chapter nine

Adverse effects of food additives

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9.1 Introduction

The increasing demand for "ready-to-eat" foods, snacks, and a continuous assortment of foodstuffs, even if they are out of season, and the change in lifestyle that has taken place during the last centuries, have led to an increase in the use of food additives.

Food additives are substances that man adds to food intentionally to provide protection against contamination with microorganisms, to prevent oxidative deterioration of oils, fats, and shortenings, to keep food appealing and tasteful, and to improve its texture, etc. By using additives, foodstuffs can be kept for long periods of time, and restricting oneself to seasonal foods is no longer necessary. Food additives thus fulfill valuable functions in our daily food and as such, cannot be left out. Currently, there may be as many as 2800 substances in use as food additives, of which the majority (about 2500) are naturally occurring flavoring substances. However, the most important additives are only a handful, and most of the additives of natural origin are used in trace amounts. In the 1970s, the US Food and Drug Administration estimated the use of sucrose, corn syrup, dextrose, and salt at 93% (w/w) of the total use of food additives. If black pepper, caramel, carbon dioxide, citric acid, modified starch, sodium bicarbonate, yeasts, and yellow mustards are included, the percentage comes to about 95%. In fact, food additives can be considered food-oriented substances.

Food additives can be divided into two broad groups: synthetic and natural substances. The latter group includes substances of plant and in some cases, of animal origin. Synthetic food additives are extensively tested for toxicity before they are allowed for use in food. Several additives of natural origin have also been tested.

Obviously, only substances that have been shown to pose no serious toxicological risks at levels anticipated for consumption are admitted for use as food additive. This also applies to substances for which there is conclusive evidence for nongenotoxic carcinogenicity from lifetime bioassays in rodents, for example, the antioxidant butylated hydroxyanisole and the sweetener saccharin. For synthetic substances, no-observed-adverse-effect levels (NOAELs) (see Sections 16.3.2.1, 17.3.2, and 19.2.2) are established. Subsequently, acceptable daily intakes (ADI) are calculated by applying safety factors (SF): ADI = NOAEL / SF. Also for the nongenotoxic food additives mentioned above NOAELs are used. Synthetic food additives are thus the safest components of the diet. In practice, the only adverse reactions to food additives are intolerances. It should be noted, however, that intolerances are not restricted to synthetic substances only.

Admitted synthetic food additives are put on a so-called positive list, indicating that only substances on that list are allowed to be used in food.

In view of the fact that the toxicological risks associated with the intake of synthetic food additives are minimal, the next sections will only deal with the most relevant toxicity data, as obtained (at the very high dose levels above the NOAEL) in experimental animals. Of each type of synthetic food additive, a few examples will be given. Intolerance reactions are discussed in Chapter 14.

9.2 *Food colorings*

Color is a property of foodstuffs that makes them visually attractive. The use of artificial colorings started at the beginning of the 19th century. At that time, there were no restrictions, and several cases of abuse have been recorded. For example, many people died from eating sweets and puddings colored with arsenic derivatives, and cheese dyed with red lead and vermilion (HgS). Foods were frequently colored to mask that they had been diluted with cheap ingredients. For example, at the turn of the century, milk was colored yellow to hide skimming and dilution with water. This practice was so widespread that people refused uncolored milk for fear of adulteration. Nowadays, food adulteration is prohibited by law, and foods are colored either by naturally occurring pigments (e.g., dried algae meal, paprika, beet powder, grape skin extract, caramel, carrot oil, ferrous gluconate, iron oxide) or by artificial food colorings (e.g., tartrazine and erytrosine). Most food colorings used are synthetic. They are cheaper, more intense, and more stable than their natural counterparts. Concerning artificial food colorings, there is a controversy whether there is an association between the intake of the colorings and intolerance reactions in children. Here, the various aspects of tartrazine, as an example of such a food coloring, will be briefly described.

9.2.1 *Tartrazine*

Tartrazine is a yellow synthetic azo dye. Several clinical symptoms have been attributed to tartrazine, including asthma, hyperactivity of children, and urticaria (hives).

Much attention has been paid to the induction of effects in asthma patients after the intake of tartrazine. A number of studies reported a high incidence of intolerance of tartrazine among aspirin(acetylsalicylic acid)-intolerant asthmatics. On the whole, however, little evidence has been found against the use of tartrazine in cases of asthma, even among those who are intolerant to aspirin.

