netherlands, Norway, Spain, Portugal, and the UK. Some of the results from this report are briefly summarised below.

Arsenic

The major source of arsenic in the diet is fish and other seafood, although the daily intake is estimated to be less than 0.35 mg. The marine environment has a great impact on arsenic levels as sea fish have arsenic levels about 10 times higher than freshwater fish. Children have a lower intake of arsenic than adults, and young children have the lowest intake.

Cadmium

None of the most commonly consumed foods were found to be high in cadmium. Cereals, fruit and vegetables are the main source of cadmium in the diet, making up about 66% of the mean cadmium intake. The other sources include meat and fish, with liver, kidney, crustaceans, molluscs and cephalopods containing comparatively higher cadmium levels. The PTWI (permitted tolerable weekly intake) is 0.49 mg for a person weighing 70 kg, and the mean intake for

most EU Member States is less than 30% of the PTWI. Children have a lower intake of cadmium than adults, and young children have the lowest intake.

Lead

None of the most commonly consumed foods were found to be high in lead, although some Member States reported high lead levels in wine, game, meat and fish. The PTWI for lead in the EU is 0.025 mg per kg of bodyweight, which is equivalent to 1.75 mg for a person weighing 70 kg. The average intake of lead was less than 25% of the PTWI in most Member States. Children have a lower lead intake than adults.

Mercury

The main source of mercury in the diet is fish, followed by fruit and vegetables. In fish and shellfish, mercury is present in the form of methylmercury, while in most other food groups it is present in its inorganic form. Methylmercury is formed from inorganic mercury by the action of microorganisms in marine and freshwater sediments. Predatory species of fish at the top of the food chain, such as tuna and swordfish, generally contain higher levels of mercury, but their contribution to total mercury intake is small as consumption levels are low. Fruit, dried fruit, mushrooms and vegetables are other sources of mercury in the diet.

The PTWI for mercury is 0.35 mg for a person weighing 70 kg. The mean intake for total mercury within the Member States is less than 30% of the PTWI. The PTWI for methylmercury is 0.112 mg per week for a person weighing 70 kg (1.6 μg per kg of bodyweight). The mean intake of methylmercury is less than 30% of the PTWI. However, for people who consume a lot of fish, such as some groups in Norway, the PTWI may be exceeded. Although children have a lower total intake of mercury than adults, they also have a lower bodyweight and so, potentially, a relatively larger intake per kg bodyweight. It is possible that, in some cases, the PTWI for methylmercury may be exceeded.

Hazard Characterisation

Effects on Health

Arsenic

Arsenic is one of the most toxic elements found, and is present in foods in organic or inorganic forms, with the latter being considered to be far more toxic than the former. Additionally, inorganic As^{3+} salts are more toxic than As^{5+} salts. Illnesses associated with excessive inorganic arsenic intake include skin, lung and heart conditions, gastrointestinal diseases and possible carcinogenic effects. As^{3+} compounds are bound by red blood cells and affect the activity of many enzymes, particularly those involved in the respiratory process. 100 mg of arsenic oxide is considered to be lethal.

Organic arsenic does not cause cancer, nor is it thought to damage DNA, but exposure to high doses may cause nerve injury and stomach problems.

The levels of arsenic in most foods are very low, with the exception of seafood. However, the majority of arsenic in seafood is present in the organic, less toxic form, and during digestion of such compounds, the arsenic is not released, or is released only very slowly. This explains why very few cases of arsenic poisoning are associated with seafood consumption, despite the high levels observed.

In a recent scientific assessment of arsenic in foods carried out by the EFSA Panel on Contaminants in the Food Chain (CONTAM), it was concluded that the PTWI of arsenic of 15 μg per kg of bodyweight established by JECFA was no longer appropriate, as data had shown that inorganic arsenic causes lung and bladder cancer in addition to skin cancer and that a range of adverse effects had been reported at exposures lower than those reviewed by the JECFA. The CONTAM Panel recommended that dietary exposure to inorganic arsenic should be reduced.

Cadmium

Human intake of cadmium occurs mostly through food or through smoking. In humans, long-term exposure may lead to kidney damage, as cadmium tends to accumulate in the kidneys. Other adverse health effects include diarrhoea, stomach pains and sickness, bone defects, immune system damage, possible infertility, possible damage to DNA and carcinogenic effects.

Cases of food-borne cadmium poisoning were reported in the 1940s in England, France, the USA, Russia, New Zealand and other countries, caused by consumption of lemonade, coffee, wine and other products that had been prepared or stored in cadmium-coated containers or in refrigerators with cadmium-coated freezers.

In 2009, EFSA's CONTAM Panel carried out a risk assessment on cadmium in food and established a tolerable weekly intake (TWI) of 2.5 μg per kg of bodyweight. Following an assessment of cadmium by JECFA, the Panel reassessed the TWI in 2011 and concluded that 2.5 μg per kg of bodyweight was still appropriate. The current average dietary exposure to cadmium for adults is close to the TWI and the exposure of some subgroups, such as children, vegetarians and people living in highly contaminated areas, could exceed the TWI.

Lead

Lead enters the human body *via* food, water and air. It is very damaging to health, particularly for infants, children and the developing foetus. Its adverse effects include disruption of haemoglobin synthesis, kidney damage, increased blood pressure, miscarriage, nervous system disruption, reduced fertility, and learning disabilities and behavioural problems in children. Lead can cross the placenta and may damage the nervous system and brain of the developing foetus.

Symptoms of chronic lead poisoning occur following daily ingestion of 2 to 4 mg for several months, whilst acute poisoning will occur after daily doses of 8 to 10 mg for a few weeks.

In an EFSA Opinion published in April 2010 on the possible health risks related to the presence of lead in food, the CONTAM Panel considered cereals, vegetables and tap water to be the main contributors to dietary lead exposure for most Europeans. The Panel concluded that current levels of exposure to lead posed a low-to-negligible health risk for most adults, but that there was potential concern over possible neurodevelopmental effects in foetuses, infants and children.

Mercury

Mercury is present in foods such as vegetables, mushrooms and, particularly, fish. It is highly toxic and can cause disruption of the nervous system, brain damage, damage to DNA and chromosomes, allergic reactions and adverse reproductive effects.

The first reported outbreak of food poisoning attributed to mercury ingestion was in 1953 in Japan. This outbreak was caused by consumption of fish containing significant amounts of methylmercury and affected people living in Minamata Bay, leading to the term Minamata disease, now often used to describe any form of food-borne mercury poisoning. Severe outbreaks of food-borne mercury poisoning also occurred in Iraq between 1955 and 1960. Over 8000 people were affected as a result of consumption of bread made from grain treated with methylmercury.

In 2004 the EFSA CONTAM Panel adopted an Opinion on mercury and methylmercury, the latter being the main mercury compound present in fish and seafood products. The opinion looked at the contribution of different foods towards overall human exposure and the risks to vulnerable groups, in particular pregnant women and children. The Panel concluded that methylmercury toxicity had been demonstrated at low exposure levels, and that exposure to this compound should be minimised.

Tin

Tin has been used since the Bronze Age and is still used widely today. It is used in the production of plastics, pesticides, wood preservatives and as a coating for metal food cans. In some countries, inorganic tin compounds are added to preserve the colour of vegetable preserves packed in glass jars. Tin can also enter foods *via* the use of tin-containing organo-pesticides.

Inorganic tin salts are poorly absorbed and generally almost completely excreted from the body *via* the stools. Organic tin compounds are thought to be more toxic. Long-term exposure to organic tin compounds can lead to nervous system disorders and sex gland atrophy. The average daily intake of tin is around 4 mg, but it is not accumulated in the body.

Sources

Heavy metals can be present in food either naturally, or as a result of human activities, such as mining, irrigation, energy extraction, agricultural practices,

incineration, industrial emissions and car exhausts. They may also originate from contamination during manufacturing, processing and storage, or from direct addition.

Plants grown in contaminated soil can accumulate heavy metals, particularly lead and cadmium. Arsenic and cadmium are concentrated in coal ash, from which they can leach into surface waters and accumulate in fish and other aquatic organisms. Mercury tends to accumulate in birds, mammals and fish. Drinking water is another possible source of heavy metals.

Stability in Foods

Heavy metals are stable elements and persist for long periods in the environment. There is no evidence to suggest that levels of heavy metals in foods are changed significantly by processing. For example, methylmercury can be found in canned fish that has undergone a severe thermal process.

Control Options

Control of heavy metal levels in foods relies largely on avoiding those food commodities that are likely to have been exposed to large concentrations of metal contaminants in the primary production environment. Examples include vegetables and produce grown in soils contaminated naturally, or by industrial activity, and large predatory fish. Many health and food safety authorities advise that children under sixteen, pregnant women, and women hoping to become pregnant should avoid shark, marlin and swordfish, and limit the amounts of tuna consumed, because of the possibility of high levels of mercury.

It is also important to ensure that heavy metal contamination cannot arise from the use of inappropriate food processing equipment. Manufacturers must ensure that all equipment is constructed from 'food grade' materials that meet the required standards.

Regulations in many countries set maximum levels for heavy metal contaminants in certain foodstuffs. It is the responsibility of manufacturers to ensure that these limits are observed, and that ingredients are sourced from reputable suppliers. It is also important to ensure that all processing water is sourced from potable supplies that are not contaminated with heavy metals.

Legislation

EU

Revised EU regulations on contaminant levels in foods were introduced in March 2007, which require tougher safety controls in food manufacturing plants, and aim to ensure a harmonised approach to the enforcement of contaminant levels across the EU.

For the heavy metals cadmium, lead, mercury and tin, maximum levels in certain foods have been established by EC Regulation No. 1881/2006, which replaces No. 466/2001 setting maximum levels for certain contaminants in food.

The limits for the heavy metals lead, mercury, cadmium and inorganic tin as set out in this Regulation are set out in Tables 2.2.3–2.2.6. Arsenic is not covered in this Regulation, but there are maximum limits for arsenic in food in the UK, as set down in the UK Arsenic in Food Regulations (as amended) 1959.

Heavy Metal Analysis

Provisions for methods of sampling and analysis for the official control of lead, cadmium, mercury, and inorganic tin in foodstuffs are laid down in EC Regulation No. 333/2007.

Table 2.2.3 Maximum acceptable levels of lead in certain foods, as set out in EC Regulation No. 1881/2006.

Foodstuffs	*Maximum levels/ mg kg^{-1} wet weight*
Raw milk, heat-treated milk and milk for manufacture of milk-based products	0.020
Infant formulae and follow-on formulae	0.020
Meat (excluding offal) of bovine animals, sheep, pig and poultry	0.10
Offal of bovine animals, sheep, pig and poultry	0.50
Muscle meat of fish	0.30
Crustaceans, excluding brown meat of crab and excluding head and thorax meat of lobster and similar large crustaceans.	0.50
Bivalve molluscs	1.50
Cephalopods (without viscera)	1.00
Cereals, legumes and pulses	0.20
Vegetables, excluding brassica vegetables, leaf vegetables, fresh herbs and fungi. For potatoes, the maximum level applies to peeled potatoes	0.10
Brassica vegetables, leaf vegetables and cultivated fungi	0.30
Fruit, excluding berries and small fruit	0.10
Berries and small fruit	0.20
Fats and oils, including milk fat	0.10
Fruit juices, concentrated fruit juices as reconstituted and fruit nectars	0.050
Wine (including sparkling wine, excluding liqueur wine), cider, perry and fruit wine	0.20
Aromatized wine, aromatized wine-based drinks and aromatized wine-product cocktails	0.20

Table 2.2.4 Maximum acceptable levels of cadmium in certain foods, as set out in EC Regulation No. 1881/2006.

Foodstuffs	*Maximum levels/ mg kg^{-1} wet weight*
Meat (excluding offal) of bovine animals, sheep, pig and poultry	0.050
Horsemeat, excluding offal	0.20
Liver of bovine animals, sheep, pig, poultry and horse	0.50
Kidney of bovine animals, sheep, pig, poultry and horse	1.0
Muscle meat of fish (excluding the species mentioned in the 2 rows below)	0.050
Muscle meat of the following fish: anchovy, bonito, common two-banded seabream, eel, grey mullet, horse mackerel or scad, louver or luvar, sardine, sardinops, tuna, wedge sole	0.10
Muscle meat of swordfish	0.30
Crustaceans, excluding brown meat of crab and excluding head and thorax meat of lobster and similar large crustaceans	0.50
Bivalve molluscs	1.0
Cephalopods (without viscera)	1.0
Cereals excluding bran, germ, wheat and rice	0.10
Bran, germ, wheat and rice	0.20
Soybeans	0.20
Vegetables and fruit, excluding leaf vegetables, fresh herbs, fungi, stem vegetables, pine nuts, root vegetables and potatoes	0.050
Leaf vegetables, fresh herbs, cultivated fungi and celeriac	0.20
Stem vegetables, root vegetables and potatoes, excluding celeriac. For potatoes, the maximum level applies to peeled potatoes	0.10

Table 2.2.5 Maximum acceptable levels of mercury in certain foods, as set out in EC Regulation No. 1881/2006.

Foodstuffs	*Maximum levels/ mg kg^{-1} wet weight*
Fishery products and muscle meat of fish, excluding species listed in the row below. The maximum level applies to crustaceans, excluding the brown meat of crab, and excluding the head and thorax meat of lobsters and similar large crustaceans	0.50
Muscle meat of the following fish: anglerfish; atlantic catfish; bonito; eel; emperor, orange roughy, rosy soldierfish; grenadier; halibut; marlin; megrim; mullet; pike; plain bonito; poor cod; Portuguese dogfish; rays; redfish; sail fish; scabbard fish; seabream, Pandora; shark (all species); snake mackerel or butterfish; sturgeon; swordfish; tuna	1.0

Table 2.2.6 Maximum acceptable levels of tin (inorganic) in certain foods, as set out in EC Regulation No. 1881/2006.

Foodstuffs	*Maximum levels/ mg kg^{-1} wet weight*
Canned foods other than beverages	200
Canned beverages, including fruit juices and vegetable juices	100
Canned baby foods and processed cereal-based foods for infants and young children, excluding dried and powdered products	50
Canned infant formulae and follow-on formulae (including infant milk and follow-on milk), excluding dried and powdered products	50
Canned dietary foods for special medical purposes intended specifically for infants, excluding dried and powdered products	50

USA

The FDA has published a booklet giving action levels established for poisonous or deleterious substances in human food and animal feed. These action levels for poisonous or deleterious substances are established to control levels of contaminants in human food and animal feed. The booklet provides action levels for the heavy metal contaminants cadmium, lead and mercury in certain foods and commodities. It was published in August 2000 and any new action levels published since then are published in the Federal Register.

The document can be accessed on the FDA website at: http://www.cfsan.fda.gov/~lrd/fdaact.html

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2.2.3.3 Melamine

Hazard Identification

What is Melamine?

Melamine ($C_3N_6H_6$, CAS No. 108-78-1) is a synthetic triazine compound and an organic base with the chemical name 2,4,6-triamino-1,3,5-triazine. It is an important industrial chemical and has been used as a component in various products since the late 1930s. The best known use of melamine is in combination with formaldehyde to produce melamine resin, a very hard-wearing thermosetting plastic. Melamine resin has been widely used to make durable tableware, worktops and whiteboards. Melamine is important in a number of other applications, including fire retardants, fertilizers, pigments and glues.

Melamine also has analogues produced by successive deamination reactions. These are ammeline, ammelide and cyanuric acid. It is now generally accepted that melamine in food, especially in combination with an analogue, can have potentially serious health consequences for animals and for humans.

Occurrence in Foods

Melamine contamination in food first became a food safety issue when the chemical was detected in pet foods linked to kidney failure in thousands of dogs and cats in North America in 2007. However, since then it has become apparent that a similar incident affecting an estimated 6000 dogs in Asia in 2004 and first attributed to mycotoxin contamination, was also likely to have been caused by melamine. Other earlier incidents in Asia, the EU and South Africa have also since come to light. An investigation of the 2007 incident found that melamine and its analogue cyanuric acid were present in wheat gluten and rice protein concentrate imported from China by the pet food producer and used as a thickening and binding ingredient. Levels of between 0.2 and 8% were detected in batches of the two ingredients. This would correspond to a dose of around 400 mg per kg per day for animals fed with the contaminated pet food. Since then, melamine has been found in animal feed samples and in animal tissues at low concentrations. It has also been found in beverages, including coffee and orange juice, at levels of up to 2 mg kg^{-1}, but this is thought to be as a result of migration from plastic cups at high temperatures. Very low levels of melamine are thought to be occasionally present in some processed foods as a result of migration from packaging or processing equipment. It is also possible that melamine could be generated at very low levels as a by-product of processing.

In 2008, melamine was found in dairy products from China, especially powdered milk used to make infant formula associated with widespread kidney disease in babies. Samples of dairy products (including infant formula) were reported to contain melamine at concentrations between 0.09 mg kg^{-1} and 6196 mg kg^{-1}. Melamine has also been found in liquid milk in China (highest concentration 8.6 mg kg^{-1}) and a wide variety of other products made using

Chinese-sourced milk powder. These include chocolate and milk-based confectionery, biscuits and other bakery products, coffee and tea whiteners and milk-based beverages. It was also detected in Chinese fresh eggs at concentrations of 3.1 to 4.7 mg kg^{-1}. Contaminated foods have been found all over the world, particularly in other Asian countries, but also in the EU, the USA, Canada and Australia. Reported contamination levels are highly variable, ranging from 0.38 mg kg^{-1} to 945 mg kg^{-1} in dairy products and from 0.6 mg kg^{-1} to 6694 mg kg^{-1} in processed foods and food ingredients. A great many food products were withdrawn from sale as a result of melamine contamination.

There is little information on the likely dietary exposure that would result from such levels in processed foods, but the EFSA has estimated that chocolate with high levels of contaminated milk powder could result in an exposure of 1.35 mg per kg of bodyweight per day, more than six times the current WHO TDI of 0.2 mg per kg of bodyweight for children and adults. It is thought that infants in China, fed exclusively on contaminated formula, could have been exposed to melamine at levels up to 200 times higher than the TDI.

Hazard Characterisation

Effects on Health

Melamine and its analogues are not particularly toxic compounds when considered individually. An oral LD_{50} of more than 3000 mg kg^{-1} has been reported for rats. Both animals and infants affected by melamine-contaminated foods suffered from kidney damage, especially kidney stones and in some cases kidney failure. However, toxicology studies have shown no acute renal toxicity caused by melamine, although high doses have diuretic properties in animals. It is not genotoxic, or teratogenic, and does not cause skin irritation except at high doses. However, some animal studies of chronic toxicity have shown that kidney and bladder calculi (stones) can form when high levels (1% or more) of melamine are included in the diet over a long period. These calculi can result in bladder cancer in rats as a result of irritation.

Following the widespread deaths among dogs and cats fed with melamine-contaminated feed in 2007, an investigation into the toxic mechanism involved was undertaken. The results of this study suggest that the effects observed in animals were caused by the formation of crystals in the urine, leading to kidney and bladder calculi, blocking the renal tubules in severe cases and causing potentially fatal kidney failure. The crystals were composed of a stable, insoluble melamine/cyanuric acid complex, which formed a lattice-like structure held together by hydrogen bonds. It has been reported that the two compounds are absorbed separately in different regions of the gut, because melamine is a base and has a much lower pK_a value than that of cyanuric acid. The insoluble complex is then thought to form and produce the crystals within the kidneys.

The mechanism responsible for the kidney problems experienced by infants fed on contaminated formula in China during 2007–2008 is now thought to be

somewhat different. Cyanuric acid was not present in significant quantities in the urinary tract calculi obtained from the affected children. Instead the stones were found to consist of melamine and uric acid. It is thought that these stones formed in infants rather than adults because they typically have higher levels of uric acid in their urine. There is still some uncertainty about the exact toxicological mechanisms involved.

The EFSA initially applied a TDI of 0.5 mg per kg of bodyweight for melamine, based on the data available, but this figure was reduced in 2010 to 0.2 mg per kg of bodyweight in line with the WHO TDI set in 2008. The WHO has also recommended a TDI for cyanuric acid of 1.5 mg per kg of bodyweight.

Incidence and Outbreaks

The 2008 incident involving contaminated infant formula in China is reported to have affected at least 294 000 children (source WHO). Some 51 900 of these required hospital treatment and at least six deaths have been associated with the contamination.

Sources

Melamine is reported to be widely available in China as a by-product of the plastics industry. Media reports suggest that it was added to certain food ingredients and to milk because of its very high nitrogen content. This would give a falsely high result in tests designed to determine protein content and cause the material to be assigned a higher quality rating and commercial value. It has been estimated that the addition of 1 g of melamine to 1 litre of milk would raise the apparent protein content by approximately 0.4%. If this is indeed the case, then melamine is an adulterant and has been deliberately added to milk, wheat gluten and rice protein concentrate in a fraudulent attempt to increase profits and disguise watered-down, or poor-quality products.

Melamine in food may also come from other sources, especially plastic packaging, or processing equipment, but usually only at levels not harmful to health. It is also produced in animals as a metabolite of the insecticide cyromazine, which is widely used to prevent insect damage to fruit and vegetables.

Stability in Foods

There is little information as yet as to the stability of the melamine–cyanuric acid complex, but its poor solubility and its survival at high temperatures during pet food processing and powdered-milk production suggests that it is relatively stable. However, the complex is thought to dissociate at low pH.

Control Options

Since melamine at detectable levels is likely to have been added as an adulterant, its presence should not be acceptable under any circumstances.

Sourcing Raw Materials

Since melamine contamination appears at present to be associated mainly with food ingredients from China, food manufacturers should exercise caution when sourcing ingredients. Traceability to the point of origin is essential. Materials such as milk powder, dried egg products and high-protein ingredients should be purchased only from known low-risk sources.

Testing and Analysis

The only practical control for melamine in foods at present, other than careful sourcing, is testing and analysis of all ingredients that carry a risk of contamination.

A number of chemical methods have been developed, based on GC or HPLC. However, both the EC and the FDA recommend a GC-MS method for the analysis of melamine in foods. A number of commercial laboratories can analyse samples for clients using this method.

Recently, a method based on ELISA has been developed and is available commercially. This is suitable for screening ingredients and can be carried out by smaller laboratories.

Legislation

In the EU, melamine can be used as a component in plastics and has been assigned a specific migration limit of 30 mg per kg of food for materials in direct contact with foodstuffs.

