CHAPTER 10

NATURALLY OCCURRING ORALLY ACTIVE DIETARY CARCINOGENS

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1 INTRODUCTION

In an attempt to provide the reader with handy information and key references in a comprehensible and concise manner on such a broad topic, it is necessary to provide some definitions and assumptions that were used to narrow the subject area covered. To begin with, the title of the chapter contains some terms that are open to interpretation and hence should be defined. “Natural” in the context of this chapter will include all compounds that are found in the food supply, exclusive of synthetic compounds that are either intentionally added or incidentally found in food. This would exclude many synthetic direct and indirect food additives, pesticides, animal drug residues, and most of the so-called environmentally persistent chemicals, such as DDT. Hence, “naturally occurring” includes all nonsynthetic substances that occur through nonanthropogenic activity (i.e., plant- or fungi-derived contaminants) and those that are formed endogenously in the food as a result of natural chemical processes caused by some type of human activity (i.e., cooking). “Orally active” is used in the context of carcinogenesis and excludes the consideration of all compounds that are bereft of evidence of carcinogenic activity via the oral route of administration. Hence, there are many compounds which may be found in food that have carcinogenic activity upon some parenteral route of administration, such as inhalation (e.g., acetaldehyde), but either lack carcinogenic activity upon oral administration or have not been so tested. Since carcinogenic metabolic pathways are so heavily dependent on the route of administration, oral exposure is the only relevant route for food. Given that cancer formation is a multifactorial, multistage process, defining the term “carcinogen” is not so straightforward. For the purposes of this chapter, a rather simple definition was employed. A “carcinogen” is any solitary compound (i.e., not a mixture or physicochemically unidentified substances), exposure to which is capable of increasing the incidence of either benign or malignant neoplasm. Excluded from consideration are all the “co-carcinogens”, “promoters”, and other amplifiers of the carcinogenic process. For example, certain macro-components of the diet such as alcohol and certain fats are excluded, as well as naturally occurring trace compounds that can occur in the diet, such as the diterpene esters from certain plants. Additionally, compounds that are known to exert their carcinogenic action via a secondary mechanism (i.e., nongenotoxic carcinogens), such as hormone disruptors (e.g., phytoestrogens and thioureas), are also excluded from consideration. In general, effects of nongenotoxic carcinogens occur at high doses, above some threshold level that exceeds human exposure. Finally, somewhat arbitrarily, certain inorganic ions and compounds that are thought to produce cancer in humans (e.g., arsenic) are excluded from consideration.

The general format for each section in the chapter loosely adheres to the following: (1) chemical identification of the specific compound, which may include its structure; (2) dietary natural source(s) of the compound, mostly limited to the U.S.; (3) available information on source level(s) of the compound; (4) evidence of carcinogenicity in laboratory animals, including species and primary carcinogenic target organ identification; (5) mention of mutagenicity evidence, primarily from the Ames’ Salmonella assay due to its universal reproducibility;
(6) mention of epidemiological carcinogenicity evidence; and (7) the International Agency for Research into Cancer (IARC) carcinogen classification, if available. Each section ends with a brief statement on the relevance of the experimental data to human carcinogenic risk.

2 **ALKENYLBENZENES**

Safrole is one of the many allylic and propenyl benzene derivatives which occur naturally in many spices, herbs, and vegetables. Additional characteristics of this class of compounds are ring methoxy and/or ring methylenedioxy substitutions. Most of these compounds are found in the essential oils of plants (i.e., the oils obtained from plant materials by steam distillation, solvent extraction, or physical expression). Many of these compounds (including synthetic versions) are used as flavor additives and fragrances. A good review of the physical and chemical properties of safrole and related compounds can be found by Woo et al. (1988).

About 30 alkenylbenzenes and related compounds have been found naturally. Eleven have been assessed for carcinogenicity, with six testing positive (see Table 1 for chemical name, structure, natural sources with associated references, and references for carcinogenicity studies). The earliest evidence of tumor induction by safrole came in 1961 from three independent investigations, which revealed hepatic tumors (or preneoplastic changes) in rats receiving high doses of safrole in the diet (0.5 to 1%). This discovery contributed to the banning of its use (along with isosafrole and the synthetic compound, dihydrosafrole) as a food additive in the U.S., which included its use as a flavoring agent for root beer (at levels up to 20 mg/l).

Safrole is a major component (from 80 to 93%, depending on the plant variety) of sassafras oil, and is also present in lesser amounts (usually <1 to 10% of the oil) in sweet basil, nutmeg, mace, star anise, ginger, black pepper, and cinnamon leaf. Although banned as a food additive, sassafras bark is still being used as a herbal tea or folk medicine. According to Segelman et al., a single, commercially bought herbal tea bag contained the equivalent of 200 mg safrole. β-Asarone was once used as a bitter flavor in liqueurs and vermouth at levels up to 10 to 30 ppm, prior to banning by the Food and Drug Administration (FDA) in 1967. Its food use is still permitted in some countries, and calamus drugs containing β-asarone are currently being used in Europe. Estragole, the major constituent of the essential oils of tarragon and basil, has been used in gourmet vinegars and in certain foods such as candy, chewing gum, and ice cream at levels ranging from 2 to 50 ppm. Sesamol is a minor component of sesame seed oil, being found at 4.3 to 45 ppm, depending on the processing method.