With regard to hyperactivity of children, there is a controversy regarding the association between tartrazine and the hyperactivity. So far, studies on this potential problem have not provided conclusive evidence for such an association. A similar controversy links a possible association between tartrazine and urticaria. In this case too, no relationship has been found.

9.3 *Preservatives*

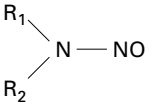
Preservatives keep food edible for long periods of time by preventing the growth of microorganisms such as bacteria and fungi. Although the public perceives preservatives in particular as hazardous, they are not only harmless at the levels ingested but in fact beneficial in that they reduce or prevent the risks due to bacterial and fungal contamination (see Part 1, Chapter 2).

9.3.1 *Nitrate and nitrite*

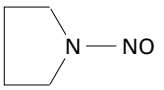
Nitrates and nitrites are used to preserve meats. For example, they contribute to the prevention of growth of *Clostridium botulinum*, the bacterium that produces the well-known highly potent botulinum toxin. The adverse effects after intake of nitrates and nitrites are methemoglobinemia and carcinogenesis, the latter resulting from the formation of nitrosamines.

Bacteria in the oral cavity can reduce nitrate to nitrite. Nitrite oxidizes (ferrous) hemoglobin to methemoglobin, which cannot bind oxygen. This may lead to a state of anoxia. The consumption of meat with high levels of nitrate and nitrite as well as of other dietary nitrate sources, such as drinking water and spinach, has resulted in life-threatening methemoglobinemia, especially in young children. Newborns are (transiently) deficient in NADH-reductase, the major system responsible for methemoglobin reduction.

Nitrite (either ingested directly or indirectly via the reduction of nitrate) also reacts with secondary amines under the formation of a variety of nitrosamines, e.g., dimethylnitrosamine, diethylnitrosamine, and N-nitrosopyrrolidine.



$R_1 = R_2 = CH_3$ Dimethylnitrosamine
$R_1 = R_2 = C_2H_5$ Diethylnitrosamine



N – nitrosopyrrolidine

Nitrosamine formation can take place in food and *in vivo*. The acidic conditions in the stomach favor nitrosamine formation. Nitrosamines are mutagens as well as carcinogens. They induce cancer in a variety of organs, including the liver, respiratory tract, kidney, urinary bladder, esophagus, stomach, lower gastrointestinal tract, and pancreas. Nitrosamines need biotransformation for their activation. The bioactivation of nitrosamines is mediated by cytochrome P-450. It involves oxidative N-dealkylation, followed by a sequence of rearrangements to yield the alkylating alkylcarbonium ions (see Figure 9.1).

It should be noted that a decrease in the incidence of botulism may be accompanied by an increase in the formation of carcinogenic nitrosamines, as a result of an increase in the nitrite level of the meat (products).

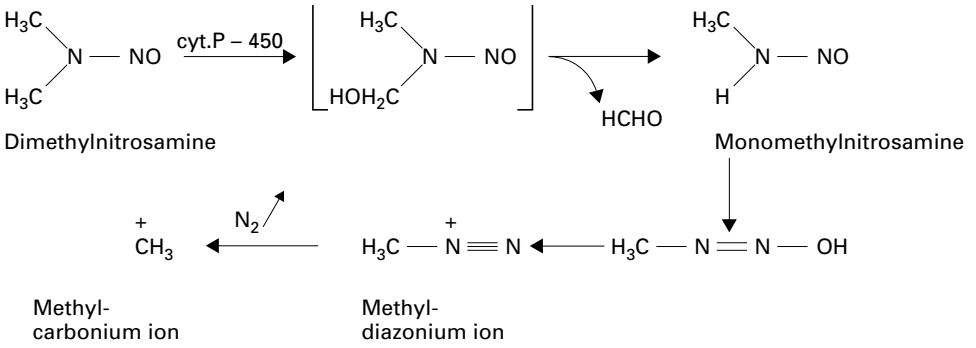


Figure 9.1 Metabolic activation of dimethylnitrosamine.

9.4 *Antioxidants*

Antioxidants are used to protect oils, fats, and shortening against oxidative rancidity and to prevent the formation of toxic degradation products and polymers.

Many foods may undergo oxidation, but particularly those containing fats are susceptible to changes in color, odor, taste, and nutritional value. Unsaturated fatty acids are readily peroxidized in the presence of molecular oxygen. The peroxidation products may induce toxic effects. Also, in biological systems peroxidation of lipids may have severe adverse consequences. Peroxidation of polyunsaturated fatty acids is believed to be involved in disturbing the integrity of cellular membranes, the pathogenesis of hemolytic anemia, and pulmonary and hepatic injury. Secondary peroxidation products, e.g., hydroxynonenal, can form adducts with DNA.