Melamine is not a permitted additive or ingredient in food and therefore limits have not been set in food legislation before now. However, following the incident in China both the EC and the FDA have applied a maximum acceptable limit of 2.5 mg kg^{-1} for melamine in imported foods, particularly foods containing powdered milk from China, and 1 mg kg^{-1} in infant formula. In 2010, the Codex Alimentarius Commission adopted the same recommended limits.

The legislation position could change as more information becomes available and should be regularly reviewed.

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2.2.3.4 Perchlorate

Hazard Identification

What is Perchlorate?

Perchlorate is a chemical that occurs naturally and is also manufactured. The perchlorate anion consists of a chlorine atom surrounded by four oxygen atoms, and it is a very strong oxidising agent.

Perchlorate is very soluble in water, stable under most environmental conditions and very mobile in most media. Perchlorate has been recognised in the USA as an emerging contaminant, mainly associated with industrial activity and space exploration. Owing to this, there has been increasing interest in the levels of perchlorate in soil, groundwater, drinking water, irrigation water and food.

Occurrence in Foods

During 2004, the FDA conducted an initial survey investigating the perchlorate levels in a variety of products, including bottled water, milk, lettuce, tomatoes, carrots, spinach, and melons. Produce samples were collected particularly from regions known to have perchlorate-contaminated water supplies, such as Southern California and Arizona. Bottled water and milk samples were collected from throughout the entire USA.

A further study conducted in 2005, extended the scope of the investigation to include additional samples of previously examined produce, together with fruits, such as apples, grapes and oranges, and their juices, vegetables such as cucumbers, green beans and greens, and seafood. In addition, grain products such as wheat flour, cornmeal and rice were sampled. On this occasion, the samples were obtained from a broader range of locations.

The results from these studies indicated that perchlorate was present in almost all samples of milk tested, at levels from 1.91 to 11.3 ppb. Perchlorate was found in varying amounts in lettuce, tomatoes, carrots, spinach, and melon, and in oatmeal, wholewheat flour and a single sample of cornmeal. Although some samples were found to contain relatively high perchlorate levels, they were not deemed by the FDA to represent a risk to public health.

Hazard Characterisation

Effects on Health

Exposure to high doses of perchlorate has been found to interfere with iodine uptake into the thyroid gland. Perchlorate appears to remove an iodine ion from a protein that transports the iodine to the thyroid, leading to iodine deficiency. This, in turn, disrupts thyroid development and function, and may lead to a reduction in thyroid production. The thyroid plays an essential role in regulating metabolism. In the developing foetus and in infants, thyroid hormones are essential for normal growth and development of the nervous

system. Pregnant women and their unborn children are therefore at the greatest risk of iodine deficiency.

Although no studies have indicated that perchlorate-induced changes to thyroid function occur, even at doses as high as 0.5 mg per kg of bodyweight per day, a recent report has suggested that a significant association might be present between perchlorate exposure and reduced thyroid function in women with low urinary iodine levels.

A report to assess the health implications of perchlorate, published by the NAS in 2005, recommended an RfD for perchlorate of 0.7 µg per kg of bodyweight per day. Inhibition of iodine uptake, the precursor to hypothyroidism, was used to derive the reference dose, which has now also been adopted by the EPA. The RfD has been set so that it protects those most at risk from perchlorate; namely, the foetuses of pregnant women who might have hypothyroidism or iodine deficiency. The RfD is equivalent to a level of 24.5 ppb of perchlorate in drinking water, based on a daily consumption of 2 litres.

As yet, there is no established standard for perchlorate in bottled water.

Sources

Naturally occurring perchlorate is found in the soil, particularly in dry areas, in nitrate fertiliser deposits in Chile (Chile saltpetre), and in potash in the USA and Canada. Ammonium perchlorate is also manufactured in the USA, where it is used as an oxidising agent in missile and rocket fuel. The compound is also used in fireworks and airbag inflators. The highest levels of perchlorate contamination are found in water and soil near military installations and around the industrial plants where the chemical is manufactured.

Perchlorate is thought to enter plants when they are irrigated with perchlorate-containing water, or when they are cultivated in soil containing natural perchlorate or perchlorate-containing fertilisers or water.

Stability in Foods

Perchlorate is very soluble in water, stable under most environmental conditions and very mobile in most media. Because of its high water solubility and stability, it tends to accumulate in foods that have a high water content, such as cucumbers, melons and tomatoes, when they are grown in soils contaminated with perchlorate or irrigated with perchlorate-contaminated water.

Control Options

Control is currently centred on reducing contamination of soil and water with perchlorate. Biological remediation appears to have the most promise for dealing with contaminated sites. Some bacteria possess perchlorate reductase enzymes, which could possibly be used to treat contaminated water, although systems involving the use of these microorganisms have not yet been commercialised and are not currently used by USA water authorities. Commercial

anion exchange systems also offer promise for treating perchlorate-contaminated water.

Legislation

The FDA has not established a standard for perchlorate levels in bottled water, and current legislation does not require bottled water manufacturers to test for perchlorate.

Currently, there is no drinking water standard for perchlorate in the USA. However, the EPA has established an Interim Health Advisory Level for subchronic exposure. The levels are not legally enforceable Federal Standards and are subject to change when new information becomes available. The Interim Drinking Water Health Advisory Level of 15 $\mu g\,l^{-1}$ is based on recommendations of the National Research Council (NRC) of the National Academies as reported in "Health Implications of Perchlorate Ingestion" (NRC, 2005). The NRC recommended, and EPA adopted, an RfD of 0.7 μg per kg of bodyweight per day. This dose is based on the finding that ingestion of up to 0.0007 mg of perchlorate per kg of bodyweight can occur without adversely affecting the uptake of iodine into the thyroid. Details of the Interim Drinking Water Health Advisory for Perchlorate can be found at the following website: http://www.epa.gov/ogwdw/contaminants/unregulated/pdfs/healthadvisory_perchlorate_interim.pdf

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2.2.4 VETERINARY RESIDUES

2.2.4.1 Antibiotics

Hazard Identification

What are Antibiotics?

The term *antibiotics* is now used to describe a broad and diverse range of chemical compounds that destroy, or limit, the growth of microorganisms. Antibiotics may have activity against bacteria, fungi, or protozoa, though not viruses, and are used widely as veterinary drugs in food animals by the farming industry. There are many classes of compound with antibiotic properties, but some of the major groups in use are the β-lactams (including the penicillins), macrolides, ionophores, quinolones, lincosamides and tetracyclines.

Antibiotics may be administered to food animals for two reasons. They may be used, at relatively high doses, as therapeutic agents to treat clinical infections, or they may be administered at low, sub-therapeutic doses as 'growth promoters'. The use of antibiotic growth promoters in intensive livestock farming has been shown to be an effective means of increasing the growth rate of food animals and improving the quality of meat by raising the protein content. It is not entirely clear how this effect is achieved, but it seems likely that antibiotic growth promoters in animal feed suppress some of the bacteria in the gut and allow more of the energy in the feed to be diverted to the growth of the animal. A further benefit of antibiotic growth promoters is said to be improved control of disease caused by bacterial pathogens, including *Salmonella* and *Campylobacter*, in intensively reared livestock.

The use of antibiotics in food animals has both direct and indirect implications for food safety. Some antibiotics and their metabolites may be toxic to humans, or may cause serious reactions in sensitive individuals (*e.g.* penicillins). Therefore antibiotic and antibiotic metabolite residues in meat, milk and other animal products may be a direct risk to human health. However, many experts currently consider that the development of antibiotic resistance in pathogenic bacteria that can cause disease in animals and humans (zoonoses) is a much more serious potential threat to human health, and the use of antibiotic growth promoters is widely thought to have contributed to reported increases in the prevalence of antibiotic resistance. The farming industry is a significant consumer of antibiotics, and it has been estimated that as much as 60–80% of antibiotics produced in the USA are administered in feed to healthy livestock at non-therapeutic levels. Many of these antibiotics are closely related to compounds that are administered to humans in clinical settings, and include tetracyclines, macrolides, streptogramins, and fluoroquinolones.

Occurrence in Foods

Antibiotic residues are most likely to be found in foods of animal origin, such as meat, poultry, fish, eggs and honey. They are usually present as a result of the use of therapeutic veterinary drugs to control infection and disease in food animals. Antibiotics are frequently used to treat mastitis in cows, and therefore antibiotic residues may be present in milk. Antibiotic residues in milk can pose significant problems to the dairy industry, as many of the antibiotics used may inhibit the starter cultures used in cheese and yoghurt production.

The use of antibiotic growth promoters in animals is unlikely to give rise to detectable residues in meat and other animal products unless they have been administered at levels much higher than are permitted.

The use of veterinary drugs for therapeutic use is highly regulated within the EU and in the USA, and only certain drugs that have met stringent safety requirements are permitted (see Control Options). However, residues of antibiotics not authorised for food use may sometimes be found in certain foods. An example of this is the occasional detection of chloramphenicol residues in honey imported from China. Chloramphenicol is suspected of involvement in a form of anaemia in humans and is banned from food-animal use worldwide. Nitrofurans are also banned from food use in most of the world, but have been regularly detected in poultry and farmed crustaceans imported from East Asia and South America.

It is difficult to estimate current dietary intake of antibiotic residues from animal-derived products, but it is likely to be very low.

Hazard Characterisation

Effects on Health

The control of veterinary medicines in the EU and the USA is sufficiently strict that potentially toxic antibiotic residues are now very unlikely to be found in commercially produced animal products. Furthermore, most of the permitted antibiotics used are not considered to present a risk to human health at the levels likely to be found in meat, fish, milk, or eggs. However, there are still some concerns over the possible presence of penicillin and its derivatives. A number of individuals are sensitive to penicillins, and exhibit an immunopathogenic response that can be life-threatening. This makes it essential that maximum residue limits (MRLs) for this class of drugs are strictly adhered to. In addition, some hypersensitive individuals may develop a reaction to low levels of tetracyclines, also used in veterinary medicine.

Of much more concern is the possible role of antibiotic growth promoters in the development of antibiotic resistance in zoonotic bacterial pathogens. There is now considerable evidence that the use of medically important antibiotics as growth promoters in food animals may have contributed significantly to a reported rise in antibiotic resistance in several pathogenic bacterial species that cause zoonotic infections, notably *Salmonella enterica* serotypes, *Campylobacter jejuni*, *Escherichia coli* and enterococci. For example, *Salmonella*

Typhimurium definitive phage type (DT) 104 is a strain first isolated in the UK in 1988. At that time it already showed resistance to ampicillin, tetracycline and other antibiotics, but since 1988 it has spread all over the world and is often isolated from food animals. Many isolates are now resistant to other antibiotics, including fluoroquinolones, some of which have been used as growth promoters. Human infections caused by these bacteria now have very limited treatment options. The prevalence of fluoroquinolone-resistant *Campylobacter* in poultry is also increasing, especially in countries that permit the use of these antibiotics as growth promoters. The incidence of human infections caused by these pathogens is reported to be rising, especially in the USA.

The increasing prevalence of antibiotic resistance in zoonotic pathogens is now a global problem and many experts believe that the practice of using antibiotic growth promoters in food animals must be banned worldwide, as it is in the EU. There are fears that, unless action is taken, antibiotics will soon no longer be effective as a treatment for many bacterial infections in animals and humans.

Sources

It is now thought that all antibiotic residues found in food are present as the result of being administered to animals for therapeutic reasons, or as growth promoters. There is little or no evidence to support suggestions that some antibiotics, such as chloramphenicol, can be produced naturally by microbial action in the soil.

Stability in Foods

Many studies have been carried out investigating the effects of processing on the stability of antibiotic residues in food, with very variable results reflecting the wide range of chemical compounds concerned. For example, the penicillins and tetracyclines are known to be heat sensitive and may degrade during cooking or canning processes, although the degree of degradation is variable and depends often on the nature of the food containing the residues. In addition, the implications of this to food safety are uncertain, since the nature of the degradation products is unknown in most cases. It is possible that some degradation products may be more toxic than the antibiotic from which they are derived.

Control Options

Control of antibiotic residues in food is focused on the strict regulation of the veterinary medicines administered to food animals.

Primary Production

To safeguard human health, MRLs at the time of slaughter can be determined for veterinary medicines in order to set permissible limits for antibiotic residues

in foods. The limits depend on the toxicity of the drug in question. Establishing an MRL also requires the setting of a minimum withdrawal period. This is the time that passes between the last dose administered to the animal and the time when the level of residues in the tissues, milk or eggs are lower than, or equal to, the MRL. Neither the animal nor its products can be used for human consumption until the withdrawal period has elapsed. The withdrawal period is set out in the data sheet for the medicine and on the product packaging instructions. In the EU, only those drugs with established MRLs are permitted for use in food animals. MRLs are set with very large safety margins. For example, the calculation of the MRL value is based on the acceptable daily intake (ADI) for the drug in question. The calculation of the ADI includes an extremely large safety factor, and the MRL calculation assumes an average daily intake of 500 g of meat, 1.5 litres of milk, 2 eggs and 20 g of honey.

The use of sub-therapeutic doses of antibiotics as growth promoters was banned in the EU on 1 January 2006 (EC Regulation No.1831/2003). The addition of sub-therapeutic levels of antibiotics to animal feeds is currently still permitted in the USA and in other important meat-producing countries.

The effectiveness of these controls is closely monitored in the EU by the use of extensive surveillance programmes.

Alternatives to Antibiotic Growth Promoters

A number of alternatives to the use of antibiotics as growth promoters in food animals have been suggested. These include the addition of digestive enzymes to animal feed to help break down certain feed components, the addition of probiotic microbes to animal feed, and the introduction of more effective infection controls, such as improved biosecurity measures.

In Sweden, where the use of antibiotic growth promoters was banned as long ago as 1985, it has been demonstrated that antibiotics are not necessary to produce healthy food animals in modern farming systems if accommodation, husbandry practices and feed quality are of a sufficiently high standard. However, Swedish production costs are still higher than those of other countries.

Legislation

EU

Before a veterinary medicinal product intended for food-producing animals can be authorised in the EU, the safety of its pharmacologically active substances and their residues must be evaluated and included in Table 1 (Allowed Substances) of the Annex to Commission Regulation (EU) No. 37/2010, which can be found at: http://ec.europa.eu/health/files/eudralex/vol-5/reg_2010_37/reg_2010_37_en.pdf

Assessment of the safety of residues is carried out by the Committee for Medicinal Products for Veterinary Use (CVMP). Once the substances have been assessed, and following the adoption of a Commission Regulation confirming the classification of the substances, those substances that may be used

in veterinary medicinal products are listed in Table 1 of the Annex to Commission Regulation (EU) No. 37/2010.

Information on legislation regarding MRLs for antibiotic residues and residues of other medicinally acceptable veterinary drugs for food-producing animals can be found on the European Medicines Agency website at: http://www.ema.europa.eu/

EC Regulation No. 470/2009 of 6th May, 2009, lays down Community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin. The Regulation repealed EEC Council Regulation No. 2377/90.

Details of the Regulation can be found at: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:152:0011:0011:EN:PDF

All feed additives placed on the market in the EU must be approved under the auspices of EC Regulation No. 1831/2003, which is designed to ensure that all additives used are safe, not only for the animals consuming them, but also for those involved in their handling and ultimately, for the human consumer. The European Food Safety Authority (EFSA) is the body responsible for the scientific assessment of the feed additives. EC Regulation No. 1831/2003 can be found at: http://eur-lex.europa.eu/LexUriServ/site/en/oj/2003/l_268/l_26820031018en00290043.pdf

USA

Maximum tolerance levels for residues of animal drugs in food have also been laid down by the United States Food and Drug Administration (FDA). These levels can be accessed online at the following link: http://www.access.gpo.gov/nara/cfr/waisidx_02/21cfr556_02.html

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FDA Center for Veterinary Medicine. http://www.fda.gov/cvm/antimicrobial.html

Antibiotic growth promoters in food animals. http://www.fao.org/docrep/article/agrippa/555_en.htm

2.2.4.2 Hormones

Hazard Identification

What are Hormones?

Hormones are naturally produced chemicals that occur in the bodies of all animals, including humans. They can be proteins or steroids, and they act as chemical messengers. They are produced in specific hormone-producing organs or glands (the endocrine system) and circulate around the body until they reach the sites where they exert their effects. Although only produced in small amounts, they control essential body functions such as growth, development and reproduction.

Although normally produced naturally, hormones are sometimes used therapeutically. For example, insulin is a protein hormone that is administered to control Type 1 diabetes in humans. Certain hormones are also used as growth promoters to make young livestock develop and gain weight more rapidly and to increase yields. Hormone administration to cattle and sheep increases their growth rate and reduces the amount of feed needed before an animal is ready for slaughter. In dairy cattle, hormones can also be used to increase milk production. Thus, hormones are administered to animals mainly for economic purposes.

The use of hormones in food animals is controversial and there are concerns that the practice may have implications for human health.

Occurrence in Foods

Hormones are not permitted for use in meat- or milk-producing animals in the EU. However, they are permitted in the USA, where they can be used in cattle and sheep. There are currently six different kinds of hormones, all steroids, approved for use in food production in the USA. These hormones are estradiol, progesterone, testosterone, zeranol, trenbolone acetate and melengestrol acetate. Estradiol and progesterone are natural female sex hormones, testosterone is a natural male sex hormone and zeranol, trenbolone acetate and melengesterol acetate are synthetic hormone-like chemicals that make animals gain weight faster. These hormones are permitted for use in cattle and sheep, but not in poultry or pigs.

The use of recombinant bovine growth hormone (rbGH) is also permitted in the USA for use only in dairy cattle. RbGH, also known as recombinant bovine somatotropin, is a protein hormone used to increase milk production in dairy cows. This hormone is not permitted for use in the EU. As long as hormones are used as directed and correct treatment and withdrawal times are adhered to, the likelihood of unwanted hormone residues in meat and milk is low.

There are also reported to be significant levels of certain natural hormones in some plant-based foods. For example, potatoes and wheat have been reported to contain progesterone, and testosterone has been found at detectable levels in wheat and oils.

Hazard Characterisation

Effects on Health

The main concern over the use of steroid hormones for promoting growth in meat-producing animals is whether these hormones present any risk to human health. Lifetime exposure to oestrogen is associated with an increased risk of breast cancer, and excess exposure to anabolic steroids may result in a precocious puberty effect. Steroid hormones in food were suspected of causing early puberty in girls in some studies. However, exposure to higher than natural levels of steroid hormones through hormone-treated meat has not been documented. Studies have suggested that if correct treatment and slaughter practices are followed, the levels of these hormones may be slightly higher in treated animals, but still within the normal range of natural variation known to occur in untreated animals. Given the increased levels of other endocrine-disrupters in the environment, it is very difficult to attribute any increase in hormone-related cancers solely to hormone residues in meat.

With respect to milk from rbGH-treated dairy cows, scientists at the FDA Center for Veterinary Medicine have concluded that drinking milk with slightly higher levels of rbGH has no effect on human health, as the amount of rbGH present is insignificant compared with the amount of growth hormone produced naturally in the human body. Furthermore, because rbGH is a protein hormone, it is likely to be broken down during digestion.

There are, however, slight concerns over the effects of rbGH on the treated animal. The growth hormone acts by triggering cells to produce growth factors that cause an increase in growth rate and milk production. Milk from rbGH-treated cows has been found to contain slightly elevated levels of insulin-dependent growth factor-1 (IGF-1). Studies have indicated that higher levels of IGF-1 than normal are present in the blood of women with breast cancer, but it is unclear whether the higher levels are associated with increased breast cancer risk. Scientists at the FDA have concluded that IGF-1 in milk is unlikely to present any human food safety concern, particularly as it is a protein likely to be digested in the stomach.

There are also concerns that, because of increased milking, rbGH-treated cows may become more prone to mastitis, an infection of the udder. Growth hormone treatment has also been shown to cause increased lameness and injection site reactions in cattle. It has also been noted that there is a possible association between hormone use in large-scale beef cattle production and undesirable effects in wild fish species living in rivers exposed to waste water from these farms.

Sources

The source of natural hormones in meat may be endogenous production by the endocrine system of the animal itself, or administration as a growth promoter. Synthetic and recombinant hormones can only originate from the latter source.

Stability in Foods

Some steroid hormones, including trenbolone and melengestrol acetate have been shown to persist to some extent in animal dung, soil and water and so may cause environmental contamination. There are few documented reports on the stability of hormones in foods, but steroids are generally quite heat stable. For example, progesterone has been reported to survive heating at 56 °C for 30 min. It is therefore possible that steroid hormones might not be completely inactivated by typical meat-cooking processes or milk pasteurisation. It has been reported that pasteurisation destroys approximately 90% of residues of the protein hormone rbGH in bovine milk.

Control Options

Effective control of hormone residues in meat and milk depends on the careful administration of hormone preparations on the farm.

Primary Production

It is essential that hormones are used as directed, and that correct treatment and withdrawal times are adhered to. With these controls in place the likelihood of unwanted hormone residues in meat and milk is low. Steroid hormones are generally administered in the form of a pellet that is implanted beneath the skin of the ear. The ears of animals are then discarded at slaughter. Improper use of hormone-containing pellets, for example implantation into muscle tissue, results in higher levels of hormone residues in edible meat cuts. FDA regulations prohibit their use in this manner. Melengestrol acetate can also be added to animal feeds.

Recombinant growth hormone is administered as an injection beneath the skin of the animal. The hormone is available in single-dose packages to reduce the risk of accidental overdose.

Legislation

EU

The use of substances having a hormonal action for growth promotion in farm animals was prohibited in 1981 in the EU (Directive 81/602/EEC). This prohibition applies to Member States and imports from third countries. The legal instrument in force is Directive 96/22/EC as amended by Directive 2003/74/EC.

Recently, the EFSA was asked by the EC to perform a review of scientific data on potential risks to human health from hormone residues in bovine meat and meat products. In accordance with the request, the Panel on Contaminants in the Food Chain reviewed the scientific literature between 2002 and 2007 before drafting an opinion, which was published in July 2007 and concluded that there were no grounds to call for revision of previous risk assessments.

The full text of the opinion can be found at the following website: www.efsa.europa.eu/en/science/contam/contam_opinions/ej510_hormone.html

USA

The FDA permits the use of the following hormones and synthetic hormone-like growth promoters in food production in the USA: estradiol, progesterone, testosterone, melengestrol acetate, trenbolone acetate, and zeranol. These substances are permitted for use in cattle and sheep, but not in pigs or poultry. Maximum tolerance levels for hormone residues in food have been laid down by the FDA.

