Chapter 6

Food Additives and Contaminants

Introduction

The food we consume daily contains many different substances, some natural, some added intentionally and some present due to contamination. Substances intentionally added to food, 'food additives', are not as recent an innovation as is often supposed; the use of salt as a preservative and spices to disguise poor food has been common for centuries. However, such treatment of food with additives has only reached the current scale relatively recently, with something of the order of 2500 food additives currently in use. The use of food additives on such a wide scale is now beginning to be questioned by some toxicologists especially as the long-term effects of the substances in question often are not known. The general public also now questions the use of some of these additives and in response to this food manufacturers have begun to supply certain foods which are additive-free or contain only 'natural' colouring agents.

Food additives, grouped according to their use with some examples, are shown in Table 6.1. It can be seen that as well as the colouring agents and preservatives there are other types of additive whose function is less obvious. In Europe, permitted food additives are given a number, the E number, which also appears on the packaging of the food.

Food additives have many functions but primarily they allow the consumer to buy food at his convenience and the producer to 'improve' the quality. Preservatives clearly serve a public health function in reducing the likelihood of bacterial and fungal infections affecting food. The best known of such bacterial infections is food poisoning from *Salmonella* contamination. Preservatives reduce biological and chemical degradation and so allow food to have a longer shelf life. However, colours and some of the other agents added to food are of less obvious benefit to the consumer and may be more important to the manufacturer. Enhancing the attractiveness of food is the main reason given for their use but many consumers have become sceptical and have demanded additive-free food or the use of 'natural' additives. Although this may satisfy consumers who believe that natural substances are intrinsically safe, natural products can be as least as toxic as synthetic ones (see Chapter 9). Each 'natural' food additive needs to be

Table 6.1. Classes of food additives and examples.

Colouring agents	Tartrazine
Anti-oxidants	Butylated hydroxytoluene
Stabilizers	Vegetable gums
Anti-caking agents	Magnesium carbonate
Flavours	Cinnamaldehyde
Preservatives	Sodium nitrate
Emulsifiers	Polyoxyethylene sorbitan fatty esters
Acids/Alkalies	Citric acid
Buffers	Carbonates
Bleaches	Benzoyl peroxide
Propellants	Nitrous oxide
Sweetners	Saccharin
Flavour enhancers	Monosodium glutamate

assessed individually. As well as preservatives other additives may also have a useful function, such as artificial sweeteners which reduce the sugar intake of people with problems such as obesity or diabetes.

As can be appreciated from Table 6.1, food additives comprise a wide range of chemical types from the simple inorganic compounds used as preservatives to the complex organic molecules used as colouring agents and flavours.

In the past, toxic food additives were inadvertently used, such as butter yellow (4-dimethylaminoazobenzene), a dye used to colour butter, which proved to be a carcinogen capable of causing liver tumours in experimental animals.

Clearly food additives have to be tested for toxicity before they can be used and before humans are exposed to them. These tests usually consist of lifetime exposure of experimental animals to the substance at several concentrations, but with the maximum concentration several times greater than that expected to be consumed by humans. However, such testing may not always be predictive as experimental animals may not show the same type of behavioural or immunological effects as does man and absorption, distribution and metabolism can also be different. Also, the administration of relatively large amounts of a substance to experimental animals may lead to accumulation because of saturation of metabolic or excretory pathways. These kinds of problems were encountered with saccharin and clearly make the interpretation of toxicological data difficult. Although the quantities of food additives consumed by humans are very small, their consumption may occur over a lifetime and is chronic although it may be sporadic rather than continuous. This is difficult to simulate in the laboratory animal.

At the present time there is little reliable data on the toxicity of food additives in man but there is much concern on the part of the public and there have been many anecdotal reports of problems relating to food additives, particularly allergic reactions. The incidence of such intolerance to food additives in the population at large is uncertain, most data referring to those patients who have symptoms such as urticaria. In such patients up to half may be responsive to food additives but the figures show wide variation. There may also be cross-reactivity between additives and also with naturally

occurring food contaminants such as between salicylates and tartrazine (see below). However certain substances have been removed from the permitted list of additives due to animal data indicating toxicity. One example is that already mentioned, butter yellow. A more recent example is that of the synthetic sweeteners cyclamate and saccharin (see below), both of which suffered from what was interpreted as adverse animal toxicity data and were banned in the USA.

Tartrazine

One well-known example of a food additive, currently in use where there are possible problems in man is the food colour tartrazine, also known as E102 in European countries. This is one of the most widely used colouring agents and also the colour most frequently implicated in intolerance studies especially in pharmaceutical preparations. It is an orange dye used as a colour in drinks such as orange juice but also in a wide variety of other foodstuffs and also in pharmaceutical preparations.