The peroxidation of lipids consists of the following steps (LH = lipid):

initiation:	LH	$\xrightarrow{\text{catalyst}}$	$L^{\cdot} + H^{\cdot}$
	$LH + O_2$	$\xrightarrow{\text{catalyst}}$	$L^{\cdot} + {}^{\cdot}OOH$
propagation:	$L^{\cdot} + O_2$	$\longrightarrow$	$LOO^{\cdot}$
	$LOO^{\cdot} + LH$	$\longrightarrow$	$LOOH + L^{\cdot}$
termination:	$LOO^{\cdot} + LOO^{\cdot}$	$\longrightarrow$	$LOOL + O_2$
	$LOO^{\cdot} + L^{\cdot}$	$\longrightarrow$	LOOL
	$L^{\cdot} + L^{\cdot}$	$\longrightarrow$	LL

In the *initiation* step, the unsaturated lipid LH undergoes hydrogen abstraction under the formation of a lipid radical $L^{\cdot}$. This process can be catalyzed by light, heat, traces of transition metals, and enzymes. The carbon-centered radical tends to be stabilized by intramolecular rearrangement to form a conjugated diene, which readily reacts with molecular oxygen (O_2) to yield a lipid peroxide radical, $LOO^{\cdot}$. This, in turn, is capable of inducing the initiation of lipid peroxidation by abstracting a hydrogen atom from another lipid molecule, also leading to the *propagation* of the oxygenation reaction. The *termination* step is characterized by the combination of two radicals. Lipid radicals can combine to form dimers, polymers, alcohols, and peroxides. Under normal oxygen tension, the rearrangement of two lipid peroxide radicals ($LOO^{\cdot}$) is most likely to yield LOOL and O_2.

Lipid hydroperoxides undergo degradation, leading to the formation of secondary peroxidation products, such as alkanes (e.g., ethane and pentane), aldehydes (e.g., malondialdehyde and hydroxynonenal), ketones, alcohols, and esters.

The purpose of using food antioxidants is to protect food from organoleptic deterioration, decrease in nutritional value, and formation of toxic products by removing radicals. Two types of antioxidants can be distinguished: radical scavengers and synergists.

Radical scavengers, like the phenolic substances butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), interfere with the propagation step, thereby terminating the lipid peroxidation:

$$\begin{aligned} AH + L^{\cdot} &\longrightarrow A^{\cdot} + LH \\ AH + LO^{\cdot} &\longrightarrow A^{\cdot} + LOH \\ AH + LOO^{\cdot} &\longrightarrow A^{\cdot} + LOOH \\ AH = \text{phenolic antioxidant} &\qquad LH = \text{lipid} \end{aligned}$$

The antioxidants themselves are converted to resonance-stabilized intermediate radicals $A^{\cdot}$, which is illustrated for BHA in Figure 9.2. The resulting phenoxy radical $A^{\cdot}$ may either be regenerated to the parent antioxidant AH by reducing agents or further oxidized to a stable quinone, or combine with other phenoxy or lipid peroxy radicals.

Synergists may either regenerate the parent radical scavenging antioxidants from phenoxy radicals ($A^{\cdot}$) formed in the interference with the propagation step, or act as a sequestering agent for transition metals, active catalysts in the initiation, and propagation steps of lipid peroxidation.

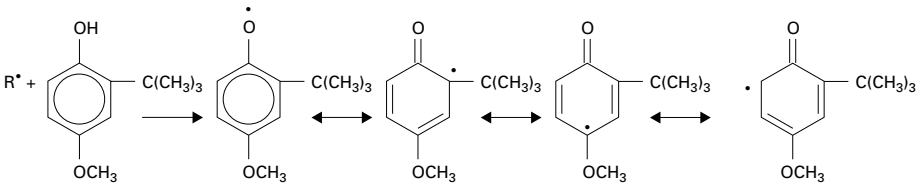


Figure 9.2 Radical scavenging by BHA; $R^{\cdot}$ = lipid free radical.

Well-known radical scavengers are α-tocopherol (vitamin E), BHA, BHT, ascorbic acid (vitamin C) and gallate esters (propyl-, octyl- and dodecylgallate). Synergistically-acting antioxidants include ascorbic acid, citric acid, and ethylenediaminetetraacetic acid (EDTA).