These can be accessed at the following website: http://www.access.gpo.gov/nara/cfr/waisidx_02/21cfr556_02.html

Meat from animals is regularly monitored for residues of synthetic hormones by the Food Safety Inspection Service (FSIS) of the United States Department of Agriculture (USDA). Meat is also monitored for the presence of the illegal synthetic oestrogen, diethylstilbestrol.

Estradiol, progesterone and testosterone are all sex hormones produced naturally by animals and no regulatory monitoring of these hormones is possible, as it is difficult to differentiate administered hormones from those produced naturally in the body of the animal. Therefore, for naturally occurring hormones, the permitted residue levels are quoted in terms of an amount above the concentrations of hormone naturally present in untreated animals.

Use of recombinant bovine growth hormone (bovine somatotropin) is permitted in the USA, but only in dairy cattle.

World Trade Organization Dispute

The use of hormones in meat-producing animals has been a major source of contention between the EU and the USA. The import of hormone-raised beef into the EU was first banned during the 1980s. The USA, and later Canada, took the case to the World Trade Organization (WTO) for settlement of the dispute. The WTO ruled that the USA and Canada could fine the EU for not abiding by world trade rules. Retaliatory trade restrictions and duties were then imposed by the USA and Canada on the EU. The EU responded by issuing a new Directive on 22 September 2003, based on a full scientific risk assessment conducted between 1999 and 2002. The new Directive supported the continuation of the ban (Directive 2003/74/EC).

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Human Safety of Hormone Implants used to Promote Growth in Cattle – A Review of the Scientific Literature. http://www.wisc.edu/fri/briefs/hormone.pdf

Section 3: Allergens

CHAPTER 3.1

Food Allergy

Hazard Identification

What is Food Allergy?

Food allergy can be defined as an adverse, immune-mediated reaction to food. Often, people will refer to any adverse reaction to food as an 'allergy'. However, it is important to remember that true food allergies involve the immune system and are almost invariably mediated through immunoglobulin E (IgE).

The majority of food allergies are caused by proteins, which sensitise and then elicit an allergic reaction in sensitive individuals. Food allergy needs to be differentiated from food intolerance, a condition that has no immune system involvement and includes reactions to certain food components, such as lactose, amines and histamine. Adverse reactions that lack an immunological mechanism are sometimes referred to as non-allergic food hypersensitivity reactions. Food intolerances can sometimes be controlled by limiting the amount of a particular food eaten, but with food allergies, much stricter avoidance of the food is necessary. Only food allergy, and not food intolerance, can lead to the potentially fatal reaction of anaphylaxis.

Gluten intolerance or coeliac disease is also not to be confused with gluten or wheat allergy, even though the symptoms may be similar. Although coeliac disease is an immune system response, it is not mediated through immunoglobulin E, as all other true food allergies are. Unlike wheat allergy, coeliac disease is mediated through immunoglobulin A (IgA) and immunoglobulin G (IgG), and sufferers will develop gliadin-specific IgA and IgG antibodies. Coeliac disease does not cause the potentially fatal anaphylaxis associated with true food allergies if gluten is eaten.

Allergy-like food poisoning has also been confused, in some cases, with food allergy. The reaction occurs as a result of ingestion of histamine from products such as spoiled tuna, mackerel, other fish and occasionally cheese. Histamine is

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Published by the Royal Society of Chemistry, www.rsc.org

one of the primary mediators of allergic reactions and is released from the cells of the body during a true allergic reaction. In the case of allergy-like food poisoning, the histamine is ingested and then elicits the allergy-like symptoms.

Mechanism of Allergenicity

Immunoglobulins, such as IgE, are produced by the body's immune system as a defence against invading microorganisms. Sometimes, the body also mounts an IgE response against certain agents, such as pollen, dust, house mites and food, and it is this response that gives rise to allergic reactions such as hay fever and food allergy.

There are two stages to the development of IgE-mediated allergies. The first is the sensitisation stage, in which an individual on first exposure to an antigen (usually a protein) will undergo a series of metabolic reactions resulting in the production of specific IgE (an antibody normally only produced in response to parasitic infections such as malaria).

The second stage involves elicitation of an allergic reaction. IgE becomes associated with specific receptors on the surface of special blood cells packed with inflammatory mediators, such as histamine. On the next exposure to the specific antigen, the cell-bound IgE reacts with the antigen, causing the cells to release the inflammatory mediators, which then trigger the symptoms associated with the allergic response, such as difficulty in breathing, gastro-intestinal upsets and skin itchiness, *etc*. These symptoms normally occur within a very short time following exposure to the antigen.

The majority of food allergens are proteins. Sensitisation can occur through ingestion of the allergen, or through inhalation of certain allergens such as birch or grass pollen. Owing to the similarities between certain allergens, cross-reactions can occur in some unfortunate individuals, who might find themselves allergic to more than one type of allergen. Cross reactions are particularly common between pollen or latex and some fruits and vegetables, giving rise to the syndrome known as pollen–fruit or latex–fruit syndrome.

Another sub-set of food allergies is known as ''exercise-induced allergy''. In this case, the allergic response occurs only when the specific food is eaten just before or after exercise.

Prevalence

The overall and worldwide prevalence of IgE-mediated food allergies is not precisely known. About 1–2% of adults and between 5 and 7% of children are believed to suffer from some type of food allergy, and it is believed that these numbers are increasing. The prevalence is higher amongst children, who often grow out of allergies, such as cows' milk or egg allergy. Prevalence also depends on country; for example, peanut allergy is particularly common in the USA, where peanut butter is a very widely consumed food. Mustard allergy is particularly common in France, and celery allergy is very common in Switzerland, Germany and France.

Currently, legislation in the EU requires that the following allergens must be declared on food labels: cereals containing gluten, crustaceans, milk, eggs, fish, peanuts, soya beans, tree nuts, celery, mustard, sesame seeds, lupin, molluscs and all their products, and sulfur dioxide. Legislation in the USA requires that the following eight types of allergen be declared: cows' milk, eggs, peanuts, tree nuts, wheat, soya, fish and shellfish. (For more details please see Chapter 3.4 on Allergen Legislation).

This section of the Food Safety Hazard Guide covers the 14 major food allergens currently designated by EU legislation, although it is clear that allergies can be caused by many more foods than these.

Allergen Nomenclature

An allergen is termed "major" if it is recognised by IgE from at least 50% of a cohort of allergic individuals, but does not carry any connotation of allergenic strength; otherwise, allergens are termed "minor". The allergen designation is based on the Latin name of the species it originates from, and is made up of the first three letters of the genus followed by the first letter of the species finishing with an Arabic number, *e.g.* Ara h 1 is an allergen from peanuts (*Arachis hypogea*), and Gly m 1 is an allergen from soya (Glycine max.).

Hazard Characterisation

Effects on Health

The main symptoms of IgE-mediated food allergy are given in Table 3.1.1.

Dose–Response

The amount of allergen required to elicit an allergic response varies tremendously between individuals and between allergens. In some cases, the dose required to elicit a response can be minute (measured in micrograms), and even kissing someone known to have eaten the allergen is sometimes enough to cause a reaction. Inhalation of vapours from cooking of the allergen can also cause life-threatening reactions for some individuals. For this reason, people with food allergies are generally advised to avoid the offending food completely.

Table 3.1.1 The main symptoms of IgE-mediated food allergy.

System affected	*Symptoms*
Gastrointestinal	Nausea, vomiting, abdominal cramping, diarrhoea.
Respiratory	Wheezing, asthma, rhinitis.
Cutaneous	Itching, urticaria (hives), eczema, atopic dermatitis, angioedema, rash.
Other	Hypertension, increased heart rate, tongue swelling, anaphylactic shock, oral allergy syndrome, laryngeal oedema.

Management of Food Allergy

Typically, the prevention of IgE-mediated food allergy involves avoidance of the offending food and strict observance of food labels. For management of specific food allergies, please refer to the relevant sections.

Sources of Further Information

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CHAPTER 3.2

Specific Allergens

3.2.1 CELERY

Hazard Identification

Celery (*Apeum graveolens*) grows wild in the EU, around the Mediterranean and in Asia, West of the Himalayas. It is also widely cultivated as a vegetable, which is consumed raw, cooked, or dried in spice mixtures. Celery is grown for its wide, fleshy stalks as well as its large, edible tuber, known as celeriac. Celery stalks are commonly used in soups, stews and in salads, and celeriac is used mainly as a cooked vegetable, but is becoming increasingly popular grated into raw salads. Celery is also grown for its seeds, which contain a valuable essential oil used in the flavouring, perfumery and pharmaceutical industries. Celery seeds are used as a flavouring, either whole or ground into a powder, which is mixed with salt to form celery salt. Celery salt is also sometimes made from celeriac.

Celery is one of the most common foods to cause oral allergy syndrome (OAS, where symptoms are confined to the mouth, causing tingling of the mouth and lips, facial swelling *etc*.) in adults in countries such as Switzerland, Germany and France.

Allergenicity

Allergy to celery root (celeriac) is more common than allergy to celery stalks. The principal allergen in celery is designated Api g 1, and it appears to be resistant to heat, so that its allergenicity is retained even after extensive thermal treatment. Cooking, therefore, does not reduce the allergenicity of celery or its products. Celery spice and raw celery are equally allergenic.

Allergy to celery is often associated with allergy to tree and grass pollen. Individuals who develop allergy to birch pollen tend to be allergic to the birch

The Food Safety Hazard Guidebook, 2nd Edition
Richard Lawley, Laurie Curtis and Judy Davis

Published by the Royal Society of Chemistry, www.rsc.org

pollen allergen, designated Bet v 1. Proteins related to Bet v 1 are found in other plants and in the edible tissues of a number of fruits and vegetables, including celery. When people who have a Bet v 1-type allergy eat certain fruits and vegetables, such as celery, they often experience a reaction confined to the mouth. Because allergy to celery is frequently associated with birch and/or mugwort pollinosis, the term birch–mugwort–celery syndrome has been established.

Allergy to other vegetables, such as carrots and bell peppers, is also associated with celery allergy, as is allergy to certain other members of the *Apiaceae* family, such as parsley, aniseed, cumin and coriander.

Prevalence

Allergy to celery is particularly common in EU countries, such as Germany and France. It is also the most common pollen-related food allergy in Switzerland, where about 40% of patients with food allergy are allergic to celery root, and severe anaphylactic reactions have been observed. In France, about 30% of severe allergic reactions to food were thought to be caused by celery.

There is evidence that birch pollen and celery allergy are highly related in the Central EU, while in the Southern EU, celery allergy is most frequently related to mugwort pollen.

Hazard Characterisation

Effects on Health

The most common symptom associated with celery allergy is OAS. During challenge testing with celery, 50% of patients developed local reactions in the mouth and 50% developed systemic reactions. Other symptoms include:

- Itchiness and redness of the skin and skin swelling.
- Stomach cramps and nausea.
- Wheeziness, asthma and tightness of the chest.
- Anaphylactic shock.

The symptoms associated with celery allergy are frequently more severe compared with allergic reactions associated with other fresh vegetables.

Dose–Response

The threshold dose needed to elicit an allergic reaction has not yet been established; however, in a study of patients undergoing oral challenge with celery, almost a half developed symptoms of allergy at a dose of 700 mg.

Management of Celery Allergy

Avoidance of celery, celeriac and all foods containing celery is the best way to manage the condition. The main difficulty arises in the extensive use of celery extracts in spices. The dried powder from celeriac is used as a flavouring

ingredient in numerous processed foods, such as soups, stews, salad dressings and spice mixtures. Care should be taken when reading food labels. Owing to its high allergenic potential, celery has now been included as one of the major allergens that have to be labelled in pre-packed foods sold in the EU. This is not currently the case in the USA.

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3.2.2 CEREALS

Hazard Identification

Cereals are a worldwide staple and provide 72% of the protein in the human diet. The majority of cereals belong to the grass family (*Gramineae*). Wheat is the predominant cereal grain and represents about a third of world cereal production, followed by rice and maize. Among cereals, wheat is the most frequent cause of allergy. As with other food allergies, it is the protein fractions that are responsible for causing the allergic reaction. The proteins found in wheat are similar to those found in related cereals such as rye, barley and spelt. Cereals that have been shown to cause type 1 hypersensitivity reactions are wheat, rice, maize, barley, oat, rye and buckwheat.

Allergenicity

Cereal allergy is an adverse reaction involving production of immunoglobulin E (IgE) antibodies in response to one or more of the protein fractions found in the cereal kernel. These include gliadin, glutenin (gluten), albumin, and globulin. The majority of allergic reactions to wheat are caused by the albumin and globulin fractions, although gliadin and gluten may also be responsible, though far less frequently. Allergic reactions to wheat are caused by ingestion of wheat-containing foods or by inhalation of flour containing wheat.

Individuals with wheat allergy will often also be allergic to related cereals, such as barley, rye and spelt, and possibly oats. Some wheat allergens are the same proteins as the allergens found in grass pollen.

Heating does not appear to reduce the allergenicity of wheat. In fact, it has been shown that the baking process actually increases the resistance of the allergens in wheat flour to proteolytic enzymes, allowing the allergenic proteins to reach the digestive tract un-degraded, where they can elicit an immunological response. Therefore, baked bread appears to be potentially more allergenic than raw flour.

Wheat allergy is not to be confused with coeliac disease, although the symptoms may be similar. Coeliac disease, also known as gluten enteropathy, was, until recently, known as gluten intolerance. It is a hereditary disorder of the immune system, during the course of which, eating gluten causes damage to the lining of the small intestine. This results in malabsorption of nutrients and vitamins. Unlike wheat allergy, coeliac disease is mediated through immunoglobulin A (IgA) and immunoglobulin G (IgG), and sufferers will develop gliadin-specific IgA and IgG antibodies. Coeliac disease does not cause the potentially fatal anaphylaxis associated with true food allergies if gluten is eaten.

Allergy to wheat and gluten-containing cereals can occur in any individual, but coeliac disease is hereditary.

Prevalence

Cereal allergy occurs in both children and adults, although young children are likely to outgrow it, while individuals who develop cereal allergy in later life are

more likely to retain it. It is far more common in children than in adults, but there are few data indicating exactly how prevalent cereal allergy actually is. It is probably less common than peanut, tree nut, shellfish, fish, milk, egg and soya allergies. A study of wheat allergy in Australia suggested that there was a prevalence of about 0.25% amongst young adults.

Among cereals, wheat is the most frequent cause of allergy. In a study conducted with 31 cereal-allergic children, it was found that 26 (84%) were allergic to wheat, five to oats, five to maize, four to barley, four to rye and one to rice.

In certain sub-groups, cereal allergy may be more common than in the general population. For example, in the baking industry, it is reported that wheat allergy is responsible for occupational allergy in up to 30% of individuals.

A specific type of allergy, known as wheat-dependent exercise-induced anaphylaxis, is linked to physical exercise after consumption of wheat. This type of allergy is more often reported in adults with no previous history of wheat allergy in childhood.

Hazard Characterisation

Effects on Health

Allergic reactions to wheat generally start within minutes and up to a few hours of eating wheat (or inhaling it). The most common symptoms are:

- Itching of the skin, hives, urticaria, eczema.
- Angioedema (swelling of the skin, lips and throat).
- Abdominal cramps, nausea, vomiting, diarrhoea.
- Asthma, wheezing, allergic rhinitis.

In severe cases:

- Blood pressure drop, collapse.
- Anaphylactic shock.
- Exercise-induced anaphylaxis.

Dose–Response

It is unclear how much wheat is needed to cause a reaction in sensitive individuals; however, a recently reported challenge protocol in Germany used doses of between 4 mg and 3.5 g of wheat flour, suggesting that only small quantities of wheat would be required to induce symptoms.

Management of Cereal Allergy

As with most food allergies, avoidance is the best way to treat allergy to wheat and other gluten-containing cereals. As wheat is such a widely used ingredient in common foods, avoidance can be difficult. Wheat is frequently present as an invisible ingredient. However, to comply with recent allergen legislation, it is

required that all pre-packed foods containing wheat and other gluten-containing cereals are labelled as such in both the EU and in the USA.

Wheat is used for making bread, biscuits, crackers, pastry, breakfast cereals, pasta and thickening agents. It is also used to make alcoholic beverages such as beer, lager and whisky. Ingredients to look out for and avoid include breadcrumbs, bran, cereal extracts, gluten, couscous, semolina wheat, wheat germ, wheat malt, gelatinised starch, modified starch, soya sauce and vegetable gums and starches.

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3.2.3 CRUSTACEANS

Hazard Identification

Shellfish can be divided into two broad groups, namely crustacean and molluscan shellfish. Crustacean shellfish include species such as crabs, prawns, shrimps, lobsters and crayfish, whilst the molluscs encompass species such as mussels, oysters, winkles, octopus and squid. Allergic reactions reported from consumption of crustacean shellfish tend to be far more frequent and more severe than those reported from consumption of molluscan shellfish.

Crustacean shellfish are widely consumed throughout the world. Little accurate consumption data is available, but intake varies considerably between different areas depending upon local customs and availability. In the EU, Iceland is believed to have the highest consumption of crustacean shellfish, followed by Portugal, Norway, Spain, Sweden, France, Italy and the UK.

Allergenicity

Crustacean shellfish allergy is relatively common, and is thought to be caused by a protein known as tropomyosin, which is very similar in the majority of crustaceans. Tropomyosin has been found to be a 'pan-allergen' with extensive sequence identity between crustaceans, which causes significant serological and clinical cross-reactivity between crustaceans such as shrimp, prawn, lobster, crab and crayfish. Thus, a person who is allergic to one type of crustacean shellfish is quite likely to be allergic to others. Tropomyosin is also found in certain insects, such as cockroaches, dust mites and chironomids (used as fish food), and people allergic to crustacean shellfish may also be allergic to these. Tropomyosin is a water-soluble heat-resistant protein and cooking does not destroy its allergenicity, although some allergens may leach out into the cooking water, making this allergenic too.

Allergy to molluscan shellfish is less common than allergy to crustacean shellfish, and people who are allergic to crustacean shellfish are not necessarily allergic to molluscan shellfish, although a small proportion may be. Serological and clinical cross-reactivity between molluscan and crustacean shellfish is most commonly seen between crustaceans, such as shrimp, lobster and crab and the mollusc squid, in which the major allergen, Tod p 1 is also a tropomyosin.

Prevalence

Allergy to crustacean shellfish is the third most common allergy after peanuts and tree nuts. It is thought that about 1% of the population may be affected, although the frequency varies tremendously throughout the world. Scandinavian countries, for example, appear to have higher rates of allergy to crustacean shellfish than other Northern EU countries. It has been estimated that approximately three-quarters of people allergic to one type of crustacean

shellfish are also allergic to others. Allergy to molluscan shellfish is less frequent than allergy to crustacean shellfish by a factor of about three.

Food allergy to crustacean shellfish has been reported in both children and adults, and, although little is known about the persistence of shellfish allergies, evidence suggests that they are not outgrown.

Hazard Characterisation

Effects on Health

As with most allergies, symptoms vary depending upon the sensitivity of the individual. Common symptoms include:

- Itching of the lips, mouth and throat.
- Swelling of the lips, tongue, throat and palate.
- Urticaria, itchy skin, and swelling beneath the skin.
- Nausea, vomiting and diarrhoea.
- Asthma, difficulty breathing, wheeziness, and sore and runny eyes.
- Anaphylaxis.

Shellfish are the third most common cause of anaphylaxis after peanuts and tree nuts.

Symptoms can occur after ingestion of shellfish, when shellfish are handled, or even by inhalation of steam from cooking shellfish. The route of exposure often determines whether food or respiratory allergy develops. In crustacean-processing facilities, respiratory allergy to crustacean shellfish can be a significant problem owing to inhaled allergen.

Shrimp has been implicated in cases of food-dependent exercise-induced anaphylaxis, and many cases of fatal food-induced anaphylaxis caused by crustacean shellfish ingestion have been reported in the literature.

Dose–Response

There is very little evidence in the literature relating to the minimum amount of shellfish needed to cause an allergic reaction, although it is likely to be very small, as inhalation of shellfish allergens in the steam from cooking water has been known to elicit an allergic reaction in some people. It is also likely that the dose needed to elicit an allergic reaction following crustacean shellfish ingestion will be very low. Allergic reactions to 14 g of shrimp or 32 mg of shrimp extract have been reported, suggesting that the dose of protein provoking the reactions was less than 32 mg.

Management of Crustacean Shellfish Allergy

Once a diagnosis of crustacean shellfish allergy has been confirmed, the only way to successfully manage the allergy is by complete avoidance of any form of crustacean shellfish, and of crustacean shellfish-derived ingredients. As one of

the recognised major allergens, crustacean shellfish should always be labelled on pre-packaged foods in the EU and the USA.

As crustacean shellfish is a relatively expensive ingredient, it is rarely undeclared on the label, or used as an unexpected ingredient. Stocks and soups may contain shellfish extract to enhance flavour, and surimi may contain shellfish extract. Foods to avoid include paella and many South East Asian dishes. People with crustacean shellfish allergy are also advised to avoid the food supplement, glucosamine, as this is made from the shells and exoskeletons of shellfish.

People with crustacean shellfish allergy need to be especially careful when eating out, as very sensitive individuals have been known to suffer anaphylactic shock from breathing in airborne particles of crustacean shellfish originating from cooking fumes. For the same reason, sensitive individuals should avoid open fish markets.

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3.2.4 HENS' EGGS

Hazard Identification

Hens' egg allergy is one of the most common immediate food allergies in children in the EU and the USA, but it also affects some adults too. It is caused by the proteins found in hens' eggs. Hens' eggs cannot be replaced by other eggs, such as those from ducks, turkeys, geese or quail as these are also known to cause allergic reactions in people who are sensitive. The correct name for the chicken is *Gallus gallus domesticus*, and therefore, the designated allergen names all start with the letters Gal.

Allergenicity

Eggs are made up of about 60% egg white and 35% egg yolk. The egg white appears to be slightly more allergenic than the egg yolk. Over 50% of the egg white is composed of the protein ovalbumin, the rest is made up from ovotransferrin, ovomucoid, ovomucin and lysozyme. Other minor proteins include ovoflavoprotein, ovodin, ovomacroglobulin and cystatin. The major egg white allergens are ovomucoid, with the designated allergen name of Gal d 1, and ovalbumin, designated allergen name Gal d 2.