The toxic effects ascribed to tartrazine are the induction of hyperkinetic behaviour or purposeless activity in children, and of urticaria or skin rashes. Hyperkinetic behaviour is difficult to diagnose and distinguish from restlessness which may be due to other factors such as hunger, boredom or inappropriate treatment by adults. The causation of this syndrome by food additives is somewhat controversial as some studies have shown an improvement in behaviour after switching to diets, such as the Feingold diet, which are free from artificial colours and flavours, whereas other studies have shown no improvement. One double-blind cross-over study of 15 hyperkinetic children found some improvement when the Feingold additive-free diet was used. On the one hand, a major change in dietary habits might be expected to cause behavioural changes; on the other hand, another double-blind cross-over study using objective laboratory and classroom observation failed to find any effect of the Feingold diet. Yet, another trial on 22 hyperkinetic children found a statistically significant improvement in the mother's ratings of their children's behaviour but not in objective tests. According to Juhlin, the one study carried out to the most rigorous scientific standards where objective, non-involved observers were used showed no effect of diet on behaviour.

Urticaria due to intake of tartrazine, however, is more widely accepted as an adverse effect and has been demonstrated in a number of studies. There is histamine release and the symptoms are the appearance of red weals on the skin and itching. A number of other food colours and other types of food additive may also cause urticaria and there may be cross reactivity between other colours such as erythrosine and Sunset Yellow. A challenge of patients whose urticaria had improved on a colour-free diet with 0.15 mg of tartrazine resulted in 3 out of 13 developing urticaria within three hours of exposure. Asthma may also be a symptom of hypersensitivity to tartrazine: a study showed that 11 per cent of asthmatics reacted to an orange drink containing colouring agents.

Tartrazine sensitivity is also often related to aspirin intolerance. Indeed, between 10 per cent and 40 per cent of aspirin-sensitive patients respond to tartrazine with reactions ranging from severe asthma to urticaria and mild rhinitis. The mechanism underlying tartrazine sensitivity is unknown but does not seem to involve a reagenic antibody or the prostaglandin synthesis system. A range of antigenic substances in the diet are absorbed from the gastrointestinal tract but most individuals become immunologically tolerant via a regulatory system which prevents adverse reactions to food constituents and additives. However, some individuals seem predisposed to allergic diseases and do not become immunologically tolerant, hence developing adverse reactions to dietary constituents.

Tartrazine is metabolized by the gut flora giving rise to several metabolites (Figure 2.23) and the urine of animals fed tartrazine has recently been shown to be mutagenic.

Although tartrazine is probably the food colour most commonly implicated in reports of adverse reactions, several others may also cause adverse effects including the 'natural' food colour annatto. Indeed, in one study 26 per cent of patients with chronic urticaria were shown to be responsive to annatto.

Saccharin

This artificial sweetener, first used in the nineteenth century, has been extensively scrutinized over the years and at one stage was banned from use in the USA. As expected of a food additive, saccharin has low acute toxicity, with an LD_{50} of between 5 and 17.7 g kg^{-1} in experimental animals. It is not metabolized and volunteers taking large amounts for several months suffered no ill effects. Two early long-term studies confirmed its safety. Then two studies showed it to be weakly carcinogenic, but these studies have since been criticized as inappropriate. Increased consumption of saccharin and a report showing another sweetener to be carcinogenic prompted further studies to be carried out. In one, saccharin and cyclamate were studied as mixtures with doses up to 2500 mg kg^{-1}. Bladder tumours were observed and as a result cyclamate was banned. Still further studies were carried out but proved inconclusive. Finally, a comprehensive study carried out by the Canadian authorities showed that saccharin could produce bladder tumours in rats and saccharin was suspended from use by the Canadian and US authorities in 1977. In the USA it was banned under the Delaney Clause of the Food, Drug and Cosmetic Act which prohibits the use of any food additive which has been shown to produce cancer in laboratory animals. There was a public outcry against this banning because saccharin was the only general purpose artificial sweetener approved for use and therefore available to diabetics and those with an obesity problem, as well as to other members of the public wishing to reduce their sugar intake. The result was a moratorium on the ban to allow further evidence to be examined. Epidemiological studies mostly showed no increased incidence of bladder tumours but some studies did indicate a slight increase of bladder tumour risk. The absence of detectable metabolism of saccharin after chronic low level

dietary exposure and negative mutagenicity data were taken to indicate that saccharin was not a classical electrophilic carcinogen. Therefore, any carcinogenicity was probably due to the unmetabolized parent compound acting by some epigenetic mechanism.

It was found in experimental animals that levels of up to 5 per cent in the diet caused no detectable increase in bladder cancer but levels of 5–7.5 per cent did cause a significant tumour increase. However, pharmacokinetic studies have now shown that the plasma clearance of saccharin is saturated at the higher exposure level, giving higher tissue concentrations than would be predicted from a linear extrapolation of data from lower dose studies. Consequently, such high-level exposure in animals may be inappropriate as regards normal human exposure. The saccharin case illustrates the wider social aspects as well as the scientific considerations involved with toxicology. There are value judgements to be made and risk must be balanced against benefit. These issues will be addressed in the final chapter.