9.4.1 *Butylated hydroxyanisole*

The acute toxicity of BHA is relatively low. Its oral LD_{50} in rats is 2.5 to 5 mg/kg body weight. Owing to many years of use without adverse effects (except for a few cases of allergic reactions), BHA was given the Generally Recognized As Safe (GRAS) status by the US Food and Drug Administration. In the early 1980s, experimental data became available on the induction of tumors by BHA in rodents (rats, hamsters, and mice). Changes such as hyperplasia, papillomas, and carcinomas were observed in the forestomach (an organ that is absent in man). These changes were time- and dose-dependent (Figure 9.3).

The International Agency for Research on Cancer (IARC) evaluated that there was sufficient evidence for carcinogenicity of BHA in experimental animals to classify BHA as a (IARC) class 2B carcinogen (i.e., possibly carcinogenic for humans). However, no conclusive evidence for genotoxicity has been found. This means that BHA does not directly interact with DNA. Such carcinogens are known as nongenotoxic carcinogens and they are assumed to have threshold doses. Induction of hyperplasia is believed to play an essential role in the mechanism underlying the tumorigenicity of BHA. Recently, however, a

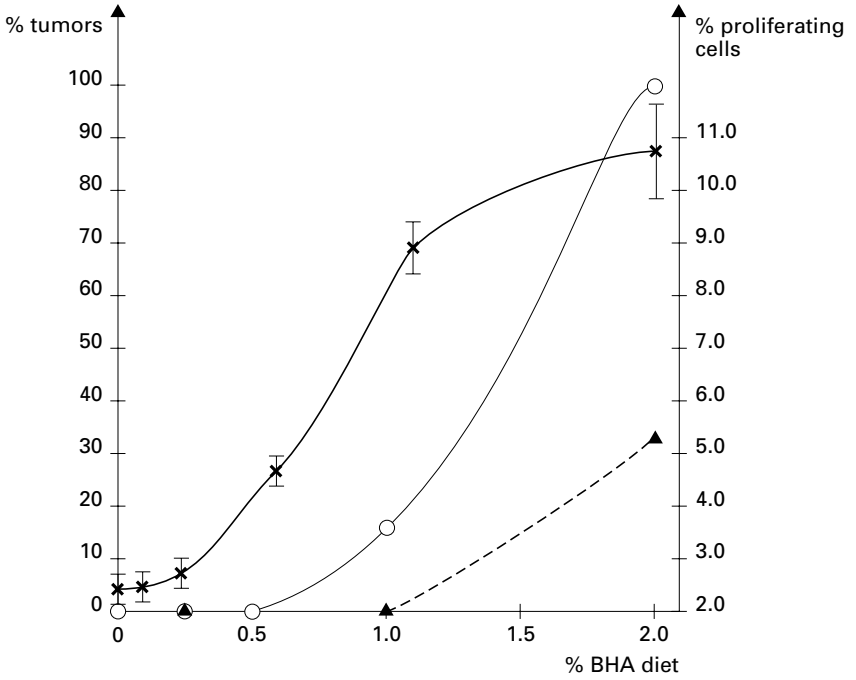


Figure 9.3 Dose-response curves for dietary BHA-induced cell proliferation (at 9 days x), carcinomas (at 2 years ▲) and papillomas and carcinomas (at 2 years o). Source: Clayson et al., 1991.

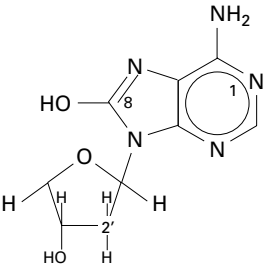
proposal has been made for the mechanism underlying the tumorigenicity of BHA, in which its biotransformation plays an essential role.

The main metabolic pathways of BHA in all species studied, including man, are glucuronidation and sulfation (Figure 9.4). Both conjugation reactions lead to detoxication and elimination of the ingested BHA. A minor metabolic pathway in several species, including man, is oxidative O-demethylation to tertiary butylhydroquinone (TBHQ, Figure 9.4). O-demethylation is relatively more important at lower dose levels. TBHQ also undergoes glucuronidation and sulfation.