The proteins found in egg yolk include lipovittelin, phosvitin, egg yolk specific lipoprotein and apovittelin I and IV. It has been proposed that egg allergy in children is caused by egg white proteins and in adults by livetins in the egg yolk.

Both of the major egg white allergens, ovomucoid and ovalbumin are resistant to denaturation and enzymic digestion, but cooked egg appears to be less allergenic than raw egg.

It is thought that sensitisation occurs through ingestion of egg proteins in the diet. Even minute amounts of egg protein in human milk are sufficient to sensitise an infant, with a reaction occurring when the child eats food that contains egg. Consumption of poultry meat rarely causes a reaction. However, inhalation of allergenic proteins, which sometimes occurs in people who keep birds as pets, can cause sensitisation.

Prevalence

Egg allergy is one of the most common allergies found in children, with a prevalence of about 2%. The majority outgrow their allergy before adulthood, leaving less than 1% of the adult population allergic to hens' eggs. Early sensitisation to hens' eggs however, may predispose some children to later development of asthma.

Hazard Characterisation

Effects on Health

In sensitised individuals, ingestion of egg or egg-white proteins will elicit an immediate response. The following symptoms have been observed:

- Itching of the mouth and pharynx.
- Eczema, pruritis and dermatitis, and urticaria.
- Nausea and vomiting.
- Rhinoconjunctivitis.
- In very rare cases, anaphylaxis.

Dose–Response

The minimum dose required to elicit an allergic reaction has been reported as 1 mg of liquid egg. The majority of those sensitive to egg allergy will respond to doses in the milligram to gram range. Reportedly, 5% will respond to doses below 5 mg, whereas about 50% will require doses of about 100 mg before symptoms are observed. As with most allergens, the threshold dose varies for each individual.

Management of Egg Allergy

Avoidance of eggs and all egg-derived products is the recommended way to treat this allergy. As the threshold dose varies so greatly between individuals, some may not need to avoid egg derivatives used as only very minor ingredients in foods, such as egg yolk lecithin.

All pre-packed products containing eggs or egg-derived ingredients must now be labelled as such in the EU and the USA. Egg-derived ingredients to look out for include albumin, ovalbumin, vitellin, globulin, ovomucoid, *etc*. Prepared foods commonly containing eggs or egg derivatives include cakes, desserts, pasta, biscuits, mayonnaise, sauces and chocolate. Some childhood vaccines are also prepared in egg yolks and parents of very sensitive children need to be aware of this.

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Published

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3.2.5 FISH

Hazard Identification

Finfish is one of the most common causes of food allergy. It is a real food allergy resulting in IgE-mediated symptoms, and is not to be confused with the toxic reactions that occur after histamine ingestion from spoiled fish (which will usually cause a reaction in everyone who has eaten the fish, see section 2.1.29).

The allergy is caused by ingestion of almost all fish because it involves a protein found in the muscle of the majority of fish species. Although not complete, the list of fish causing allergy includes cod, mackerel, herring, sardine, anchovy, bass, haddock, hake, plaice, sole, salmon, tuna, trout, Alaska pollock, eel, catfish, perch, and carp. Although finfish and shellfish allergies are not linked by a common allergen, individuals may be allergic to both types of seafood.

Allergenicity

The major fish allergen is parvalbumin, a protein that is conserved across all species of fish. As the parvalbumins are similar in all species, individuals allergic to one type of fish are likely to be allergic to all others. Parvalbumin is heat stable and therefore, cooking is unlikely to remove the allergenicity from fish. In addition, other proteins in fish, apart from parvalbumin, have been shown to be allergenic. The designated allergen name for parvalbumin from cod is Gad c 1 (from the Latin name for cod, *Gadus callarias*), and the designated allergen name for the allergen from salmon is Sal s1 (from the official name *Salmo salar*). A few people who are allergic to fish also react to frog, as frog muscle also contains the protein parvalbumin.

Allergy to cartilaginous fishes also exists, but it is possible that there may be differences between these allergies and allergy to bony fish. The cartilaginous fish include sharks, rays, dogfish and skate.

Prevalence

The prevalence of fish allergy varies, but it is generally thought to affect between 0.1 and 0.2% of the population. Both children and adults are affected, and fish allergy generally persists throughout the lifetime of an individual. Fish allergy is more prevalent in countries and parts of the world where fish constitutes a major part of the diet.

Hazard Characterisation

Effects on Health

As with most allergens the severity of the reactions varies depending upon the sensitivity of the subject and on how much of the allergen is consumed.

The first symptoms are generally itchiness and sensitivity of the mouth and throat, which can be followed by other reactions, such as:

- Nausea, vomiting, stomach pains and diarrhoea.
- Hives, itching, swelling and reddening of the skin.
- Eczema, asthma and hayfever, accompanied by runny and itchy eyes and nose.
- Swelling of the airways.
- Anaphylactic shock.

Dose–Response

Doses as low as 5 mg of cod have been reported to elicit an allergic reaction. Allergic reactions to fish have also been reported after inhalation of allergens in the steam from cooking fish, and after kissing someone who had previously consumed fish. Cross contamination from frying oil containing minute amounts of fish protein is also a problem. Manual handling of fish can also cause eczema or asthma in sensitive individuals.

Management of Fish Allergy

Once a diagnosis of fish allergy has been confirmed, the only way to successfully manage the allergy is by complete avoidance of fish in any form, and fish-derived ingredients. As one of the recognised major allergens, fish should always be labelled on pre-packaged foods in the EU and the USA.

The following foods may contain hidden fish: surimi, pâté, Worcestershire sauce, Caesar salad dressing, oyster sauce, tapenade, pizza toppings, kedgeree, caponata, bouillabaisse, gumbo, paella, fruits de mer, frito misto (mixed fried fish dish), fish sauce (Nuoc Mam and Nam Pla), gentleman's relish, sushi, and animal fat. Some fish or animal oils may also contain minute amounts of fish protein. Gelatine obtained from fish skin and bones and used in foods is not considered a problem for fish-allergic consumers.

Special care should be taken by people allergic to fish when they eat out in restaurants, as cross-contamination of foods can easily occur, for example, from the frying oil.

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3.2.6 LUPIN

Hazard Identification

Lupins are protein-rich legumes, closely related to the peanut. They belong to the genus *Lupinus*, which includes more than 450 species. The widely grown garden species are poisonous, but others, including blue, white and yellow lupins, are low-alkaloid varieties, which can be used to produce whole-seed flours. The consumption of lupin has been documented since ancient times and the seeds are commonly served as snacks in many EU countries. Recognition of the nutritional and food processing qualities of lupin has led to its increasing use as an ingredient in food formulations and particularly in bakery products. It is only relatively recently that the use of lupin in foods has been permitted in the UK, when lupin was recognised as a novel food.

The species most widely cultivated for food is *Lupinus alba* (white lupin) and it is products derived from this plant that are generally linked to allergic and anaphylactic reactions. Almost all cases of food allergy to lupin have been associated with consumption of lupin flour; however, dried lupini (lupin seeds), eaten whole, are a traditional snack in some Mediterranean countries and have also been reported to cause severe allergic reactions. Lupin flour is used in biscuits, pasta, sauces and in dietetic products produced as milk and soya substitutes.

Allergenicity

Most of the allergenic proteins of lupin are α- and β-conglutins, with a lesser presence of γ- and δ-conglutins. The major allergenic protein is believed to be β-conglutin, corresponding to the allergen Lup an 1, a protein with a similar sequence to a major allergen in peanut, Ara h 1. Cross-reactivity between lupin and peanut has been widely reported. However, the proteins responsible for this cross-reactivity have yet to be clearly identified and characterised, although a major cross-reactivity against peanuts has been observed in the γ-conglutin protein of lupin.

Lupin allergens, as with other legumes, are relatively resistant to thermal, chemical and proteolytic degradation. In one study investigating the effects of processing on the allergenicity of lupin, it was found that lupin allergens were resistant to boiling for 60 min, microwave heating for 30 min and extrusion cooking. However, a reduction in allergenicity was reported after autoclaving at 138 °C for 20 min, and an absence of IgE binding was observed after autoclaving for 30 min.

Prevalence

Lupin allergy is a relatively new allergy in the UK. It started to be recognised shortly after 1996, when the inclusion of lupin flour into wheat flour was permitted in the UK. In 1997, lupin was also permitted in France and the first

reports of anaphylaxis began shortly after this. By 2002, lupin had become the fourth most frequent cause of severe food-related anaphylaxis reported to the French Allergy Vigilance Network. Lupin allergy is far more common in France and other EU countries than it is in the UK, most probably as a result of the more widespread use of lupin flour in mainland EU countries. It is likely that the prevalence of lupin allergy in the UK will rise in line with the increasing use of lupin flour in food products. At the moment, it appears that the main at-risk population is peanut-allergic individuals, who represent about 0.7–1.5% of the EU population, as a result of potential cross-reactivity.

The majority of cases of lupin allergy have involved individuals who were allergic to peanuts. However, some cases of lupin allergy have been observed in non-peanut-allergic individuals. Clinical studies on lupin-allergic individuals appear to suggest that allergy to lupin is associated with multiple sensitisations and IgE cross-reactivity to other legumes, such as soya beans, peanuts, chickpeas and peas. Sensitisation to lupin *via* inhalation has also been reported in individuals with no immunological reactivity to other legumes.

Hazard Characterisation

Effects on Health

The symptoms of lupin allergy vary from very mild to severe. The most common mild symptoms include:

- Oral itching and urticaria.
- Angioedema of the skin.
- Allergic rhinitis and asthma.
- Abdominal discomfort.

Severe reactions, exhibited by those more sensitive include:

- Swelling of the airways and respiratory difficulties.
- Sudden drop in blood pressure.

The more severe reactions are classified as anaphylaxis and require immediate medical attention. A number of cases of lupin anaphylaxis have been reported.

Dose–Response

There is little information in the literature regarding the lowest doses of lupin known to cause an allergic reaction. One study found that doses of lupin flour ranging from 265 to 1000 mg were capable of eliciting allergic reactions in peanut-sensitive children. Another study reported a case of anaphylaxis and deteriorating lung function in a peanut-allergic teenage girl after oral challenge with a dose of 965 mg of crude lupin flour extract. This approximates to the amount of lupin present in 100 g of bread produced using 10% lupin flour.

Management of Lupin Allergy

As with other allergies, the best way to manage lupin allergy is by complete avoidance of products containing lupin seeds or flour. The products most likely to contain lupin allergens are lupini, bakery products, pasta and certain meat products, in which lupin is included for its emulsifying properties. Individuals with a known allergy to peanuts are likely to be most at risk. There is no indication that food-processing procedures reduce lupin allergenicity. There is a possibility that lupin allergy has been under-reported as, until recently, lupin was a hidden ingredient in various bakery and meat products.

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3.2.7 COWS' MILK

Hazard Identification

Hippocrates first observed and wrote about negative reactions to cows' milk around 370 BC, since when, the prevalence, awareness and understanding of this allergy has increased. Milk allergy is one of the major allergies in infants and is caused by the proteins present in cows' milk. Contrary to popular opinion, goats or sheep's milk cannot generally replace cows' milk for those who are sensitive. This is because of the similarity between the casein and whey proteins in cows' milk and those in the milk of goats and sheep.

Allergenicity

Most milk proteins are potential allergens and milk contains about 30–35 g protein per litre. The major allergens recognised in milk are casein, β-lactoglobulin (a protein that is absent from human milk), α-lactalbumin and α-lactoglobulin. Although it may be reduced, the allergenicity of milk cannot be removed by simple thermal processing. Low-heat treatment, like pasteurisation at 75 °C for 15 s, ensures the microbial safety of milk, but does not cause significant reduction in its allergenicity. Strong heat treatment (121 °C for 20 min) largely destroys the allergenicity of the whey proteins, but it only reduces the allergenicity of the caseins. Homogenisation has no effect on the allergenicity of milk proteins.

Casein appears to be the most potent allergen when it comes to skin tests, and β-lactoglobulin appears to be the most potent in oral challenges.

The blood proteins present in cows' milk are also present in meat (beef). These proteins are not the most important allergens of milk, but for around 10% of milk-allergic patients, allergy to milk goes together with allergy to beef. Some of these people may tolerate well-cooked beef.

Prevalence

There are no definitive data on the prevalence of allergy to milk. However, in Western countries, it is believed to affect about 2–3% of children under the age of two years. In general, children lose this sensitivity as they grow up, with 90% losing it by the age of three. In a very few cases, milk allergy may persist and occur in adults. It is interesting to note that the pattern of sensitisation to milk proteins is not the same now as it was in 1990. For example, the prevalence of sensitisation to casein has dramatically increased, possibly in line with the much wider use of casein as a food ingredient.

Hazard Characterisation

Effects on Health

Cows' milk allergy differs from most other allergies, such as allergy to nuts or crustaceans, in that the allergy generally develops before the age of three, and the majority of sufferers become tolerant to milk within a few years. Thus, the distribution of symptoms tends to be different from that of other allergies, with more cases of atopic dermatitis associated with milk allergy.

The majority of milk-allergic children demonstrate two or more types of symptoms in at least two different organs. Up to three quarters have skin symptoms, such as atopic dermatitis, eczema, and urticaria. Just over half have gastrointestinal symptoms such as vomiting, diarrhoea, constipation, and abdominal pain. About 20–30% have symptoms associated with breathing problems, such as hayfever-like symptoms from the nose and eyes, and recurrent wheezing.

Systemic symptoms, such as anaphylactic shock, may occur in up to 10% of subjects. In infants with cows' milk allergy, who are exclusively breast-fed, severe atopic eczema is the predominant symptom.

Symptoms can occur within a few minutes and up to an hour after milk exposure. These reactions are called immediate reactions. Reactions occurring after one hour are called delayed reactions. In some cases, symptoms even occur after a few days have passed. These late reactions are generally limited to atopic eczema and gastrointestinal disorders like constipation.

Dose–Response

The lowest dose of milk protein capable of eliciting an allergic reaction during challenge studies has been reported to be in the range of 0.6 mg to 180 mg. The minimum amount of milk reported to cause an allergic reaction is 0.02 ml cows' milk.

Management of Milk Allergy

Giving cows' milk formula as a first feed to babies with a family history of atopy may possibly lead to development of cows' milk allergy. Mothers in this situation should be advised accordingly.

Complete avoidance of cow's milk protein is the best way to manage the allergy. For babies and young infants, a hypoallergenic formula (*i.e.* one that has been extensively hydrolysed) is recommended if breast-feeding is not possible. Hydrolysis degrades the large allergenic milk proteins into smaller peptides that have lost their allergenicity. In rare cases, an amino acid-based formula may be required (amino acids are the building blocks of proteins and peptides). Partially hydrolysed formulas are not well tolerated, as large protein fragments may still be allergenic. In older children, soya milk or soy-milk formula may offer an alternative. However, it has been shown that about 25% of individuals allergic to cows' milk will also be allergic to soya milk.

The advice of a clinical dietician may help to ensure an adequate diet and to avoid 'hidden' cows' milk proteins in commercial foods.

Casein and caseinates are widely used as extenders in foods such as sausages, soups and stews. Both casein and whey are used in high-protein powdered drinks. Other ingredients to look out for that may indicate the presence of milk include, butter, butterfat, butter oil, ghee, cheese, yoghurt and ice cream. Foods that may contain 'hidden' milk proteins are so numerous it would be difficult to list them all, therefore, strict observance of food package labels is essential, as pre-packed foods containing cows' milk and its derivatives have to be labelled by law.

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3.2.8 MOLLUSCS

Hazard Identification

The molluscs are a large and diverse group that includes over 100 000 species living in the sea, in freshwater and on land. They range in size from less than one mm to almost 20 m (the giant octopus), and can weigh up to 900 kg (the giant squid). Molluscs of importance from a food perspective can be divided into three main groups:

1. Bivalve molluscs – mussels, oysters, clams, scallops.
2. Gastropod molluscs – winkles, whelks, periwinkles, limpets, snails.
3. Cephalopod molluscs – octopus, squid, cuttlefish.

Molluscs are frequently grouped together with crustaceans under the term shellfish; however, molluscs represent a completely separate phylum (*Mollusca*), whilst crustacean shellfish are classified under the phylum *Arthropoda*. Worldwide, the most popular mollusc food appears to be oysters with an annual consumption of more than 3 million tonnes. Squid is very popular in the EU, particularly in Spain, Portugal and the Mediterranean, and is the most frequently consumed seafood product in Japan. Consumption of mussels is very popular in France and Spain and along the coastal regions of Asia. Use of molluscs as an added ingredient in processed foods is fairly limited, although they can be found in soups, sauces and stocks, and in products such as surimi.

Molluscs have only relatively recently been included in the list of potential food allergens that must be declared on food labels in the EU.

Allergenicity

The principal allergen in many molluscs is the protein tropomyosin, which is also the major allergen in many crustaceans. Tropomyosin is, however, only a minor allergen in snails. Tropomyosin is stable to heat and is water soluble. Other non-tropomyosin allergenic proteins have been reported in molluscs, including oysters, abalone, limpets, scallops, squid, whelks, and snails.

Only a limited degree of cross-reactivity appears to exist amongst individuals allergic to molluscs, and individuals allergic to one type of mollusc may not necessarily react to other types. It has been found that mollusc tropomyosins do not always have IgE-binding epitopes in the same regions, supporting the suggestion that cross-reactivity between molluscs is more limited than cross-reactivity between crustaceans. In addition, cross-reactivity between molluscs and crustaceans is often restricted to a few species of the two groups. In one study, only 14% of individuals with shellfish allergy reported allergic reactions both to one or more crustaceans and one or more molluscs.

Mollusc allergens do not cross-react with fish allergens; however, it is possible that individuals reacting to fish infested with the parasite *Anisakis* might also react to molluscs.

Tropomyosin is a heat-resistant protein and so heat treatment and food processing will not reduce allergenicity in molluscs. Certain reports suggest that heating may in fact increase mollusc allergenicity, and one study indicated that the Maillard reaction might increase IgE-binding capacity after heating scallop tropomyosin.

Prevalence

Prevalence of allergy to molluscs varies throughout the world, most likely following regional patterns of consumption, so that it may be proportionately more important in regions of high consumption, such as Spain, France, Hong Kong and Singapore. Questionnaire-based studies of self-reported mollusc allergies indicate that the prevalence varies from about 0.15%, found amongst school children in France to about 0.4%, found in individuals in the USA.

Little information exists regarding the age of onset of mollusc allergy, although a number of reports indicate that many of the reactions occur in school-age children and young adults, with the youngest reported being a three-year-old boy allergic to grand keyhole limpet. The later onset of mollusc allergy compared with milk or egg allergy is most likely due to the later introduction of molluscs into the diet.

Hazard Characterisation

Effects on Health

Symptoms of mollusc allergy are largely similar to those of allergic reactions reported to other foods. Common symptoms include:

- Itching of the lips, mouth and throat.
- Swelling of the lips, tongue, throat and palate.
- Urticaria, itchy skin, and swelling beneath the skin.
- Nausea, vomiting and diarrhoea.
- Asthma, difficulty breathing, wheeziness, and sore and runny eyes.
- Anaphylaxis.

A number of reports of anaphylaxis and death have been documented in individuals allergic to molluscs.

Case reports suggest that asthmatic symptoms are very common in allergic reactions to snails when exhibited by individuals sensitive to dust mites. Certain molluscs, including cuttlefish, squid, abalone, oyster and snails have been implicated in cases of food-dependent exercise-induced anaphylaxis.

Dose–Response

Very little data exists on the dose of mollusc needed to elicit an allergic response. The fact that mollusc cooking vapours have been known to trigger allergic respiratory reactions suggests that the dose in airway exposure may be

very small. One report indicated that a dose of 100 g of canned oysters was able to cause exercise-induced anaphylaxis, whilst fatal anaphylaxis was reported following ingestion of three snails by one individual. One double-blind placebo-controlled food challenge study suggested that doses of dried snail in the low 100 mg range were sufficient to elicit allergic reactions.

Management of Mollusc Allergy

As with other allergies, the best strategy for managing mollusc allergy is avoidance. Because of the possibility of cross-reaction, it may be sensible to eliminate all molluscs from the diet unless a negative skin prick test indicates otherwise. Foods that might contain molluscs as hidden ingredients include fish soups and stocks, surimi, and Chinese dim sum soup.

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3.2.9 MUSTARD

Hazard Identification

There are various varieties of mustard belonging to the *Brassicaceae* family. Mustard powder is typically a mixture of *Sinapsis alba* (white mustard) and *Brassica juncea* (oriental mustard). The mustard seeds are ground to form a powder that is used as a condiment and as flavouring in numerous dishes. The whole seeds are often used in pickling solutions to add flavour, and mustard oil is occasionally used in cooking. Because of its use as a flavouring, mustard can often act as a masked allergen, giving rise to serious allergic reactions. France is the largest EU producer of mustard and also the biggest consumer, ahead of Germany and the UK. This explains the high prevalence of mustard allergy in France. In addition, the mustard varieties *Brassica nigra* and *Brassica juncea* are extensively cultivated in India.

Allergenicity

The major allergen of white mustard is designated Sin a 1, and that of oriental mustard Bra j 1. These allergens are heat stable and resistant to digestion by proteolytic enzymes, such as trypsin and proteases. Therefore, roasting mustard seeds has little effect on their allergenicity. Also, their resistance to proteolytic enzymes means that they have a high resistance to digestion in the stomach and will pass unchanged into the GI tract.

Numerous members of the *Brassicaceae* family are used as food plants, including cabbage, cauliflower, broccoli, watercress, horseradish and turnips. However, cross-reactions involving clinical symptoms between mustard and other *Brassicaceae* family members are rare. Cross-reactions with ragweed pollen have been reported.

Prevalence

As an emerging allergen, the prevalence of allergic reactions to mustard is on the rise. In the EU, it is particularly common in France, the largest producer and consumer of mustard, and most of the published research has been conducted by French researchers. Regional differences in prevalence have been reported. In the eastern part of France, a prevalence of 0.8 to 1% of food allergies is attributed to mustard; in the centre of France it is 3% and in the South of France, 8.9%. In Spain, 1.5% of food allergies are attributed to mustard.