Food Contaminants

As well as intentional food additives, foodstuffs may also contain contaminants. These might be toxic bacterial or fungal products, toxic degradation products from food constituents, such as pyrolysis products resulting from cooking, or they might be substances inadvertently added to the food. There is now great interest in toxic and especially carcinogenic compounds produced as a result of cooking such as the mutagenic compounds Trp 1 and Trp 2, and carcinogenic nitroso compounds produced from dietary amines.

Two examples of naturally occurring but toxic food contaminants are botulinum toxin and aflatoxin. Botulism will only be briefly discussed here as it is covered in more detail in Chapter 9 under natural products.

Botulism

Botulism is the syndrome caused by botulinum toxin from the bacterium *Clostridium botulinum*. This anaerobic bacterium may contaminate tinned or bottled food and the toxin is extremely potent. Heating destroys the toxin.

Aflatoxin

The aflatoxins are a group of mycotoxins produced by the mould *Aspergillus flavus*. This mould may grow on foodstuffs such as damp peanuts and stored crops, particularly under hot, humid conditions, and the resulting contamination can be a serious problem in some tropical countries. Tainted crops are difficult to sell to countries such as the USA and UK which have strict criteria on levels of mycotoxins. Consequently, the tainted crops may then be sold within the

poorer producing country or may find their way to famine victims as part of the relief effort.

Animals fed on meal derived from contaminated feed such as peanuts may develop tumours. The toxins were in fact discovered as a result of the loss of turkeys suffering liver damage after being given mouldy feed. Also, traces of aflatoxin have been detected in peanut butter, especially that made from peanuts not treated with chemicals to prevent mould growth and consequently sold in health food shops labelled as 'natural'.

Aflatoxin B_1 is a very potent liver carcinogen and hepatotoxin; a level of 1 ppb in the diet may be sufficient to cause liver tumours. Levels of aflatoxin in the diet are higher (ppm as opposed to ppb) in Africa than in other parts of the world and this explains the higher incidence of liver cancer in certain parts of Africa. The mechanism of toxicity of aflatoxin B_1 involves metabolism to a chemically reactive intermediate (an epoxide) which binds covalently to protein but which also interacts with nucleic acids. This chemically reactive intermediate may be responsible for both the liver necrosis and the liver tumours.

Ptaquiloside

See Chapter 9 for a discussion of this naturally occuring carcinogen found in edible bracken fern shoots.

The Spanish Oil Syndrome

Non-natural substances may also sometimes contaminate food and there have been several examples of this such as Epping jaundice which has already been mentioned in chapter 5. A more recent and tragic example of this was the contamination of cooking oil in Spain.

In May 1981 an unusual outbreak of a pulmonary disease was reported around Madrid. The unusual syndrome included severe pulmonary oedema which was not prolonged, exanthema and eosinophilia. Overall there were more than 20 000 cases of the syndrome and 351 fatalities (Figure 6.1). A toxic substance was suspected and finally a connection was established between the disease and the use of cheap cooking oil. Action by the Spanish Government to replace the oil with pure olive oil decreased the numbers of cases reported. There was a correlation between the consumption of cheap oil, especially that sold by certain salesmen, and the development of the syndrome.

The disease appeared after a latent period of at least 1–2 weeks, longer in some cases, and an apparent dose-response relationship was noted in one report. However, the association between the intake of oil and the syndrome is circumstantial as the effects have not been reproduced in experimental animals and the precise causative agent has not been identified. The syndrome had an acute phase with mainly acute pulmonary interstitial oedema, and a chronic phase which was mainly neuromuscular with muscular atrophy, skin lesions and weight loss. Vasculitis was also observed which affected many blood vessels.

Figure 6.1. A headline reporting the disaster which followed the use of rape-seed oil contaminated with aniline as a substitute for olive oil in Spain in 1981.
From *The Sunday Times,* August 23 1981, with permission.

The toxic oil was rape-seed oil which had been denatured by the addition of aniline, as required by law in Spain for imported rape seed oil so that it cannot be used for cooking. However, refining of this oil was undertaken and the resulting oil sold as suitable for human consumption. This had been practised previously without the toxic effects being seen, and consequently it seems that the particular batch of oil responsible for the syndrome may have been refined differently or was different in some other way. It was mixed with other oils in some cases and so may have become contaminated. Identifying the toxic constituents so far has not been possible. The failure to understand the mechanism underlying this major public health disaster highlights the difficulties of studying food additive/contaminant problems. These are often due to factors beyond the control of the toxicologist. In this case, the problem of obtaining samples of oil reliably associated with the syndrome and the absence of an animal model have greatly hampered the research.

This tragedy also illustrates how a large number of people may be affected by a toxic contaminant in a foodstuff. A more subtle toxic reaction to a food additive than the one described here could affect many more people before it was detected.

Questions

1. Write short notes on the toxicological aspects of the following:

   (a) aflatoxin;
   (b) ptaquiloside;
   (c) botulinum toxin.

2. What particular problems are associated with the safety evaluation of food additives? Illustrate your answer with reference to saccharin.

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