In the proposed mechanism, BHA-induced tumor formation is believed to result from a sequence of reactions. This includes O-demethylation of BHA, oxidation of TBHQ to tertiary butylsemiquinone (TBSQ), and tertiary butylquinone (TBQ), conjugation of TBQ with glutathione (GSH) and ultimately formation of reactive oxygen species in the redox cycling of the TBQ-glutathione conjugate. Redox cycling leads to the formation of superoxide anion radicals ($O_2^{\cdot}$). These radicals can spontaneously dismutate to hydrogen peroxide (H_2O_2). In the so-called Haber-Weiss reaction $O_2^{\cdot}$ and H_2O_2 can react to form hydroxyl radicals ($OH^{\cdot}$):

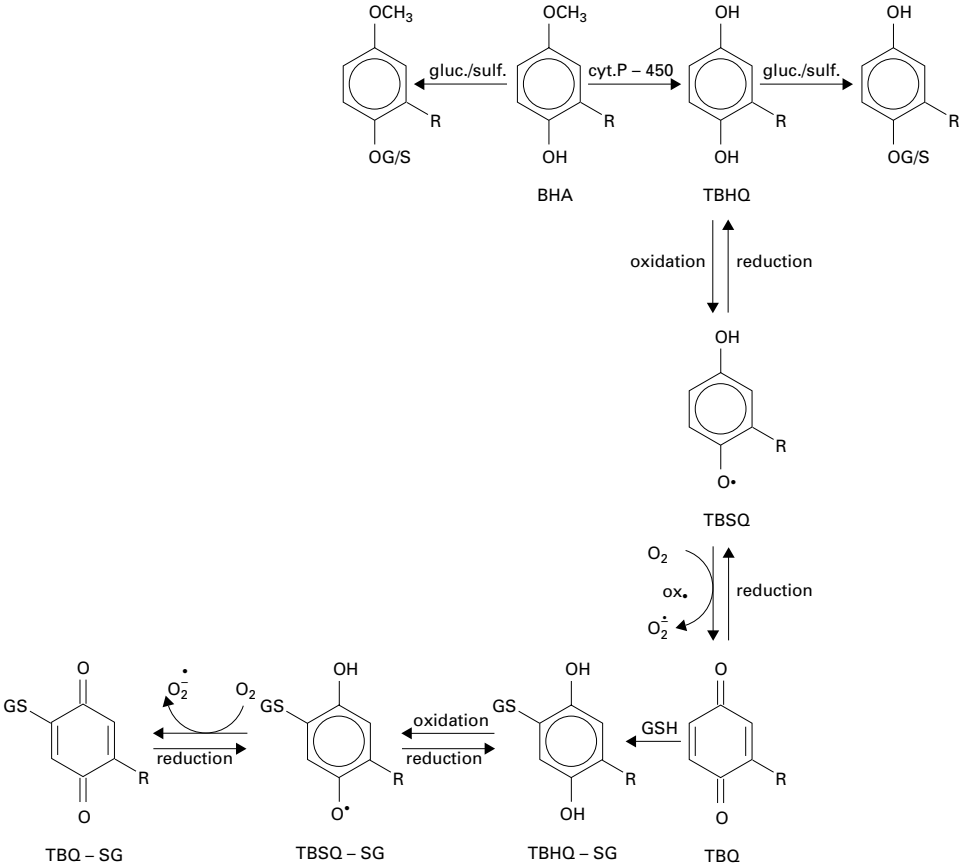
$$O_2^{\cdot} + H_2O_2 \xrightarrow{Fe^{2+}} OH^{\cdot} + OH^{-} + O_2$$

Hydroxyl radicals readily react with biomacromolecules, such as proteins, DNA and RNA. The formation of 8-hydroxy-2′-deoxyguanosine in DNA has been reported to lead to the induction of mutations and to tumor development.



8 – OH – 2′ – Deoxyguanosine

The consequences for the assessment of the risk of BHA-induced genotoxicity are not yet clear. The above findings contribute to the elucidation of the mechanism underlying the carcinogenicity of BHA (hazard identification). To assess the risk of genotoxicity more knowledge of the kinetics of the sequence of steps is needed.



R = tertiary butyl – $C(CH_3)_3$
G/S = glucuronate/sulfate
BHA = butylated hydroxyanisole
TBHQ = tertiary butylhydroquinone
TBSQ = tertiary butylsemiquinone
TBQ = tertiary butylquinone
SG = glutathione conjugate

Figure 9.4 Metabolic inactivation and activation of BHA.