India is another country where production and consumption of mustard is high. Prevalence of allergy to mustard is also very high in India. Because mustard is introduced into the diet at an early age, prevalence of mustard allergy is high in infants and children. There are no data indicating whether the allergy is outgrown.

Hazard Characterisation

Effects on Health

The initial clinical features are atopic dermatitis, urticaria and/or angioedema. Other typical symptoms include:

- Asthma and wheeziness.
- Abdominal pain and diarrhoea.
- Dizziness, low blood pressure, and anaphylactic shock.

Contact dermatitis has also been reported in workers involved in salad production, and contact urticaria for workers in food factories.

Many incidents of anaphylactic shock to mustard have been documented, indicating the seriousness of this allergy, but no deaths have been recorded.

Dose–Response

The dose of mustard required to elicit an allergic response is unclear. In studies, individuals have been shown to react to between 40 and 440 mg of a mustard condiment containing about 33% of seeds. Based on these findings, the smallest dose of mustard needed to elicit a response is approximately 14 mg.

Management of Mustard Allergy

As with all other food allergies, the best way to manage this allergy is by avoidance of all food products containing mustard. Because of its use as a seasoning and condiment, this is not always easy.

Foods to avoid include spicy sauces, curry sauces, mayonnaise, vinaigrette, crackers, flours, dried soups, and some baby foods. The whole seeds are used in pickling spices, so products such as baby gherkins and some pickled onions may be contaminated with mustard. Care should be taken when eating out in restaurants and at fast-food stands. Hot dogs are likely to be contaminated, as the individual preparing and serving the product will probably have handled mustard at some point.

All pre-packed food containing mustard must be labelled in the EU under the provisions of recent allergen legislation. This is not the case in the USA.

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3.2.10 PEANUTS

Hazard Identification

Peanuts are unrelated to tree nuts such as almonds and hazelnuts, and actually develop in a seed-pod below ground, which explains their alternative name—goundnuts. They are also sometimes called monkey nuts. Botanically, peanuts are a member of the legume family, which includes peas, soya beans and lentils.

Peanuts are one of the most common causes of food allergy and can cause severe reactions, including anaphylaxis. Very tiny amounts of peanut can cause a reaction in people who are sensitive. An adverse reaction to peanuts is a true food allergy response, involving an over-reaction of the immune system and production of IgE antibodies.

Allergenicity

Peanuts are harvested as shelled products containing the fruit surrounded by a skin and formed into two halves. Peanut proteins make up about 25% of the fruit, and it is these proteins that are responsible for peanut allergenicity. Peanut proteins are thought to contain numerous allergenic fractions, many of which remain unidentified and uncharacterised. Neither roasting nor other heat treatment of peanuts seems to reduce the allergenic response. In fact, roasting peanuts may actually increase their potential allergenicity. On the other hand, when peanuts are boiled in water, their allergenicity is reduced. This is because some of the allergenic proteins leach out into the cooking water.

Prevalence

Peanuts are a common cause of food allergy in the USA, where consumption of peanuts is very high. Peanut allergy is also becoming increasingly common in the UK in line with the increasing popularity of peanut products. Although exact numbers are unknown, some studies suggest that one person in 200 might be affected to some degree, although a recent study in children, carried out in 2002, indicated that as many as one in 70 children across the UK was allergic to peanuts. At one time, it was thought that peanut allergy was lifelong in all cases, but recently it has been shown that about 20% of young children outgrow their peanut allergy.

It is thought that the increased incidence of peanut allergy is the result of increased dietary exposure to peanuts at an earlier age than previously occurred. Susceptible infants can probably become sensitised through breast-feeding, *via* certain ointments used for skin lesions, or *via* the respiratory system following exposure to peanut allergen. Sensitisation may even occur *in utero*. Atopic individuals with asthma seem to be more at risk of developing food allergies.

Hazard Characterisation

Effects on Health

The symptoms of peanut allergy can vary tremendously, from very mild to severe. The most common mild symptoms include:

- Tingling in the mouth and lips and facial swelling.
- Nausea and colicky pain, accompanied by a feeling of tightness in the throat.
- Urticaria or nettle rash.

Severe reactions, exhibited by those more sensitive to peanuts include:

- Swelling of the airways and obstructed breathing.
- Sudden drop in blood pressure.
- Collapse and unconsciousness.

These symptoms result from the widespread release of pre-formed histamine and other inflammatory mediators from mast cells and basophil cells. The more severe reactions are classified as anaphylaxis and require immediate medical attention. The onset of anaphylactic reactions is generally extremely rapid and can proceed very quickly to unconsciousness.

A recent analysis was carried out of 32 fatal cases of food-related anaphylaxis reported to a national registry, established by the American Academy of Allergy, Asthma, and Immunology, with the assistance of the Food Allergy and Anaphylaxis Network. The 32 individuals could be divided into two groups. Group 1 had sufficient data to identify peanut as the responsible food in 14 (67%), and tree nuts in seven (33%) of the cases. In group 2 subjects, six (55%) of the fatalities were probably due to peanut, three (27%) to tree nuts, and the other two cases were probably due to milk and fish. The sexes were equally affected; most victims were adolescents or young adults, and all but one subject were known to have a food allergy before the fatal event. In those subjects for whom data were available, all but one was known to have asthma, and most of these individuals did not have epinephrine available at the time of their fatal reaction. In this series, peanuts and tree nuts accounted for more than 90% of the fatalities.

Dose–Response

The amount of peanuts required to elicit an allergic reaction has not been extensively studied, although sensitive individuals can react to minute amounts (100 μg to 50 mg). Some case studies report reactions to extremely low doses of peanut. For example, children have been reported to exhibit symptoms after contact with a table, reportedly wiped clean of all visible peanut butter; other cases have been documented as being caused by kissing someone who had previously eaten peanuts, or by sharing drinks. Symptoms were even reported

by a patient when a jar of peanut butter was opened in their presence. Even being close to someone eating peanuts can be sufficient to cause a reaction in some individuals.

Management of Peanut Allergy

Complete avoidance of peanuts and all peanut products is the best way to manage peanut allergy, although this may not be straightforward. The presence of 'hidden' peanut products in processed foods is always a risk for sensitised individuals. Food labels must always be read carefully as peanuts and their products may appear under different names, such as groundnuts, monkey nuts, earth nuts, mixed nuts, peanut butter, peanut oil, groundnut oil and arachis oil. Products such as cakes, biscuits, desserts, ice cream, cereal bars, satay sauces, breakfast cereals, ready meals (particularly Thai, Indonesian, Chinese and Indian meals), curry sauces, salad dressings, marzipan and praline and vegetarian products such as veggie burgers, *etc*. may all contain hidden peanut products.

Eating out in catering establishments and buying unwrapped foods also pose a risk, as no labelling laws exist to cover these situations. Care is needed in preparation and storage of food to ensure that no cross-contamination occurs.

It is probably wise for children who are allergic to peanuts to avoid other nuts, sesame seeds, nut mixes and possibly other legumes to prevent further sensitisation.

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3.2.11 SESAME

Hazard Identification

Sesame (*Sesamum indicum*) is an oilseed plant originating in India and cultivated in Africa, Asia, the Middle East, the Balkans, Latin America and the USA. It belongs to the *Pedaliaceae* family. Sesame seeds have an oil content of between 50 and 60%. In contrast to other vegetable oils, such as sunflower or groundnut oils, sesame seed oil for food use is always cold-pressed to preserve its delicate flavour. The production and consumption of sesame seeds have increased dramatically over the past few years, in line with the increasing prevalence of sesame seed allergy.

Sesame seeds are used whole or can be crushed to form a paste used as an ingredient in many foods. The oil is used for cooking and in salad dressings. Sesame seed oil is also often used in cosmetic and pharmaceutical products.

Allergenicity

The major allergens in sesame belong to the seed storage proteins and are very resistant to processing and proteolysis. At least four proteins in sesame are thought to be responsible for the allergenicity. These are, a 7S vicilin-type globulin, two seed storage proteins of sesame (Ses i 3, and Ses i 2) and a 2S albumin.

Homology between Ses i 3 and the peanut allergen Ara h 1 has been found. Allergy to poppy seed and/or sesame seed has also been reported to occur with simultaneous sensitisation to nuts and flour. Common allergenic structures have also been identified in sesame, poppy seed, hazelnut and rye. In patients with sesame allergy, associated allergy to almond, Brazil nut, walnut and pistachio has also been reported.

Sesame oil has reportedly been the cause of a number of incidents of anaphylactic shock. This is probably because, for culinary purposes, sesame oil is used unrefined, to retain its delicate flavour and aroma. Therefore, tiny traces of allergenic proteins are likely to remain in the oil.

Prevalence

Sesame allergy was almost unheard of twenty years ago, but today it is increasingly common. In Australia, the prevalence amongst children was reported to be 0.42%, and in the UK, a figure of 0.04% amongst adults has been suggested, although it is likely to be much higher. In fact, the first survey of the Allergy Vigilance Network, launched in 2000, indicated that 4% of life-threatening food allergies were caused by sesame seeds.

Sesame allergy is far more common in Japan and China, the main global producers of sesame, and where sesame seed is a common constituent of the diet. The prevalence is increasing dramatically in countries such as Australia and France, and particularly in Israel, where sesame seed pastes in the form of

tahini, hummus and halva are common snack foods. Sesame was found to be a major cause of IgE-mediated food allergy in Israel, and it is second only to cows' milk as a cause of anaphylaxis in that country. The increasing use of sesame in food products, including food preparations for infants, may also explain the increase in sesame allergy in extremely young children.

Sesame allergy is also a cause of occupational allergy in people involved in the production of speciality breads and pastries containing sesame. Many people with sesame allergy are also allergic to nuts.

The natural course of sesame allergy is unknown; however, it is reported that only 15% of infants diagnosed at the age of 10–12 months outgrew their allergy within two years. In adults, there are no examples of recovery from allergy to sesame.

Hazard Characterisation

Effects on Health

The predominant clinical features of sesame allergy in children are asthma and atopic dermatitis. About half of affected adults have been reported to experience anaphylactic shock, with loss of consciousness in some cases. In general, the principal symptoms are:

- Skin rash, urticaria, hives, itchiness, angioedema and skin swelling.
- Hay fever, asthma, coughing, wheeziness and tightness of the chest.
- OAS, nausea, vomiting, diarrhoea, stomach cramps.
- Dizziness, drowsiness, low blood pressure, collapse, anaphylaxis.

Symptoms generally occur within a few minutes to up to two hours after ingestion of sesame-containing products. The incidence of gastrointestinal symptoms with sesame allergy is low compared with other symptoms experienced.

Dose–Response

Doses of as little as 100 mg of sesame seeds or 3 ml of sesame seed oil have been reported to elicit an allergic response in sensitive individuals. In general, however, the threshold dose for most people is around 2–10 g of sesame seeds or sesame seed flour.

Management of Sesame Allergy

Complete avoidance of sesame seeds, flour and oil is the recommended course of action for anyone found to have an allergy to sesame. As the allergy appears to be particularly prevalent in individuals already known to be susceptible to allergies, such as those with eczema or other food allergies, it is recommended that sesame be excluded from the diet of infants with a history of atopic dermatitis or atopic family history. Because the incidence of sesame allergy has

increased so dramatically in infants and young children, it has also been suggested that sesame be added to the list of allergenic foods to be avoided in the first year of life.

Sesame can be present as a hidden ingredient, especially in margarines and salad dressings, where the label merely states "vegetable oil". However, the requirements of recent EU labelling legislation are that it is mandatory to include sesame on the label of pre-packed foods that contain it as an ingredient. In the USA, sesame is not yet among the list of allergenic ingredients that have to be labelled by law.

Common foods containing sesame include sesame-topped burger buns, tahini, halva, salad dressings, sauces, falafel, Turkish cakes, Chinese foods, breads, muesli bars, and mixed seed products. Sesame oil is also commonly used in cosmetics, such as lipsticks and moisturising creams.

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3.2.12 SOYA

Hazard Identification

Soya beans (*glycine max*.) are one of the most common causes of food-related allergic reactions. It is the protein fraction of soya that causes the reaction and, unfortunately, this protein fraction is found in many soya products, including soya flour, soya milk, soya meal, soya protein isolate, soya protein concentrate, tofu, miso, textured vegetable protein and many more. Soya derivatives are very commonly used as food ingredients in numerous processed foods. For example, soya products are widely used as texturisers, emulsifiers and protein fillers. Soya bean lecithin is also used as an emulsifier (E322).

Allergenicity

Soya bean allergy appears to occur in both infants and adults, but it is generally accepted that it is less severe and less frequent than peanut allergy. As with all the other food allergies, soya allergy does not appear on first exposure to the allergen, symptoms only occur upon re-exposure to soya. The first contact only sensitises the individual to soya. It is still unclear exactly which components of soya are responsible for allergenicity, but so far, at least fifteen different allergenic proteins have been found in soya. People who are allergic to soya are frequently also sensitive to tree pollen, such as birch.

Some fermented soya foods appear to be less allergenic than unfermented soya products, most likely because fermentation may cause the degradation of allergenic proteins.

The major known allergens in soya are the 7S seed storage globulin, the 11S seed storage globulins, the Bet v 1 homologue and an inactive papain-related thiol protease. Some of the designated allergen names of soya allergens, as given by the Allergen Nomenclature sub-committee of the International Union of Immunological Societies are Gly m 1 (hydrophobic soya bean protein), Gly m 2 (disease response protein), Gly m 3 (a profilin), Gly m 4 and Gly m Bd 30K. The Kunitz-trypsin inhibitor has also been recognised as an important allergen in people suffering with baker's asthma. However, this is a respiratory rather than a food allergen.

Processing of soya beans may alter their allergenicity. For example, the Bet v 1 allergen is found in textured soya protein but is absent from roasted soya beans and fermented soya products, such as soy sauce.

Prevalence

Epidemiological data on soya allergy are poor and the data relating to identity of soya bean allergens are inconsistent. Studies suggest that the prevalence of this allergy is between 0.3 and 1.0%, with a slightly higher prevalence in children than in adults. The higher prevalence in children is most likely the result of infant exposure to soya bean-based infant formula, or to pre-sensitisation in the

womb. Many infants outgrow soya allergy, so the prevalence is therefore lower in adults.

Hazard Characterisation

Effects on Health

The symptoms of soya allergy range from relatively mild symptoms to severe symptoms that require emergency treatment. Soya is considered one of the most important food allergies and it elicits a true food allergy response involving over-reaction of the immune system and production of IgE antibodies.

There are significant differences in the reported reactions to the molecular allergens of soya in different parts of the world. It appears that different allergens are involved in Japan compared with those in North America and the EU, although the basis for these differences remains unclear.

Symptoms range from mild, including OAS, nausea and vomiting, diarrhoea, urticaria and itchy skin, to severe reactions requiring treatment, such as a sudden drop in blood pressure, asthma, breathing difficulties and anaphylaxis.

There are numerous reports of incidents in which soya has been implicated in causing allergic reactions. For example, in Sweden, researchers examined cases that came to light after a young girl suffered an asthma attack and died after eating a hamburger that contained only 2.2% soya protein. The researchers evaluated 61 cases of severe reactions to food, of which five were fatal, and found that peanut, soya and tree nuts caused 45 of the 61 reactions. Of the five deaths that occurred, four were attributed to soya. The four children who died from soya had known allergies to peanuts but not to soya. The amount of soya eaten ranged from 1–10 g, which is typical of the levels found when soya protein is used as a meat extender in ready-made foods such as hamburgers, meatballs, spaghetti sauces, kebabs and sausages or as an extender in breads and pastries.

Dose–Response

There is very little information concerning the threshold dose of soya required to elicit an allergic response, but one report suggested it was in the region of 1 g of soya bean in dry matter, far higher than the threshold level reported for peanut. There have been a number of reports describing asthmatic symptoms suffered by workers handling soya flour, suggesting that powder inhalation can also elicit allergic reactions.

Management of Soya Allergy

The best way to manage soya allergy is by employing an exclusion diet and vigilant avoidance of foods that may contain soya ingredients. As soya is recognised as one of the major allergens, both in the EU and in the USA, any pre-packed food products containing soya should be labelled as such. Strict observance of all food labels is therefore recommended.

Foods that may contain soya include bakery products, breakfast cereals, ice cream, margarine, chocolate, pasta, processed meats, ready meals, vegetarian convenience foods, tofu, tempeh, miso, and soya protein concentrates and isolates. Food additives that may contain soya include hydrolysed vegetable protein, certain flavourings and lecithin (E322). Studies indicate that most individuals allergic to soya protein are able to consume refined soya oil safely, as virtually all traces of protein are removed during the refining process.

Eating out in catering establishments and buying unwrapped foods also pose a risk, as no labelling laws exist to cover these situations. Care is needed in preparation and storage of food to ensure that no cross-contamination occurs.

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InformAll Database. http://foodallergens.ifr.ac.uk/

Food Allergy Info (Institute of Food Research). www.foodallergens.info

Allergy UK. http://www.allergyuk.org/

3.2.13 SULFITE

Hazard Identification

Sulfites and sulfiting agents are compounds containing the sulfite ion (sulfur and oxygen), most often in combination with sodium (sodium sulfite) or potassium (potassium sulfite). Sulfites release the irritant gas sulfur dioxide, which acts as a preservative and bleaching agent. As well as occurring naturally in some foods and in the human body, sulfites are added to certain foods to act as a preservative, as they inhibit microbial growth, maintain food colour and increase shelf-life. Foods to which sulfites are commonly added include wines, beer, and dried fruit. They are also used to bleach food starches, such as potato starch, and are used in the production of some food packaging materials such as cellophane.

The levels of sulfites found in foods range from under 10 mg kg^{-1}, as in frozen doughs and corn syrups, to 60 mg kg^{-1} in fresh shrimps and mushrooms, and up to 100 mg kg^{-1} in dried potatoes and wine vinegar. The highest levels of sulfites (up to 1000 mg kg^{-1}) can be found in dried fruits, wine, molasses and lemon and lime juices.

Allergenicity

It is still unclear why sulfites elicit an allergic reaction in some people but not in others. Sulfur dioxide is an irritant gas and so reflex contraction of the airways has been proposed as one possible mechanism, as the majority of sulfite-allergic individuals exhibit asthma-like symptoms. IgE involvement has also been demonstrated in some subjects who exhibit a positive skin prick allergy reaction to sulfites, and a few subjects have a partial deficiency of the enzyme sulfite oxidase that helps to degrade sulfur dioxide. Sulfite allergy is unlike other food allergies, in that it is not triggered by a protein.

Prevalence

The true prevalence of sulfite allergy in the general population is unknown. Figures for the prevalence amongst asthmatic individuals vary. Prevalence of sulfite allergy in steroid-dependent asthmatic children is estimated to be between 20 and 66%, whilst prevalence in steroid-dependent asthmatic adults is lower, and estimated at between 3.9 and 4.5%. The United States Food and Drug Administration (FDA) has estimated that one out of a hundred people in the USA are sensitive to sulfites and that 5% of those with asthma are at risk of suffering allergic reactions to sulfites. The average age of individuals experiencing asthma after sulfite exposure is 40 years, and sensitivity is believed to be higher amongst women.

Hazard Characterisation

Effects on Health

The majority of sulfite-allergic individuals exhibit asthma-like reactions and bronchospasm with the following possible symptoms:

- Trouble breathing, speaking or swallowing.
- Wheezing.

A few will exhibit symptoms similar to anaphylaxis:

- Flushing, fast heartbeat and dizziness.
- Stomach upset and diarrhoea.
- Collapse.

In restaurants, the sudden choking sensation may sometimes be incorrectly attributed to aspiration of food.

Dose–Response

Among asthmatics, the amount of sulfite required to elicit an allergic reaction varies, and quantities as low as 1 to 5 mg of ingested potassium metabisulfite have been reported to provoke a reaction in sulfite-sensitive asthmatics. Threshold levels have not yet been systematically assessed and the smallest concentration of sulfites needed to provoke a reaction in a sensitive person is unknown.

Although there are no definitive data on dose–response effects, sensitive individuals have been known to react to the very small amounts of sulfite used as additives in products such as jam.

Management of Sulfite Allergy

As with all other food allergies, avoidance is the best way to manage sulfite allergy. Table 3.2.1 shows the additives that should be avoided by those with sulfite allergy in the EU.

It is therefore essential to read all food labels properly to ensure that the food is free of these additives.

Foods that might contain sulfites include beer, cider and wine, bottled lemon or lime juice concentrate, canned vegetables, condiments, deli meats, sausages, dressings, dried fruits, dried herbs, fish, fresh grapes, lettuce, fruit fillings, jams, fruit juices, glacée fruits, processed potatoes, soya products, starches, sugar syrups, sugar and vinegar. Sulfites are frequently used in restaurant foods as preservatives and an average restaurant meal may contain sulfites well in excess of 25 mg. Use of sulfiting agents in fruits and vegetables intended to be eaten raw has also been responsible for many cases of sulfite-induced bronchospasm. The use of sulfites in raw fruits and vegetables has been banned by the FDA.

Table 3.2.1 Additives to be avoided by those with sulfite allergy in the EU.

E number	*Name*
E220	Sulfur dioxide
E221	Sodium sulfite
E222	Sodium hydrogen sulfite
E223	Sodium metabisulfite
E224	Potassium metabisulfite
E226	Calcium sulfite
E227	Calcium hydrogen sulfite
E228	Potassium hydrogen sulfite
Other additives containing sulfites which are not used as preservatives, nor referred to as "sulfites"	
E150b	Caustic sulfite caramel
E150d	Sulfite ammonia caramel

Sulfites can also occur naturally in foods. For example, wine-making yeasts generate sulfur dioxide in wines and some strains produce over 100 ppm. Sulfites are also generated naturally in the human body by metabolism of sulfur-based amino acids.

Sources of Further Information

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3.2.14 TREE NUTS

Hazard Identification

Tree nuts are defined botanically as the edible kernels of the seeds of trees. Included in the category of tree nuts as potential allergens are almonds, Brazil nuts, cashew nuts, hazelnuts, pecans, pistachios, walnuts, Macadamia nuts and Queensland nuts.

Allergenicity

Tree nut allergies are common, potentially life-threatening, food allergies. The allergy frequently lasts throughout an individual's lifetime. Tree nuts may belong to different families that are unrelated to one another, and tree nuts are also not related to peanuts. Peanut allergic individuals can often eat tree nuts and those allergic to tree nuts can often tolerate peanuts. However, some allergic individuals may be allergic to both peanut and tree nuts. In addition, individuals can be allergic to some, but not all, tree nuts. Of all the common tree nuts, almond appears to cause the fewest cases of allergy.

An adverse reaction to tree nuts is a true food allergy, involving an over-reaction of the immune system and production of IgE antibodies. The major allergens in tree nuts include the 2S albumin, the 7S storage globulins, the 11S seed storage globulins, non-specific lipid-transfer proteins and the Bet v 1 homologue. Some of the designated allergen names of tree nut allergens, as given by the Allergen Nomenclature sub-committee of the International Union of Immunological Societies are: Brazil nuts – Ber e 1, Ber e 2; Walnuts – Jug r 1, Jug r 2, Jug r 3; Cashews – Ana o 1, Ana o 3; and Hazelnuts – Cor a 8, Cor a 11.

Prevalence

Food surveys suggest that tree nut allergy affects about 1% of the population. It appears to be more common in the USA than in some parts of the EU, such as Spain, although it is unclear why this should be so. Genetic or environmental factors may play a part. Tree nut allergy is not generally as common as peanut allergy, although in Germany, hazelnut allergy is more common than peanut allergy.

Hazard Characterisation

Effects on Health

Allergies to tree nuts tend to be of a more severe nature, causing life-threatening and occasionally fatal reactions. People with tree nut allergies also often suffer from reactions triggered by a number of different types of nuts, even though they do not come from closely related plant species. In general, these allergies are triggered by the major proteins found in nuts and seeds, many of which are heat resistant.

There is also a milder form of tree nut allergy (OAS), which is associated with birch pollen allergy. This condition is triggered by molecules found in tree nuts, which are very similar to pollen allergens like the major birch pollen allergen Bet v1. These molecules tend to be destroyed by cooking, which can therefore reduce the allergenicity of nuts for some consumers.

The symptoms of tree nut allergy can vary from mild to severe. The most common mild symptoms include:

- Tingling in the mouth and lips and facial swelling.
- Nausea and colicky pain, accompanied by a feeling of tightness in the throat.
- Urticaria or nettle rash.

Severe reactions, exhibited by those more sensitive to tree nuts include:

- Swelling of the airways and obstructed breathing.
- Sudden drop in blood pressure.
- Collapse and unconsciousness.

These symptoms result from the widespread release of pre-formed histamine and other inflammatory mediators from mast cells and basophil cells. The more severe reactions are classified as anaphylaxis and require immediate medical attention. The onset of anaphylactic reactions is generally extremely rapid and can proceed very quickly to unconsciousness.

Dose–Response

There is very little information concerning the dose required to elicit an allergic response to tree nuts. Sensitivity appears to be very variable and dependent on the particular individual.

Management of Tree Nut Allergy

Complete avoidance of all tree nuts and their products is probably the best way to manage this allergy. Despite the fact that allergy to one type of tree nut does not necessarily pre-suppose allergy to other types of tree nut, this may not necessarily be the case. Those allergic to tree nuts would be best advised to avoid other tree nuts, unless their tolerance has been clearly proven by reliable tests.

The types of product likely to contain tree nuts include chocolate, candies, cookies, desserts, sweets, almond paste, doughnuts, ice cream, cereals, ready meals, granola bars, trail mixes, pesto sauce, muesli, vegetarian ready meals and products, and care should be taken when checking the labels.

Eating out in catering establishments and buying unwrapped foods also pose a risk, as no labelling laws exist to cover these situations. Care is needed in preparation and storage of food to ensure that no cross-contamination occurs.

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The Calgary Allergy Network. http://www.calgaryallergy.ca/Articles/English/treenuthp.htm#chart

Food Allergy Info (Institute of Food Research). http://www.foodallergens.info

CHAPTER 3.3

Allergen Control Options

Manufacturing

It is the responsibility of food manufacturers to minimise the risks of their products to individuals with food allergies. The UK Institute of Food Science and Technology (IFST) advise that the following strategies should be adopted:

- Implementation of a HACCP plan to analyse the entire manufacturing process in relation to allergen hazards.
- In a multi-product company, wherever possible, segregate manufacturing operations involving the allergen-containing food into a separate building.
- When possible, formulate foods that are free of all unnecessary major allergens as ingredients.
- Organise raw materials supplies, storage and handling, production schedules and cleaning procedures to prevent cross-contamination of products with 'foreign' allergens.
- Ensure all personnel are fully trained to understand the necessary measures and the reasons for them.
- Comply with the relevant labelling legislation, ensuring that appropriate warnings are included on the product label warning the consumer of the presence of a major allergen.
- Have in place an appropriate recall system for any product found to contain a major allergen not indicated on the product label.

By following strict Good Manufacturing Practice, most problems can be avoided. Misformulation results from inattention or inadequate quality control. Cross-contamination stems from residues in shared equipment caused by inadequate cleaning, airborne dust, or even incorporation of rework without consideration of the allergen problem. Ideally, separate equipment should be used for products containing the specific allergen in question. For larger

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companies, designation of an allergen-only site is the most effective way to prevent any cross-contamination. If it is impossible to avoid sharing production equipment, then it is preferable to schedule the allergen-containing product at the end of the day, just before cleaning.

Allergen Control Plan

In order to develop an effective allergen control plan, every aspect of the manufacturing operation must be examined for the risk of allergens. The following is an example of a checklist providing the components of an allergen control strategy:

- Develop a list of all the raw materials used in your factory/production area, including all processing aids, additives, flavourings, *etc*. Specify which of them are allergens, or contain allergens. In the case of outside suppliers, ensure that they too have a documented allergen control plan in place. Specify that any purchased ingredients are free of undeclared allergens and that a letter guaranteeing this be supplied with each shipment.
- Compile a list of all finished products, and state which ones are produced using allergenic ingredients.
- Deal with allergen-containing incoming ingredients appropriately. Allergens should be transported in clearly marked containers and must be separated physically from non-allergenic ingredients. All incoming containers should be checked for possible damage or spillage. Allergenic ingredients should ideally be kept in an area separate from non-allergenic ingredients. The different areas should be well marked and colour coded if possible. Allergenic materials should always be stored below non-allergenic materials.
- Where bulk tanks are used, try to dedicate them to allergenic or non-allergenic materials only. Where this is impossible, ensure an appropriate and thorough sanitation programme is carried out between shipments.
- If possible, dedicate processing equipment, production lines and personnel to allergenic products, to prevent cross-contamination. Where this is not feasible, the alternatives are to segregate production to different days of the week, and if not possible, run non-allergenic products before allergen containing products; schedule long production runs of allergen-containing products to minimise changeover; and schedule cleaning to follow immediately after allergen-containing products have been run.
- In the case of rework, the ideal would be to advocate an 'exact into exact' approach, *i.e*. rework should only be used in the same product from which it was generated. Containers for rework should be clearly labelled, for example, by using colour-coded tags.
- Ensure that the correct packaging materials are used. Discard all obsolete packaging materials immediately. Packaging materials should ideally be stored in a designated area, and the accuracy of labels should be thoroughly checked.

- Cleaning and sanitation are of prime importance, particularly where equipment is shared. Wet cleaning is generally preferred as allergenic proteins tend to be soluble in hot water and detergents can help in removing proteins. Where wet cleaning is impossible, wipe downs are often needed and other approaches are available. Validation of sanitation practices on shared equipment is recommended. Various analytical kits are available, such as ELISA kits, and lateral flow devices (dipsticks), which can be used to validate sanitation practices.

Precautionary Labelling

Many manufacturers use precautionary labelling in cases where it is impossible to guarantee that the manufactured product is completely allergen-free. Precautionary statements such as ''may contain'' or ''may contain traces of'' are often used. However, these can often even further limit the allergic individual's choice of foods, with the result that some consumers choose to ignore precautionary labels putting their health at risk.

Sources of Further Information

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On the Web

Information Statement on Food Allergy – IFST. http://www.ifst.org/document.aspx?id=119

Guidance on allergen control and consumer information. Best practice guidance on controlling food allergens with particular reference to avoiding cross-contamination and using appropriate advisory labelling (*e.g.* ''may contain'' labelling) – Food Standards Agency. http://www.foodallergenslab.com/eng/legislation_uk.pdf

CHAPTER 3.4

Allergen Legislation

Pre-packed Foods

EU Legislation

In recent years the food-labelling regulations have been amended to help people suffering from allergies. The Labelling Directive (Directive 2000/13/EC) and its later amendments is the only piece of EU legislation that specifically refers to allergenic foods. This legislation came into force in November 2004, and was fully implemented on 25th November 2005. Since that date, all pre-packed food and drink has to comply with the new labelling rules.

The major difference between current and previous legislation is that the so-called ''25% rule'' has now been abolished. Manufacturers have to list product ingredients in descending order of weight, but there was a previous exclusion for ingredients if they were part of a compound ingredient that constituted less than 25% of the product. For example, if sliced salami were included in the topping of a pizza, and the salami made up less than 25% of the whole product, then there was no legal requirement to list the ingredients of the salami. This meant that consumers with food allergies would not necessarily have all the information they needed to make an informed choice as to whether the food was suitable for them.

The European Directive 2003/89/EC (European Commission, 2003), which amends Directive 2000/13/EC, came into force in November 2004. This legislation gives a list of allergenic food ingredients that now have to be indicated on the label when they, or their derivatives, are used in food sold pre-packed in the EU. The legislation includes all food ingredients, including carry-over additives, additives used as processing aids, solvents and media for additives and flavours. It also applies to alcoholic beverages.

In England, the equivalent legislation—The Food Labelling (Amendment) (England) (No. 2) Regulations 2004—came into force on 26th November, 2004.

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Similar Regulations apply to Scotland, Wales and Northern Ireland. The Regulations can be found at the following website: http://www.legislation.hmso.gov.uk/si/si2004/20042824.htm

The new rules require that for all allergenic ingredients, the source must be indicated. Thus, if vegetable oil contains peanut oil, then this has to be declared on the label. If the source of a natural flavour is allergen-based, *e.g.* from nuts, then this must also be declared, rather than "natural flavour".

There were originally 12 allergenic foods on the list of those that must be declared:

- Cereals containing gluten (*i.e.* wheat, rye, barley, oats, spelt or their hybridised strains) and products thereof.
- Crustaceans and products thereof.
- Fish and products thereof.
- Egg and products thereof.
- Peanuts and products thereof.
- Soya beans and products thereof.
- Milk and products thereof.
- Tree nuts—almonds, hazelnuts, walnuts, cashews, pecans, Brazil nuts, pistachio nuts, Macadamia nuts, Queensland nuts and products thereof.
- Celery and products thereof.
- Mustard and products thereof.
- Sesame seeds and products thereof.
- Sulfur dioxide and sulfites at concentrations of more than 10 mg kg^{-1} or 10 mg l^{-1}, expressed as sulfur dioxide

Following advice from the European Food Safety Authority (EFSA), this list of 12 potential food allergens was extended to 14 by Directive 2006/1423, which added lupins and molluscs and products obtained from them. Businesses were instructed to comply fully with the new labelling requirements from 23rd December 2008.

Whenever any of these 14 ingredients (or their products) is used in the production of foods, they must be labelled. At the moment, many other allergens, which are less common, have been omitted from the list. However, this may change, as other allergenic foods can be added to the list on the advice of the EFSA. Because different people have different tolerances to allergens, it is impossible to define an acceptable threshold limit, as is the case with setting acceptable levels for other chemicals in food.

In some cases, processing removes the allergenic risk from ingredients derived from some of the foods on the list. A list of products that were temporarily exempt from the labelling requirements of Directive 2003/89/EC was published in Commission Directive 2005/26/EC; this list has now been modified and the list of permanent exemptions was published in Directive 2007/68/EC in November 2007.

The list of permanently exempt derived ingredients is given in Table 3.4.1.

Table 3.4.1 Ingredients permanently exempt from Directive 2007/68/EC.

Ingredient	*Products thereof provisionally excluded*
Cereals containing gluten	• Wheat-based glucose syrups including dextrose • Wheat-based maltodextrins • Glucose syrups based on barley • Cereals used for making distillates or ethyl alcohol of agricultural origin for spirit drinks and other alcoholic beverages
Fish	• Fish gelatine used as a carrier for vitamin or carotenoid preparations • Fish gelatine or isinglass used as a fining agent in beer and wine
Soya bean	• Fully refined soya bean oil and fat • Natural mixed tocopherols (E306), natural D-α-tocopherol, natural D-α-tocopherol acetate, natural D-α-tocopherol succinate from soya bean sources • Phytosterols and phytosterol esters derived from vegetable oils from soya bean sources • Plant stanol esters produced from vegetable oil sterols from soya bean sources
Milk	• Whey used for making distillates or ethyl alcohol of agricultural origin for spirit drinks and other alcoholic beverages • Lactitol
Nuts	• Nuts used for making distillates or ethyl alcohol of agricultural origin for spirit drinks and other alcoholic beverages

The Directive and the UK Regulations do not specify the format in which allergen declarations must appear, other than that they have to be included somewhere in the list of ingredients. It has been suggested that allergen information on a label should be made more prominent, for example, by putting it in a box labelled ''Allergen Information''. Some manufacturers are currently doing this, but it is not yet required by law.

Advisory Labelling

Some manufacturers use phrases such as ''may contain nuts'' to indicate that small amounts of nuts might be present in a product, either in the ingredients or through accidental contamination from other processing lines. It is not a legal requirement to state that a food may contain small amounts of nut and there are concerns that the ''may contain'' labelling undermines valid allergen warnings on a food label. Attempts are being made to reduce the unnecessary use of ''may contain'' warnings and to provide clear advice to the public on why the terms are used and what they mean.

A detailed guidance document to the food allergen labelling legislation has been produced by the UK Food Standards Agency (FSA) and is available at the following website: http://www.food.gov.uk/multimedia/pdfs/allergenukguidance.pdf

USA Legislation

In the USA, regulation is by the Food Allergen Labelling and Consumer protection Act 2004, which can be found at the following website: http://www.cfsan.fda.gov/~dms/alrgact.html

The law in the USA requires that food manufacturers identify, in plain common language, the presence of any of eight major food allergens; namely, wheat, eggs, milk, fish, crustacean shellfish, peanuts, nuts and soya beans. The legislation states that the presence of the major food allergens in spices, flavourings, colourings and additives must be declared.

Non-pre-packed Foods

A wide number of establishments and organisations produce food for the general public that is not pre-packed. A number of food allergy accidents have been attributed to food sold in this way, for example from restaurants, bakeries and other food catering establishments. Many fatal allergic reactions have occurred when allergic consumers eat out. The FSA has provided some information and guidance for the catering industry, which can be found at the following website: http://www.food.gov.uk/safereating/allergyintol/

In the USA, guidance for caterers and retailers has been produced by the Hospitality Institute of Technology and Management, which can be found at the following website: http://www.hi-tm.com/Documents2005/allergens-retail-and-list.pdf

Sources of Further Information

On the Web

Allergy Labelling Guide for Small Businesses – UK Food Standards Agency. www.food.gov.uk/multimedia/pdfs/allergyleaflet.pdf

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Food Allergen Labelling and Consumer Protection Act of 2004 (Public Law 108–282, Title II) – United States Food and Drug Administration. http://www.fda.gov/Food/LabelingNutrition/FoodAllergensLabeling/GuidanceComplianceRegulatoryInformation/ucm106187.htm

Section 4: HACCP and Food Safety Management Systems

CHAPTER 4.1

HACCP and Food Safety Management Systems

Introduction

What is HACCP?

HACCP is an acronym for Hazard Analysis Critical Control Point, a science-based food safety management system that has become the preferred method of ensuring safe food all over the world. The HACCP approach to food safety is based on a detailed examination of every stage in the production process for an individual food product. The objective is to identify where and when hazards could occur and to design effective controls for each hazard. In other words, HACCP anticipates food safety hazards in a process and builds in safeguards to prevent them from occurring.

HACCP has its origins in the USA manned space flight programme of the 1960s and 1970s. It was vital that the food provided for astronauts was completely free from foodborne pathogens and other hazards, since any illness in flight would have serious consequences. NASA, in collaboration with the Pillsbury Company, therefore adapted analytical techniques used to anticipate failures in the engineering industry to develop the first HACCP system.

This early version of HACCP has since been further developed by the food industry and over the last 25 years it has been widely adopted by many food manufacturers. More recently, HACCP has increasingly become a basic requirement of complying with food safety regulations. In many countries legislation requires food businesses to employ some form of risk-based food safety management system to control hazards. In practice, this generally means a system built on HACCP principles, with the onus being on the food business to apply those principles correctly at every stage in the production and supply chain over which it has control.

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HACCP Basics

The HACCP system is based on, and defined by, seven underlying principles. However, these principles use some important terminology that needs to be defined first.

Definitions

Control measure – an action or an activity that can be used to prevent, eliminate, or reduce a food safety hazard to an acceptable level.

Corrective action – an action to be taken when loss of control at a CCP is indicated by monitoring.

Critical Control Point (CCP) – a step in the production process at which control can be applied and is essential to prevent, eliminate, or reduce a food safety hazard to an acceptable level.

Critical limit – a predetermined value for a control measure marking the division between acceptability and unacceptability.

Hazard – a biological, chemical, or physical agent in, or property of, food that has the potential to cause an adverse effect on consumer health.

Hazard analysis – the process of collecting and assessing information on the hazards and the conditions leading to their presence to determine which are significant for food safety and should therefore be addressed in the HACCP plan.

Monitoring – conducting a planned sequence of observations or measurements of control parameters to assess whether a CCP is under control.

Step – a raw material, location, procedure, operation or stage in the food production process from primary production to final consumption.

Validation – obtaining evidence that the elements of the HACCP plan are effective.

Verification – the application of supplementary information, including methods, tests and other evaluations, in addition to monitoring, to determine the effectiveness of the HACCP plan.

The Seven HACCP Principles

1. Conduct a hazard analysis (identify hazards and control measures).
2. Identify the critical control points (CCPs).
3. Establish the critical limit(s) for each CCP.
4. Establish a system to monitor control of the CCPs.
5. Establish the corrective action to be taken when monitoring indicates that a CCP is not under control.
6. Establish verification procedures to confirm that the HACCP system is working effectively.
7. Establish documentation concerning all procedures and records appropriate to these principles and their application.

The Application of HACCP

Although HACCP has been shown to be an effective means of managing food safety, there are a number of prerequisites for the successful application of the HACCP principles.

It is essential that any food business is already aware of appropriate food safety requirements and good hygiene practice before developing an HACCP plan. HACCP is not a substitute for basic hygiene and good manufacturing practice. Adequate staff training procedures should also be established in advance of applying HACCP principles.

A high degree of management commitment is necessary for the successful application of HACCP. A food safety management system that does not have the full backing of the managers implementing it is unlikely to be effective. Clear definition of individual responsibilities with regard to the HACCP system is also essential.

Finally it is essential that managers and staff have appropriate knowledge and skills to undertake a HACCP study and some training is likely to be required. Smaller businesses may lack the technical and scientific expertise needed to identify and evaluate hazards and controls. It may therefore be necessary to seek expert advice and support from trade associations, independent consultants, or enforcement officers. A great deal of information about HACCP and its application is freely available and some web-based resources are listed below.

Stages of the HACCP Process

1. *Assemble the HACCP team*

The development of an effective HACCP plan normally requires a multi-disciplinary team to ensure that appropriate product-specific expertise and knowledge is available. The team should comprise individuals familiar with all aspects of the production process, plus specialists with expertise in specific areas, such as engineering or microbiology. It may be necessary to use external sources of expertise in some cases.

The scope of the HACCP study should be determined by defining the extent of the production process being considered and identifying the classes of hazard being addressed (*e.g.* biological, chemical, and/or physical hazards).

2. *Describe the product*

It is important to have a complete understanding of the product, which should be described in detail. The description should include all relevant safety information and should cover factors such as composition, physical and chemical structure (including A_w, pH *etc.*), processing conditions (*e.g.*

heat-treatment, freezing, fermentation, curing, smoking *etc.*), packaging, shelf-life, storage and distribution conditions and use instructions. Where a range of similar products are being considered, these can be grouped together if their characteristics and processing steps are similar.

3. *Identify intended use*

The intended use should be based on the expected uses of the product by the end-user or consumer (*e.g.* is a cooking or reheating process required?). It is also important to identify the consumer target groups. Vulnerable groups, such as children or the elderly, may need to be considered specifically.

4. *Construct flow diagram*

The HACCP team should construct an accurate and detailed flow diagram of the manufacturing process being considered, covering every individual step and providing sufficient technical data for the study to progress. It should provide an accurate representation of each step from raw materials to end product and may include details of the factory and equipment layout, ingredient specifications, features of equipment design, time/temperature data, cleaning and hygiene procedures and storage conditions. The same flow diagram can be used where a range of similar products are being produced on the same line.

5. *On-site confirmation of the flow diagram*

The HACCP team should confirm that the flow diagram it has drawn up matches the process that is actually carried out in practice. The operation should be observed at each stage and any discrepancies between the diagram and normal practice should be recorded. The diagram should then be amended to take these discrepancies into account. The production process should also be observed outside normal working hours, such as during night shifts, as practice may vary between shifts. It is essential that the flow diagram is accurate, since the hazard analysis and identification of CCPs relies on the data it contains.

6. *List all potential hazards associated with each step, conduct a hazard analysis and identify control measures for each hazard*

The HACCP team should list all hazards that may reasonably be expected to occur at each step in the production process.

The team should then conduct a hazard analysis to identify those hazards that are of such a nature that their elimination, or reduction to acceptable levels is essential to the production of safe food.

Wherever possible, the hazard analysis should include consideration of:

- the likely occurrence of hazards and the severity of their adverse health effects;
- the qualitative and/or quantitative evaluation of the presence of the hazards;
- survival or multiplication of pathogenic microorganisms;
- production or persistence in foods of toxins, chemicals or physical agents;
- conditions leading to the above.

The HACCP team should then consider what control measures exist that can be applied to each hazard identified. Some hazards may require more than one control measure for adequate control and a single control measure may act to control more than one hazard.[†]

7. *Determine critical control points*

The determination of the CCPs is the key stage in a HACCP study, since the final HACCP plan will focus on the control and monitoring of the process at these points. It is vital that the HACCP team has sufficient technical data to determine the CCPs effectively and it is also important to be aware that more than one CCP may exist for a single hazard.