9.5 Emulsifiers

This group of additives includes thickening, gelatizing and stabilizing agents. They are used to improve the texture of food. Examples are agar-agar, tragacanth, sorbitol, mannitol, glycerol, gelatin and cellulose.

9.5.1 Sorbitol

Sorbitol is found in high levels in rowan berries (*Sorbus aucuparia*, Rosaceae), and also in cherries, prunes, apples, pears, peaches, apricots, and algae. It is also a synthetic substance. Sorbitol is a stabilizer as well as a sweetener.

Sorbitol acts as a diuretic and as a laxative. Large amounts of sorbitol may cause formation of gas, swelling of the belly, and diarrhea, accompanied by pain. It is metabolized for 70% to CO_2. No ADI has been estimated for sorbitol. A daily intake of 40 g is considered to be acceptable.

9.6 Flavoring agents

The most widely used flavor enhancer is salt (sodium chloride, NaCl). It is also a preservative and a nutrient. Generally, it is primarily regarded as a food additive. A well-known toxic effect of NaCl is high blood pressure.

9.6.1 Monosodium glutamate (ve-tsin)

Monosodium glutamate (MSG) is found in seaweed (*Laminaria japonica*). It is also a synthetic product. MSG is an excitatory neurotransmitter. It has been shown to cause permanent lesions of the hypothalamus in newborn rats and mice. Presumably, this is attributable to immaturity of the blood-brain barrier. Further, in young mice and rats, lesions of the retina have been reported after large doses of glutamate.

Humans have also been found to be sensitive to food to which MSG has been added as a flavor enhancer. The symptoms, known as "Chinese restaurant syndrome," include loss of feeling, general weakness, and heart palpitations.

9.6.2 Safrole

This substance is a typical member of a series of propenylbenzenes. These also include methyleugenol and estragole. The propenylbenzenes are natural and synthetic flavoring agents. Sassafras, containing high levels of safrole, used to be added to sarsaparilla root beer. Nowadays, safrole is still present in the diet as a (minor) component of various herbs and spices, e.g., cloves.

Safrole and related substances have been shown to be carcinogenic. Possible metabolic activation routes are 1′-hydroxylation, followed by sulfation, and epoxidation of the double bond in the propenyl group (Figure 9.5).

In the case of safrole, biotransformation data suggest that the 1′-hydroxy sulfate ester is the ultimate carcinogenic species capable of binding to DNA. Administration of 1′-hydroxysafrole to sulfation-deficient mice resulted in a lower tumor incidence than administration of the metabolite to normal mice.

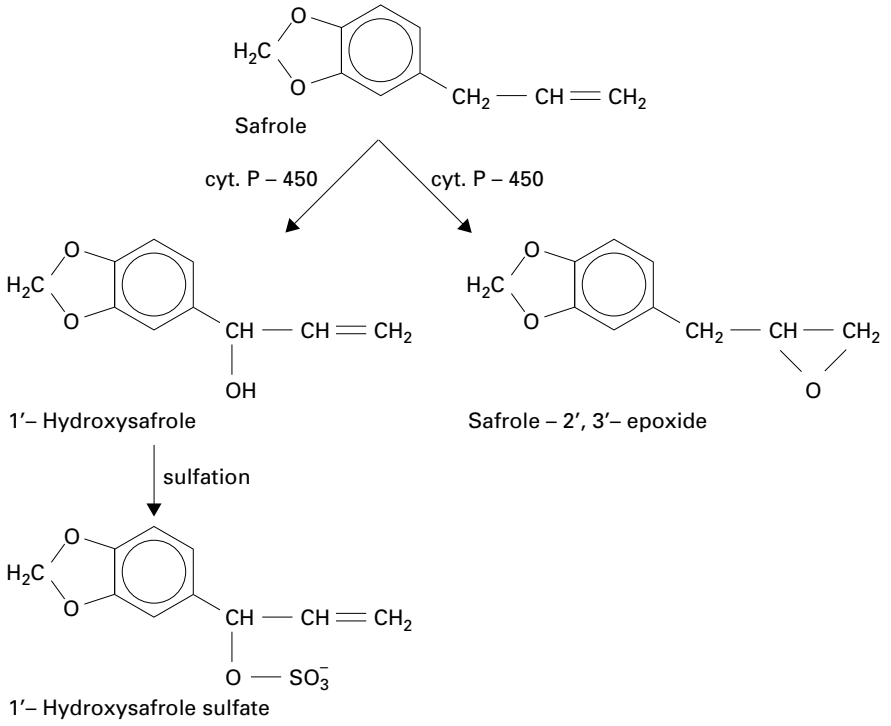


Figure 9.5 Possible routes of metabolic activation of safrole.