The determination of a CCP can be facilitated by the use of a decision tree (Figure 4.1.1) to provide a logical, structured approach to the decision making. However, the decision tree is for guidance only and its application should be flexible. Its use may not always be appropriate. Training in the effective use of the decision tree is recommended.

If a realistic hazard has been identified at a step where control is necessary for safety, and no control exists at that step, or any other, then the production process should be modified to include a control measure.

8. *Establish critical limits for each CCP*

Critical limits must be specified and validated for each CCP. More than one critical limit may be defined for a single step. For example, it is usually necessary to specify both time and temperature for a thermal process. Criteria used to set critical limits must be measurable and often include measurements of temperature, time, moisture level, pH, A_w, available chlorine, and sensory parameters, such as visual appearance and texture.

9. *Establish a monitoring system for each CCP*

Monitoring is the planned and scheduled measurement or observation of a CCP relative to its critical limits. The monitoring procedures must be able to

[†]It is important that no attempt is made to identify CCPs at this stage as this may disrupt the analysis.

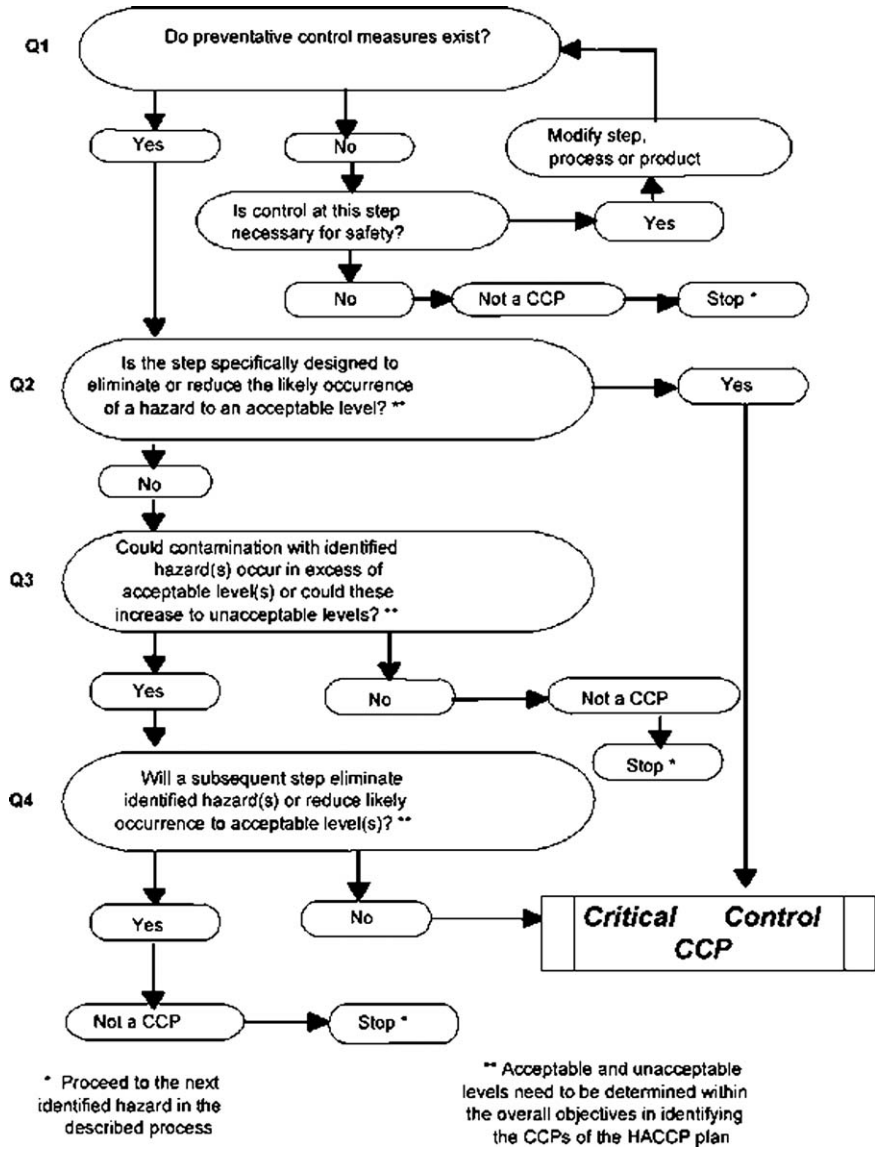

Figure 4.1.1 Example of a decision tree to identify CCPs (adapted from Codex Alimentarius Commission General Principles of Food Hygiene).

detect loss of control at the CCP and should provide this information in time to make appropriate adjustments so that control of the process is regained before the critical limits are violated. Where possible, process adjustments should be made when monitoring results indicate a trend towards a loss of control at a CCP.

Monitoring should either be continuous, or carried out sufficiently frequently to ensure control at the CCP. Monitoring procedures for CCPs must be rapid so that results are available quickly enough to maintain control at the CCP. Therefore, physical and chemical on-line measurements are usually preferred to lengthy microbiological testing.

The information derived from monitoring must be evaluated by a designated individual who has the knowledge, training and authority needed to act effectively on the basis of the data. The data must also be properly documented and recorded by that person.

10. *Establish corrective actions*

For each CCP in the HACCP plan, specific corrective actions must be developed that can be applied when the CCP is not under control. If monitoring indicates a deviation from the critical limits for a CCP, action must be taken that will bring it back under control. Actions taken should include proper isolation and disposition of any affected product and all corrective actions taken should be recorded and documented.

11. *Establish verification procedures*

Verification and auditing methods, procedures and tests, including product sampling and analysis, should be used frequently to determine whether the HACCP system is working correctly.

Responsibility for verification activities should be given to someone other than the individual responsible for monitoring and corrective actions. In some cases, this may mean that verification activities are performed by external experts.

Verification procedures should include detailed reviews of all aspects of the HACCP system and its records. The documentation should confirm that CCPs are under control and should also indicate the nature and extent of any deviations from the critical limits and the corrective actions taken in each case. Information such as customer complaints and returns may also be useful for verification.

12. *Establish documentation and record keeping*

Efficient and accurate record keeping is an essential element of the application of a HACCP system. All HACCP procedures should be documented. However, documentation and record keeping should be appropriate to the nature and size of the operation, but sufficient to ensure that the business is able to verify that controls are in place and are being properly maintained.

Examples of appropriate documentation include:

- Hazard analysis
- CCP determination
- Critical limit determination

Examples of appropriate recorded data are:

- CCP monitoring activities and results
- Deviations from critical limits and corrective actions taken
- Verification procedures performed
- Modifications to the HACCP plan

A record-keeping system should be clear and simple so that it can be easily maintained and communicated. It may be helpful to integrate HACCP records with other documentation. For example, product temperatures can be recorded on delivery invoices.

Review of the HACCP Plan

It is important to remember that a HACCP plan is a dynamic system and must be kept up-to-date at all times.

The plan must be reviewed following any changes to the production process, including changes to raw materials, processing conditions or equipment, packaging, cleaning procedures and any other factor that may have an effect on product safety. Even small modifications to the product or process can invalidate the HACCP plan and introduce potential hazards. The implications of any such changes to the overall HACCP system must be fully considered and documented and adjustments made to procedures as necessary.

Food Safety Management Standards and Codes of Practice

The widespread adoption of HACCP in the food industry has led to the development of a number of formal standards and less formal codes of practice (COPs) designed to facilitate the integration of HACCP principles and practice into the overall management of food safety. These include an international standard for food safety management systems (ISO 22000:2005), several retailer-led certification schemes and a raft of industry-specific guielines and COPs.

ISO 22000:2005

The International Organization for Standardization (ISO) has developed the ISO 22000:2005 Food Safety Management Systems Standard (*ISO 22000, Food safety management systems – Requirements for any organization in the food chain*). ISO 22000 sets out the requirements of a food safety management system covering all stages in the food chain from farm to fork, including food service and packaging manufacturers.

The aim of ISO 22000 is to provide a harmonised basic framework for food safety standards across national borders. It utilizes HACCP principles to outline methods for controlling food safety hazards and brings together many of the other key elements in an effective food safety management system.

ISO 22000 is the first in a 'family' of related standards, which includes the following:

- ISO/TS 22002-1:2009 – Prerequisite programmes on food safety. Part 1: Food manufacturing.
- ISO TS 22003:2007 – Food safety management systems for bodies providing audit and certification of food safety management systems.
- ISO TS 22004:2005 – Food safety management systems. Guidance on the application of ISO 22000:2005.
- ISO 22005:2007 – Traceability in the feed and food chain. General principles and basic requirements for system design and implementation.

Although ISO 22000 is now widely recognised as a valuable contribution to the harmonisation of food safety standards, it has so far proved to be best suited to larger businesses. Smaller food manufacturers may find the standard difficult to apply.

Certification Schemes

While all food businesses must comply with the requirements of legislation, the demands of customers, especially major retail chains and big manufacturers, have become increasingly important. These organisations often require their suppliers to comply with their own standards for food safety, which may be more stringent than those required by legislation. Some retailers have developed their own standards for food safety and audit their suppliers to ensure compliance. This has led sometimes led to suppliers undergoing multiple audits with variable requirements.

One solution to this problem has been the development of third-party food safety certification schemes. Many retailers require suppliers to gain certification under such a scheme and several are now widely accepted by big retailers in the EU and North America. These retail-led schemes publish standards, largely based on the retailers requirements. Suppliers agree to meet these requirements and are then audited against the standard by an accredited third party certification body to demonstrate compliance. The benefits of this approach should be consistent standards and less overall need for auditing.

Three of the most widely used retailer-led standards are:

- BRC (British Retail Consortium) Global Standard for Food Safety – originally drawn up by UK retailers, but increasingly used in the EU, North America and elsewhere.
- International Food Standard (IFS) – IFS is similar to the BRC Global Standard, but is specifically aimed at suppliers of French and German retailers.
- SQF (Safe Quality food) Program – founded in Australia, but now a USA-based certification program for suppliers of retailers and wholesalers in the USA and worldwide. It is administered by the SQF Institute (part of the Food Marketing Institute).

Although these standards have been widely taken up by food suppliers and their differences have become less marked, it has become increasingly clear that the global nature of the modern food industry calls for universal food safety standards that can be accepted worldwide.

This issue is being addressed by the Global Food Safety Initiative (GFSI), launched in 2000 as a collaboration between eight major retailers from the EU and the USA. It has since been joined by other retailers, food service businesses and manufacturers and is managed on a day-to-day basis by the Consumer Goods Forum. The main objective of the GFSI is to benchmark food safety certification schemes so that all recognised schemes have a "common foundation of requirements" and should give consistent results in audits.

Schemes currently recognised by the GFSI are:

Manufacturing schemes:

- BRC Global Standard Version 5
- Dutch HACCP (Option B)
- FSSC 22000
- Global Aquaculture Alliance BAP Issue 2 (GAA Seafood processing Standard)
- Global Red Meat Standard Version 3
- International Food Standard Version 5
- SQF 2000 Level 2
- Synergy 22000

Primary production (pre-farm gate) schemes:

- Canada GAP
- GlobalGAP IFA Scheme V3.0
  - General regulations
  - Fruit and Vegetables
  - Livestock base
  - Aquaculture
- SQF 1000 Level 2

Primary and manufacturing scheme:

- PrimusGFS

For food businesses supplying major retailers and big manufacturers, certification against a GFSI-recognised scheme has clear advantages. It is likely to help in gaining approved supplier status initially and may reduce auditing requirements significantly. However, no single scheme can be considered the preferred option at present and businesses seeking certification are advised to examine the characteristics and requirements of each before deciding which is most suitable for their particular operation. For example, the FSSC 22000 scheme, based on ISO 22000 and PAS 220, is likely to appeal to larger

businesses favoring sophisticated management-based food safety and hygiene control systems.

National schemes specifically aimed at small businesses have also been developed, such as the UK-based Safe and Local Supplier Approval (SALSA) scheme launched in 2007. SALSA has almost 2000 registered member companies so far and is recognised by some of the UK's major retailers.

Industry Guides and COPs

Many sector-specific food industry trade associations and professional bodies have taken a lead role in disseminating information and guidance for best practice in food safety management.

A good example of is the UK-based Chilled Food Association (CFA), which publishes regularly updated and detailed Best Practice Guidelines for the manufacture of Chilled Foods, along with guidance on other specific and topical issues.

FoodDrinkEurope (formerly the Confederation of the Food and Drink Industries of the EU) also publishes specific guidance on food safety issues for industry, notably an "Acrylamide Toolbox" containing practical measures designed to help manufacturers reduce formation of the processing contaminant in their products.

Food safety guidance produced by industry for industry is often more practical and flexible than that issued by regulatory authorities and certification schemes and will have been written by industry professionals with a clear understanding of the problems presented by implementation in a manufacturing environment and a detailed and up-to-date knowledge of the latest research and technological developments.

The Limitations of Food Safety Management Systems

While the widespread adoption of HACCP, the development of food safety standards and certification schemes and the proliferation of guidance from trade and professional bodies has certainly led to general improvements in food safety practice it has by no means eliminated serious contamination incidents and outbreaks of food-borne disease. Food businesses are well advised to guard against complacency, no matter how robust their food safety management system appears to be.

Several recent food-poisoning outbreaks have been attributed to negligence, or in some cases alleged deception, by suppliers regularly audited by third-party inspectors and in possession of food safety certification. Certification gives reasonable assurance of competence and good practice, but it makes sense for businesses to introduce occasional additional checks on their suppliers, such as in-house auditing or laboratory analysis, for more robust protection. On the other hand, outbreaks have also been traced back to manufacturers or suppliers who have conscientiously implemented best practice and acted very responsibly to manage food safety hazards. Unforeseen problems can still occur, even in the best managed establishments, and risk-free food manufacturing remains a distant goal.

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Food Safety Enhancement Program: HACCP Generic Models – Canadian Food Inspection Agency. http://www.inspection.gc.ca/english/fssa/polstrat/haccp/haccpe.shtml
Generic HACCP models from USDA/FSIS. http://haccpalliance.org/alliance/haccpmodels.html
Generic HACCP plans – The Seafood Network Information Center. http://seafood.ucdavis.edu/HACCP/Plans.htm
HACCP in Meat Plants – UK Food Standards Agency. http://www.food.gov.uk/foodindustry/meat/haccpmeatplants/

Standards and COPs

Information about ISO 22000:2005 standards. http://www.iso.org/iso/iso_catalogue/management_and_leadership_standards/specific_applications/specific-applications_food-safety.htm
The BRC Global Standard for Food Safety. http://www.brcglobalstandards.com/standards/food/
The Global Food Safety Initiative. http://www.mygfsi.com/
The Chilled Food Association. http://www.chilledfood.org/
FoodDrinkEurope. http://www.fooddrinkeurope.eu/

Section 5: Food Safety Legislation

CHAPTER 5.1

Food Safety Legislation

Introduction

The history of much modern food safety legislation can be traced back to Victorian England, when widespread adulteration of food was a serious problem. This was not only fraudulent, but was often dangerous. For example, toxic salts of lead and mercury were sometimes used to provide additional colour in sugar confectionery intended for children. The urgent need to curb these practices lead to the introduction of the Food Adulteration Act in 1860. Since then, food law has evolved steadily into the sophisticated framework of legislation that now exists to protect consumers in most parts of the world.

Food safety legislation is a very complex subject, and a detailed examination of the law as it relates to food safety hazards is beyond the scope of this book. Furthermore, the body of food safety legislation is constantly being added to and amended, so that any written work on the subject is almost certain to be out of date by the time it is published.[‡]

What follows, therefore, is a concise overview of food safety legislation in the EU and in the USA, with a brief mention of some of the international aspects of food law. It is intended to be neither detailed, nor exhaustive. The intention is to give an overall impression of the approach to food safety regulation and enforcement taken by the authorities in two of the worlds' most highly developed and complex food markets.

EU Legislation

Much of the food safety legislation now in force in the countries of the EU originates from the European Commission (EC), rather than from national

[‡] Readers are strongly advised to consult a reputable specialist legal adviser if they require more detailed information, or have specific questions on food safety legislation.

The Food Safety Hazard Guidebook, 2nd Edition
Richard Lawley, Laurie Curtis and Judy Davis

Published by the Royal Society of Chemistry, www.rsc.org

authorities. There are two main legal instruments by which the Commission can introduce new food legislation. The first of these is the Directive, which sets out an objective, but allows national authorities to determine how that objective is to be achieved, and cannot be enforced in individual Member States until implemented into national legislation. The second instrument is the Regulation, which is 'directly applicable' and becomes law in all Member States as soon as it comes into force, without the need to change national legislation. Both Directives and Regulations may be described as 'horizontal', dealing with one aspect of food, such as hygiene, across all commodities, or 'vertical', applying to particular foods.

Although the EC initiates new Directives and Regulations, an established path of consultation, amendment and review must be followed before proposed legislation can be formally adopted by the European Parliament and by the Council of Ministers. Finally, the new legislation is published in the Official Journal of the EU and then comes into force. This process can take years, especially if there are contentious issues involved. The development of new food safety and hygiene measures is now informed by the scientific analysis and evaluation of food safety hazards. It is usual for the EC to submit a request for a risk analysis to be undertaken by the European Food Safety Authority (EFSA), before legislative proposals are drawn up.

Until comparatively recently, food safety in the EU was largely regulated by a complicated system of horizontal and vertical food hygiene Directives that had evolved over many years. This system inevitably included some anomalies and duplication, and was not implemented uniformly in all Member States. The situation became increasingly unsatisfactory, particularly in view of the planned accession of a number of new member countries. Consequently, the EC carried out a comprehensive review of the EU food hygiene legislation in the late 1990s. The result was the introduction of the ''Food Hygiene Package'' of EU legislation, which came into force on 1st January 2006.

The Food Hygiene Package

The Package consists of three main Regulations, which applied immediately throughout the EU. These are:

- EC Regulation No. 852/2004 on the hygiene of foodstuffs;
- EC Regulation No. 853/2004 setting out specific hygiene requirements for foods of animal origin;
- EC Regulation No. 854/2004 setting out specific requirements for organising official controls on products of animal origin intended for human consumption.

Regulation No. 852/2004 contains general hygiene requirements for all food businesses and covers a wide range of topics, including the general obligations of businesses in regard to food hygiene, the requirements for hazard analysis critical control point (HACCP) based food safety management procedures,

hygiene requirements for premises and equipment, staff training and personal hygiene, heat processes and packaging. Regulation No. 853/2004 supplements 852/2004 by adding specific hygiene requirements for meat, milk, fish and egg production, as well as for by-products, such as gelatine. Regulation No. 854/2004 deals only with the organisation of the official controls needed for animal products in the human food chain.

The approach of the new Regulations is described as ''farm to fork'', in that it applies to all stages in the food supply chain, including farmers and growers involved in primary production—a sector not covered by previous food hygiene legislation. All food businesses must also register with the 'competent authority', so that they can be clearly identified. The inclusion of HACCP in the Regulations is another key development, clearly signifying that this is now the preferred method of ensuring food safety.

The development of guidance documents on the new legislation in individual Member States has been encouraged, and a number of these have been produced by the EC and at national level, by authorities such as the UK Food Standards Agency, and by industry bodies and trade associations.

Other EU Legislation

While the 2006 Food Hygiene Regulations provide the current backbone of food safety legislation in the EU, they do not by any means include all of the food safety requirements that food businesses need to be aware of. For example, a large number of new 'implementing regulations' have also been introduced to deal with specific topics and amendments to the Hygiene Regulations.

The Microbiological Criteria Regulation

One of the most important implementing regulations for all food businesses is EC Regulation No. 2073/2005 on microbiological criteria for foodstuffs, often referred to as the MCR, which came into force on 1st January 2006. This Regulation brought together microbiological criteria for specific foods that had previously been scattered across a number of vertical directives and presented them in a common format.

The MCR includes some of the criteria from previous legislation in unchanged form, but others have been removed and some new criteria have been introduced. The primary purpose of the criteria set out in the Regulation is the validation and verification of HACCP procedures, rather than as stand-alone food safety controls. It is important for all food businesses to be aware of the requirements of this Regulation.

Food Contaminants Regulations

On 1st March 2007, three new EU regulations came into force, dealing with a range of chemical contaminants in foods. The most important of these from a food industry point of view is EC Regulation No. 1881/2006, which replaces

No. 466/2001 and sets maximum permitted levels for certain contaminants in foodstuffs. This Regulation covers a number of contaminants, including mycotoxins, heavy metals, chloropropanols, PAH, dioxins and PCBs.

Some of the maximum permitted levels for contaminants set out in EC Regulation No. 1881/2006 have since been modified by 'amending' Acts. For example, EU Regulation No. 165/2010 sets revised maximum levels for aflatoxins in certain food commodities. Food businesses should always ensure that they are aware of any changes made to food safety regulations by subsequent amending Acts.

USA Legislation

The system of food safety legislation in the USA is quite different in structure from that of the EU. Despite this, the main objective of protecting the consumer from exposure to unsafe and unwholesome food products is much the same. The system is based on flexible and science-based federal and state laws and the basic responsibility of industry to produce safe foods. A risk-based, precautionary approach is built in to the legislative system.

Federal Legislation

The basic foundation of USA food safety legislation is determined by Congress in the form of authorising statutes, which are designed to achieve specific food safety objectives and to establish the level of public protection. These are generally broad in scope, but also define the limits of regulation. Important statutes include the following:

- Federal Food, Drug and Cosmetic Act
- Federal Meat Inspection Act
- Poultry Products Inspection Act
- Egg Products Inspection Act
- Food Quality Protection Act
- Public Health Service Act

Implementation of these statutes is the responsibility of a number of executive agencies, and is accomplished by the development and enforcement of regulations. The main federal regulatory organisations concerned with food safety are the Food and Drug Administration (FDA) and the US Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS). However, other agencies, including the Department of Health and Human Services (DHHS) and the Environmental Protection Agency (EPA), also play important regulatory roles.

Responsibility for food safety is divided largely between the FSIS and the FDA according to food sector. The FSIS is responsible for the safety of all meat, poultry and egg products, while the FDA assumes responsibility for all

other foods. In addition, the EPA has a key role in protecting consumers from risks posed by pesticides in food.

Food safety regulations are developed using a risk-analysis approach in a transparent process that encourages the participation of industry and consumers. All significant comments must be addressed in the final regulation. Once this has been published in the *Federal Register*, it can be enforced. Examples of regulations developed in this way include the HACCP regulations and the introduction of performance standards for pathogen reduction and control. All current regulations are listed in the *Code of Federal Regulations*.

In January 2011, the FDA Food Safety Modernization Act (FSMA) was signed into law by President Obama. This Act is designed to strengthen the USA food safety system and is widely regarded as the most significant change to USA food safety law for many years. It is intended to shift the focus of FDA effort towards prevention and away from reacting to contamination incidents after they occur. The FSMA provides the FDA with new responsibilities and powers of enforcement and sets out a timetable for implementation, which is to be completed within two years of enactment. Five key authorities and mandates for the FDA are set out in the Act as follows:

- Preventive controls—the FDA has a mandate to require prevention-based controls throughout the food supply. Food businesses must put in place a food safety management system and will be held accountable for preventing contamination.
- Inspection and compliance—inspection should be carried out according to risk and will be used as a means to hold industry accountable for safe food production.
- Imported food safety—the FDA will have greater powers to exercise control over food products entering the country.
- Response—mandatory recall authority for all food products is introduced for the first time.
- Enhanced partnerships—this places an emphasis on collaboration between Federal, state and local food safety agencies and on improved training and resources for food safety officials.

State Legislation

In addition to the federal system of food safety legislation, there is an additional layer of regulation at the state level. States have their own legislative assemblies that are able to pass state laws and these may then be implemented as regulations by the local authorities for health and/or agriculture. Generally, state regulations should follow national food safety policy, but there may be differences in the detail, and some states, such as California, have passed state food safety laws. Many states also have their own microbiological standards or guidelines for foods.

International Aspects of Food Safety Legislation

Although most countries have developed food legislation structures on a national, or regional basis, there has also been a degree of international cooperation. This has been achieved mainly through the activities of the Codex Alimentarius Commission, a body set up in 1963 by the World Health Organization and the Food and Agriculture Organization with the aim of promoting the coordination of food standards work carried out by national authorities and other bodies.

Since its inception, Codex has developed and agreed a series of food standards, codes of practice, guidelines and other recommendations intended to protect consumer health and ensure fair trade practices. Codex standards cover a range of topics, including maximum residue limits for pesticides, food contaminants and toxins. Codes of practice include food hygiene principles, HACCP and control of veterinary drug use. Codex has also published 'principles' covering microbiological criteria and risk assessment.

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Information for FDA-regulated industry. http://www.fda.gov/oc/industry/default.htm

Regulations & Policies page – USDA Food Safety and Inspection Service. http://www.fsis.usda.gov/Regulations_&_Policies/index.asp

Other Useful Websites for Legislation

Codex Alimentarius Commission. http://www.codexalimentarius.org/

Foodlaw Reading – Reading University site on EU and international food law maintained by Dr D.J. Jukes. http://www.rdg.ac.uk/foodlaw/

Food Standards Code pages (includes food safety standards for Australia) – Food Standards Australia New Zealand. http://www.foodstandards.gov.au/thecode/

Japanese Food Safety Commission (pages in English). http://www.fsc.go.jp/english/

Section 6: Sources of Further Information

CHAPTER 6.1

Sources of Further Information

Today's food safety professional can access an enormous amount of information on most of the topics covered in this book from a variety of sources. More information is freely available than ever before, but this availability brings its own problems. Identifying reliable and authoritative sources of technical and scientific information can be difficult and time consuming, especially when looking for material online. The following pages are intended to guide readers to some of the most reputable information resources available to them, predominantly on the internet.

Traditional Publications

The number of published scientific journals and reference books containing information relevant to food safety is constantly increasing as the body of scientific knowledge supporting food safety practice grows.

Journals

Scientific journals provide an excellent means of keeping abreast of the latest food safety research and discovery. However, with a few exceptions (see below), most require a fairly substantial subscription for full access. Nevertheless, most journals now have dedicated web pages on the internet that allow the visitor to browse the contents of each issue, and often to view abstracts and purchase individual articles online. Traditional library facilities also allow readers to search for specific articles and papers and obtain copies at a reasonable cost. Some scientific publishers are now beginning to adopt an 'open access' approach to their journals through the internet (see below), and this may mean that current papers are more freely accessible to individuals in the future.

The Food Safety Hazard Guidebook, 2nd Edition
Richard Lawley, Laurie Curtis and Judy Davis

Published by the Royal Society of Chemistry, www.rsc.org

Reference Books

A very large number of reference books and textbooks relating to food safety have been published in recent years. Many of these are excellent sources of detailed information on specific food safety issues and the best examples are regularly updated with revised editions. Most food manufacturers will have neither the time nor the budget to assemble their own libraries of specialist food safety books, but it is worth seeking out some of the broader, more practical titles. Unfortunately, many reference texts have been written from an academic perspective and may be somewhat inaccessible for the non-specialist reader, but food safety books written from a practical viewpoint are also available. Useful reference titles can be found through library catalogues, or the websites of publishers and online booksellers. It is often possible to find reviews of books prior to purchase.

The Internet

The internet has developed in recent years into a very valuable and accessible information resource for the food safety professional. Most of the organisations concerned with food safety now have their own websites, as do many scientific and professional bodies, scientific publishers and commercial organisations. The majority of these websites contain information, or links to information, that will be of value to food businesses.[§]

List of Useful Websites

The internet is a great source of food safety information, but it is also an ever-changing resource. Websites come and go and their addresses change. The following is a compendium of some of the most useful food safety information websites and online resources. The web addresses, and descriptions, were correct at the time of writing, but are subject to change.

Libraries

The British Library (fully searchable catalogue). http://www.bl.uk/
The Library of Congress. http://www.loc.gov/index.html
New York State Library. http://www.nysl.nysed.gov/

Scientific Search Engines

In recent years a number of internet search engines specifically designed to identify scientific papers and publications have been developed. Some are freely

[§]It is important to be aware of the following warning: any individual can post information on the internet, or set up a website. It is therefore critical to ensure that any information used professionally within a commercial food business is obtained from a reputable and authoritative source, and is referenced accordingly. Ideally, the reader should cross check information between at least two reliable sources wherever possible.

accessible, but are not specific to food science and technology. These search engines include:

Google Scholar
Google Scholar is a broad search engine that finds articles from peer-reviewed papers, theses, books, abstracts and articles, from academic publishers, professional societies, preprint repositories, universities and other scholarly organisations. http://scholar.google.co.uk/

Scirus
Scirus examines only science-based web pages for articles containing the search terms. It classifies results into ''journal results'', ''preferred web results'' and ''other web results'' enabling the user to view the search results by source preference. http://www.scirus.com/

Journals

The following is a list of useful journals that provide some free access to papers relevant to food safety.

Applied and Environmental Microbiology
Published by the American Society for Microbiology. For the food technologist the journal is a useful source of research on food and industrial microbiology. Papers are freely available four months after publication for the primary research journals, and one year after publication for the review journals. http://aem.asm.org/

Comprehensive Reviews in Food Science and Food Safety
A journal produced by the Institute of Food Technologists through Wiley–Blackwell publishing. Includes papers on risk management, food microbiology and food safety. All articles are freely available online. http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1541-4337

Emerging Infectious Diseases
A publication produced by the United States Centers for Disease Control and Prevention (CDC). It provides information on emerging and re-emerging infectious diseases worldwide including food-borne microbial pathogens. Access to all papers is free. http://www.cdc.gov/eid/

Eurosurveillance
An online journal published by the European Center for Disease, Prevention and Control. It is devoted to the epidemiology, surveillance, prevention and control of communicable diseases; including foodborne disease. It is published in three formats (weekly, monthly and quarterly) all of which are freely accessible. http://www.eurosurveillance.org/

Internet Journal of Food Safety
An online journal operated by foodhacccp.com. This journal publishes papers with a food safety focus. Access is free to all papers. http://internetjfs.org/

Journal of Infectious Diseases
Produced by Oxford Journals. This journal specialises in papers on the pathogenesis, diagnosis, and treatment of infectious diseases. There are some useful microbiology papers and access is free for papers over 12 months old. http://jid.oxfordjournals.org/

Morbidity & Mortality Weekly Report
A publication produced by the CDC. It provides epidemiological and statistical data on a range of health care issues. Access to all papers is free. http://www.cdc.gov/mmwr/

Government Agencies

Government agencies dealing with food safety issues usually have websites that not only inform on current food safety issues but also have good archives of reports, and other documents discussing food safety issues. Some useful sites are listed below:

European Center for Disease Control (ECDC)
The ECDC is an EU agency set up to work with health authorities in EU Member States to help develop policies on risks posed by current and emerging disease. The agency provides information on health issues including microbiological hazards associated with food. http://www.ecdc.europa.eu/

European Commission Food Safety Site
The Food Safety site of the European Commission (EC) provides information on food safety and on European food safety legislation. This site includes the Rapid Alert System for Food and Feed (RASFF), a database of food or feed products associated with 'risks', and the countries involved. http://ec.europa.eu/food/index_en.htm

European Food Safety Authority (EFSA)
The EFSA is an independent agency funded by the EU. It supplies and publishes independent scientific advice on existing and emerging risks associated with food and feed safety. The agency provides opinions and information on biological hazards, chemical contaminants, issues associated with food contact materials allergens, pesticides, genetically modified organisms (GMOs) and animal health and welfare. http://www.efsa.europa.eu/

Food Safety Authority Ireland (FSAI)
The FSAI is an independent and science-based body funded by the Irish government. It provides information and advice in the area of food safety and hygiene. It is also responsible for the enforcement of food safety legislation in Ireland. The site contains some useful publications on HACCP, and foodborne pathogens, particularly reducing the risk of *Escherichia coli* O157. Most publications are freely accessible. http://www.fsai.ie/

Food Standards Australia New Zealand (FSANZ)
The FSANZ is an independent agency set up funded by the Australian government. For Australia the agency is responsible for food safety standards. http://www.foodstandards.gov.au/

Health Canada
Health Canada is a government agency responsible for providing information and advice on food safety and nutrition. This site has good consumer information on allergens as well as other food safety information. http://www.hc-sc.gc.ca/

New Zealand Food Safety Authority (NZFSA)
The NZFSA is a government agency providing the government, consumers and the food industry in New Zealand with information, analysis and advice on food safety issues. The site includes some useful microbial hazard data sheets and risk profiles specific to a particular hazard/food combination. http://www.foodsafety.govt.nz/index.htm

UK Food Standards Agency (FSA)
The FSA is an independent organisation set up by the UK government to protect public health and consumer interests in relation to food. This site contains useful information of microbiological and chemical hazards associated with food. http://www.food.gov.uk/

UK Health Protection Agency (HPA)
The HPA provides advice on health issues including hazards associated with food. http://www.hpa.org.uk/

United States Food and Drug Administration (FDA)
A useful and comprehensive website providing information on many aspects of food safety. Includes: data on organisms associated with food poisoning *via* the ''bad bug book''; heavy metals in food; pesticides; acrylamide; dioxins; furan; and natural toxins. This website also includes microbiological methods and analytical methods for drugs and chemical residues. http://www.fda.gov/Food/default.htm

United States Department of Agriculture's Food Safety and Inspection Service (FSIS)
The FSIS is the public agency responsible for the safety of meat, poultry and egg products in the USA. The website provides a wealth of information relating to safe production, cooking and storage of these commodities, including fact sheets and published risk assessments. http://www.fsis.usda.gov/home/index.asp

Hong Kong Centre for Food Safety
The Hong Kong Centre for Food Safety is responsible for ensuring that all food sold in Hong Kong is safe and fit for consumption. The website includes some useful risk assessments, and factsheets on the safe preparation, of high risk foods including sushi and sashimi. http://www.cfs.gov.hk/eindex.html

International Organisations

The following international organisations deal with global food safety issues.

Codex Alimentarius Commission
Develops international food standards and guidelines. http://www.codexalimentarius.org/

Food and Agriculture Organization of the United Nations (FAO)
This site contains useful risk assessments on microbial hazards and expert committee reports on food additives. http://www.fao.org/

International Programme on Chemical Safety (IPCS) INCHEM
INCHEM is an inter-governmental chemical safety website which gives access to peer-reviewed information on chemicals including those found in food, such as pesticides and food additives. http://www.inchem.org/

World Health Organization (WHO)
The site contains information on microbiological and chemical risks associated with food. The website has also been used as vehicle for publishing information on avian influenza H5N1. http://www.who.int/foodsafety/en/

Universities

Science faculties within some universities providing food safety courses have published very useful information on their websites. Some examples are as follows.

Bites
A Kansas State University based website providing information and daily news on many food safety issues. http://bites.ksu.edu/

FoodRisk
A University of Maryland and FDA joint project to provide a searchable database to support food safety risk analysis. http://www.foodrisk.org/index.cfm

Nottingham Trent University
General food microbiology information can be found on this website. http://www.foodmicrobe.com/

University of California
The website of this university includes the Seafood Information Network Center (Seafood NIC) giving access to seafood safety and quality information. http://seafood.ucdavis.edu/

University of Iowa
The website provides useful food safety information easy to find and presented in a easily understandable form. http://www.extension.iastate.edu/foodsafety/

Research Institutes and Professional Bodies

American Food Safety Institute (AFSI)
An American Institute providing food safety training and certification. Their website contains some useful information for food processors on food biosecurity. http://www.americanfoodsafety.com/

Hospitality Institute of Technology and Management (HITM)
This website contains publications relevant to the food service industry. http://www.hi-tm.com/index.html

International Life Sciences Institute (ILSI)
Provides information on food safety, toxicology and risk assessment. Many peer-reviewed publications are freely available on this website. http://europe.ilsi.org/

Institute of Food Research (IFR)
A UK-based provider of scientific research and information on food. The site contains some useful fact sheets relating to food safety. http://www.ifr.ac.uk/
Institute of Food Science and Technology (IFST)
A UK-based professional body concerned with all aspects of food science and technology, including food safety. There is free information available on their website including "Information Statements" on a range of topics, including: *Campylobacter*; *Cryptosporidium*; issues around animal cloning including food safety; HIV/Aids and the food handler, avian influenza and food; and acrylamide. http://www.ifst.org/
Institute of Food Technologists (IFT)
The USA-based IFT website provides free access to many expert reports, scientific summaries, research summits and policy comments relating to food science and food safety. http://www.ift.org/

Trade Associations

Websites for trade associations often provide very useful sector-specific information on food safety to help with risk assessment. Some of these are listed here.

Chilled Food Association (CFA)
A UK-based association. Their website provides some free information on the principles of food safety when producing and storing chilled foods. http://www.chilledfood.org/
Food and Drink Federation
A UK-based association. Provides a focus for all food processing related issues including food safety. Some food safety information is available for public viewing on their website. http://www.fdf.org.uk/
Grocery Manufacturers Association (GMA)
The GMA is a USA-based trade association with a website that provides some food safety and food science factsheets, although some are available to members only. http://www.gmaonline.org/
Ice Cream Alliance
The website includes some free information on the safe handling of ice cream. http://www.ice-cream.org/
The British Sandwich Association
Website provides food safety information to subscribers only. http://www.sandwich.org.uk/
The Specialist Cheesemakers Association
Allows free access to a guide on shelf-life with regards to *Listeria monocytogenes*, including some worked examples. http://www.specialistcheesemakers.co.uk/

Organisations Publishing Official Standards for Methods of Food Analysis

Some standards organisations websites have a facility to purchase copies of descriptive methods of analysis that have been developed and published for

public scrutiny. These methods of analysis can be referenced and are usually recognised by manufacturers and retailers.

American National Standards Institute (ANSI). http://www.ansi.org
BSI Management Systems. http://www.bsigroup.co.uk/Assessment-and-Certification-services/Management-systems/
International Organization for Standardization (ISO). http://www.iso.org/

Organisations Providing Methods of Analysis Online

There are many useful methods of analysis relating to food safety that are freely available online. Some useful websites for methods are as follows:

AOAC International
Provides guidelines for the validation of microbiological methods of analysis. Provides a list of test kit methods (allergen, toxin, microbiology, biochemical, GM Organisms and antibiotic) that have been successfully validated by AOAC. http://www.aoac.org/
Bacteriological Analytical Manual Online (BAM)
Provides full free details of the FDA's preferred laboratory methods of microbiological analysis for food and cosmetics. http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm
Compendium of Fish and Fishery Product Processes, Hazards, and Controls
From the University of California website. http://seafood.ucdavis.edu/HACCP/Compendium/compend.htm
Compendium of methods for Chemical Analysis of Foods
Chemical methods of analysis from the Health Canada website. http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/chem/index_e.html
Detection and Quantification of acrylamide in foods
A draft method published by the FDA. http://www.fda.gov/Food/FoodSafety/FoodContaminantsAdulteration/ChemicalContaminants/Acrylamide/
Determination of furan in foods
A method published by the FDA. http://www.fda.gov/Food/FoodSafety/FoodContaminantsAdulteration/ChemicalContaminants/Furan/
Methods to determine melamine and cyanuric acid residues in foods
Methods listed by the FDA. http://www.fda.gov/Food/FoodSafety/FoodContaminantsAdulteration/ChemicalContaminants/Melamine/
Method for the quantitative determination of perchlorate anion in foods
Rapid method published by the FDA. http://www.fda.gov/Food/FoodSafety/FoodContaminantsAdulteration/ChemicalContaminants/Perchlorate/
Food Contact Materials
EU legislation giving rules and specific guidance for migration testing of the constituents of plastic materials and articles intended to come into contact with foodstuffs. http://ec.europa.eu/food/food/chemicalsafety/foodcontact/legisl_list_en.htm

Mycotoxins Analytical Methods
A series of factsheets giving details of analytical methods for various mycotoxins produced by the European Mycotoxins Awareness Network, a project funded by the EC. http://www.mycotoxins.org/

Pesticide Analytical Manual (PAM)
Analytical methods used by the FDA's laboratories to examine foods for pesticide residues. http://www.fda.gov/Food/ScienceResearch/Laboratory-Methods/PesticideAnalysisManualPAM/

Rapid Microbiology
A website providing free information on rapid test kits and methods for microorganisms. Also provides details of suppliers and testing laboratories by country. http://www.rapidmicrobiology.com/

Rapid Test Methods for Seafood Hazards
From the University of California website. http://seafood.ucdavis.edu/organize/rapid.html

The Compendium of Food Allergen Methodologies
From the Health Canada website. http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/allergen/index_e.html

The Compendium of Analytical Methods
Microbiological methods from the Health Canada website. http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/index_e.html

Information on Pesticide Residues

Codex Alimentarius Commission – pesticide residues in food. http://www.codexalimentarius.net/mrls/pestdes/jsp/pest_q-e.jsp

UK Pesticides Residues Committee homepage. http://www.pesticides.gov.uk/prc_home.asp

United States Environmental Protection Agency Pesticides page. http://www.epa.gov/pesticides/

Miscellaneous

ComBase
Combined database for predictive microbiology. http://www.combase.cc/

Food Safety Watch
An independent website operated by Food Safety Info supplying food safety news and information. http://www.foodsafetywatch.com

International Food Information Council (IFIC) Foundation
USA-based website for disseminating scientific information on food safety and nutrition. http://www.foodinsight.org/

ProMed-Mail
International reporting forum for outbreaks of infectious diseases and toxins, including food poisoning outbreaks. http://www.promedmail.org

Abbreviations and Acronyms

Acid-HVP	Acid-hydrolysed vegetable protein
ACMSF	Advisory Committee on the Microbiological Safety of Food
ADI	Acceptable daily intake
ALARA	As low as reasonably achievable
AOAC	Association of Analytical Communities
APHA	American Public Health Association
ASP	Amnesic shellfish poisoning
A_w	Water activity
CAC	Codex Alimentarius Commission
CAS	Chemical Abstracts Service
CCP	Critical control point
CDC	US Centers for Disease Control and Prevention
CJD	Creutzfeldt-Jakob disease
CONTAM	European Food Safety Authority Panel on Contaminants in the Food Chain
COP	Code of practice
COT	UK Food Standards Agency Committee on Toxicity
CVMP	Committee for Medicinal Products for Veterinary Use
DHHS	US Department of Health and Human Services
DSP	Diarrheic shellfish poisoning
D-value	Decimal reduction time
EC	European Commission
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EPA	US Environmental Protection Agency
ESBL	Extended-spectrum beta-lactamase
FAO	United Nations Food and Agriculture Organization
FDA	US Food and Drug Administration
FSA	UK Food Standards Agency

The Food Safety Hazard Guidebook, 2nd Edition
Richard Lawley, Laurie Curtis and Judy Davis

Published by the Royal Society of Chemistry, www.rsc.org

FSMA	Food Safety Modernization Act
FSIS	US Department of Agriculture Food Safety and Inspection Service
GAP	Good agricultural practice
GC-MS	Gas chromatography-mass spectrometry
GMP	Good manufacturing practice
HACCP	Hazard analysis critical control point
HPA	UK Health Protection Agency
HPLC	High-performance liquid chromatography
HTST	High temperature/short time
IARC	International Agency for Research on Cancer
IFST	Institute of Food Science and Technology
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IgG	Immunoglobulin G
ISO	International Organization for Standardization
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LC	Liquid chromatography
LC-MS	Liquid chromatography-mass spectrometry
LD_{50}	Lethal Dose 50%
LOAEL	Lowest observed adverse effect level
MBM	Meat and bone meal
MCL	Maximum contaminant level
MPN	Most probable number
MRL	Maximum residue level
MRM	Mechanically recovered meat
MRSA	Meticillin (methicillin)-resistant *Staphylococcus aureus*
NOEL	No observed effect level
NRC	National Research Council
NSP	Neurologic shellfish poisoning
OAS	Oral allergy syndrome
PCR	Polymerase chain reaction
PMTDI	Provisional maximum tolerable daily intake
PSP	Paralytic shellfish poisoning
PTWI	Provisional tolerable weekly intake
RfD	Reference dose
SML	Specific migration limit
SRM	Specified risk material
TDI	Tolerable daily intake
TEQ	Toxic equivalents
TLC	Thin layer chromatography
TSE	Transmissible spongiform encephalopathy
USDA	United States Department of Agriculture
vCJD	variant Creutzfeldt-Jakob disease
VNC	Viable but non-culturable
WHO	United Nations World Health Organization
WTO	World Trade Organization

Subject Index

Note: page references in **bold** refer to figures, in *italics* to tables