9.6.3 Saccharin

Saccharin (1,2-benzisothiazole-3(2H)-one 1,1-dioxide) is a well-known non-nutritive artificial sweetener. It was discovered by accident. In 1879, Constantin Fahlberg, a graduate student at John Hopkins University in Baltimore, was working on the synthesis of toluene derivatives. One day, while having lunch with unwashed hands, he discovered his bread to taste extraordinarily sweet. Soon after, the sweet taste could be attributed to one of the derivatives he synthesized: saccharin.

Saccharin has a low toxicity. Still, it has been extensively examined as a carcinogen. Early studies in experimental animals reported an increase in incidence of bladder tumors in the offspring of mother animals fed saccharin throughout pregnancy. Interpretation of these results was complicated by the presence of contaminants in the saccharin. Interpretation of later findings concerning an increase in the number of bladder tumors was complicated by the fact that in the meantime saccharin had been shown to be a promoter of other bladder carcinogens, like methylnitrosurea.

Saccharin is not metabolized, and has not been found to be genotoxic. On the basis of additional data from animal experiments and *in vitro* genotoxicity studies as well as from epidemiological studies, saccharin can be considered as a nongenotoxic carcinogen for which a threshold dose can be set. Its ADI is 2.5 mg/kg body weight.

9.6.4 Aspartame

Aspartame is another artificial sweetener. It is a dipeptide, consisting of L-aspartic acid and the methyl ester of L-phenylalanine.

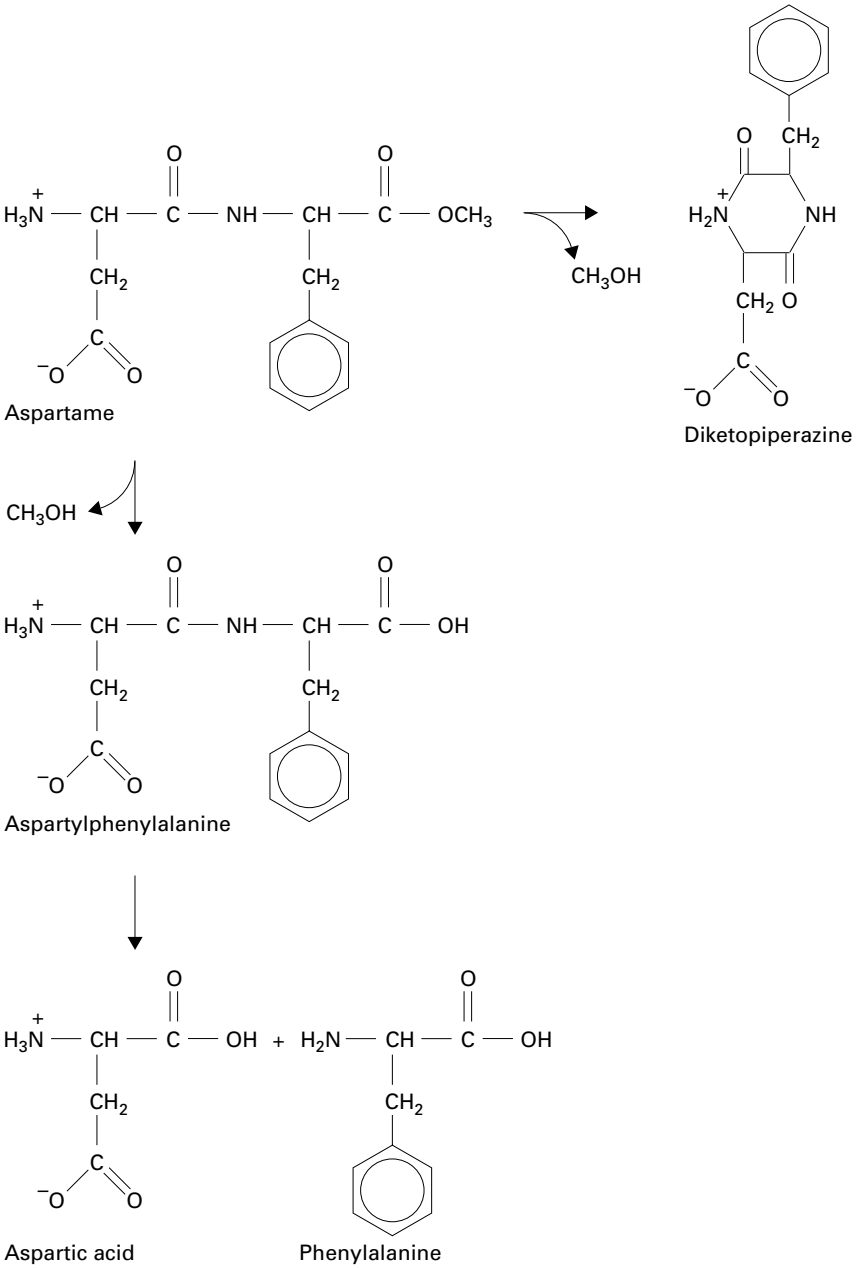


Figure 9.6 Hydrolysis of aspartame.

In the gastrointestinal tract, aspartame undergoes complete hydrolysis into its three components aspartic acid, phenylalanine, and methanol (Figure 9.6).

Although aspartame has been approved (the ADI is set at 50 mg/kg body weight per day) as a sweetener in many countries, there are still some toxicological aspects under consideration. A small number of urticarial reactions have been demonstrated. In general, it is agreed that the methanol and aspartic acid formed from aspartame by hydrolysis are safe. As far as the third component, phenylalanine, is concerned, there is the possibility of

combined action. Interactions between phenylalanine and other amino acids are suggested at the level of amino acid transport, leading to nervous disturbances as a result of decreased neurotransmitter levels. Formation of phenylalanine from aspartame can actually pose a risk for so-called homozygous phenylketonurics. These people lack the ability to hydroxylate phenylalanine, the first step in its metabolism.

9.7 Summary

The synthetic food additives, and some of the naturally occurring food additives, have extensively been screened for toxicity. Limit values have been assessed for dietary intake (by humans) on the basis of extrapolation of data obtained in experimental animals. The probability that food additives cause adverse effects in humans at the levels recommended for dietary intake is at least minimal and probably negligible. An exception should be made for those food (component)s that cause hypersensitivity or allergy.

In this chapter, therefore, the attention is focused on the examination of the type of toxic effect and the underlying mechanism after administration of food additives (at high doses) to experimental animals. A number of illustrative examples are given.

The preservatives nitrate and nitrite are known to induce acute as well as long-term adverse effects: methemoglobinemia and cancer. Bacteria in the oral cavity can reduce nitrate to nitrite. Nitrite oxidizes hemoglobin to methemoglobin, which fails to bind oxygen. The consumption of dietary sources with high levels of nitrate (e.g., drinking water) and nitrite (e.g., meat and meat products) has resulted in life-threatening methemoglobinemia, especially in children. Nitrite (either ingested directly or indirectly via the reduction of nitrate) reacts with secondary amines under the formation of a variety of nitrosamines, e.g., dimethylnitrosamine, diethylnitrosamine and N-nitrosopyrrolidine. Nitrosamines are mutagens as well as carcinogens. They induce cancer in a variety of organs, including the liver, kidney, urinary bladder, stomach, and pancreas. Nitrosamines need bioactivation, mediated by cytochrome P-450, followed by a sequence of rearrangements to yield the alkylating alkylcarbonium ions.

The synthetic antioxidant butylated hydroxyanisole (BHA) has been shown to be carcinogenic in rodents. It is a nongenotoxic carcinogen. In tests such as the Ames test, it proved to be nonmutagenic. It has been suggested that biotransformation plays an essential role in the carcinogenicity of BHA. It may undergo metabolic activation via a sequence of steps. The first step is oxidative O-demethylation to tertiary butylhydroquinone (TBHQ). This is oxidized to tertiary butylquinone (TBQ) via two one-electron steps. TBQ is nonenzymatically conjugated with glutathione to form ultimately TBQ-SG. Redox cycling of TBQ and TBQ-SG produces reactive oxygen species, the most reactive being the hydroxyl radical. The formation of hydroxyl radical–DNA adducts has been reported to lead to the induction of mutations and to tumor development.

The flavoring agents safrole, methyleugenol, and estragole are hepatocarcinogens. Data on the biotransformation of safrole suggest that the 1′-hydroxy sulfate ester is the ultimate carcinogen. Further, the adverse effects of the food color tartrazine, the emulsifier sorbitol, the flavoring agent MSG, and the artificial sweeteners saccharin and aspartame are briefly discussed.

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