Systematic structure-activity investigations of the carcinogenicity of safrole and related compounds were conducted by the Millers and coworkers (see review by Miller and Miller, 1983). They found mice were more susceptible than rats, and that the liver was the carcinogenic target organ for safrole, isosafrole, and estragole; however, the carcinogenic potency of safrole was relatively low, with liver adenomas usually produced in rats and mice fed relatively high dietary levels (0.5 to 1%). In 1976, the IARC determined that safrole and isosafrole were hepatocarcinogenic in rodents. β-Asarone was unusual in that dietary administration produced leiomyosarcomas of the small intestine of the rat, and sesamol induced rodent forestomach tumors. Mutagenicity of safrole and related compounds was determined to be of low activity, low potency, poor consistency, and with little relationship to their carcinogenic activity.

The evidence linking human exposure to naturally occurring alkenylbenzene compounds and potential carcinogenic risk is rather weak. Suggestions that exposure to these compounds may be associated with esophageal cancer are unsupported. Since these compounds occur in the food supply at low part-per-million levels, and in light of their relatively weak mutagenic
<table>
<thead>
<tr>
<th>Common Name</th>
<th>Chemical Name</th>
<th>Structure</th>
<th>Ref. (Carcinogenicity)</th>
<th>Natural Sources</th>
<th>Ref. (Sources)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Safrole</td>
<td>1-Allyl-3,4-methylenedioxybenzene</td>
<td><img src="" alt="Structure" /></td>
<td>6–9</td>
<td>Sassafras, sweet basil, cinnamon, black pepper, ginger, mace, nutmeg</td>
<td>5, 25</td>
</tr>
<tr>
<td>Isosafrole</td>
<td>3,4-Methylenedioxy-1-propenylbenzene</td>
<td><img src="" alt="Structure" /></td>
<td>10, 11</td>
<td>Rarely found in essential oils of some spices; generally similar distribution as safrole</td>
<td>5, 25, 27</td>
</tr>
<tr>
<td>Estragole</td>
<td>1-Allyl-4-methoxybenzene</td>
<td><img src="" alt="Structure" /></td>
<td>9</td>
<td>Tarragon, sweet basil, anise</td>
<td>5</td>
</tr>
</tbody>
</table>
Methyl eugenol  
1-Allyl-3,4-dimethoxybenzene

\[ \text{OCH}_3 \text{OCH}_3 \]
\[ \text{H}_2\text{C} - \text{CH} = \text{CH}_2 \]

Sweet bay, cloves, lemon grass, black pepper  
9, 28

Sesamol  
1-Hydroxy-3,4-methylenedioxybenzene

\[ \text{O} - \text{CH}_2 \]
\[ \text{O} \]

Sesame oil  
13, 26

\[ \text{OH} \]

β-Asarone  
cis-1-Propenyl-2,4,5-trimethoxybenzene

\[ \text{OCH}_3 \text{OCH}_3 \]
\[ \text{CH}_3 \text{O} \]
\[ \text{C} = \text{C} - \text{CH}_3 \]

Oil of calamus  
12, 30
and carcinogenic activity, it is likely that they make a negligible contribution in the etiology of human cancers.

### 3 Allyl Isothiocyanate

Naturally occurring allyl isothiocyanate (AITC) is found in a large number of commonly consumed vegetables. Most are found in the *Brassica* genus (which is in the Cruciferae family), which includes broccoli, Brussels sprouts, cabbage, cauliflower, collards, kale, mustard, rutabaga, and turnips. AITC is also found in garlic, onion, and horseradish. Normally, AITC and other isothiocyanates are found in the seeds, roots, and leaves of plants as glycosides, which must be enzymatically hydrolyzed to release their active form. For example, the natural form of AITC in mustard seeds is the glucosinolate sinigrin. During processing, cooking, or even maceration, in the presence of water and the enzyme myrosinase, AITC is released and constitutes more than 90% of the resulting volatile mustard seed oil. Food use of synthetically prepared AITC and naturally occurring volatile oil of mustard is quite extensive as flavoring agents at low concentrations (usually not greater than 88 ppm) in pickled products, condiments, and spice flavors. At least 50 chemically different glucosinolates have been identified in the nearly 300 plants in the Cruciferae family.

AITC (food grade, greater than 93% purity) was tested for carcinogenicity by gastric intubation in mice and rats under the National Cancer Institute/National Toxicology Program (NCI/NTP) Bioassay Program. No evidence of tumors was seen in mice, but an increased incidence of urinary bladder tumors (a rare rodent neoplasm) was observed in male rats only. Equivocal evidence of mammary tumors was seen in female rats. Evidence of genotoxicity in short-term tests has been evaluated by the IARC and was found to be limited. Based on the available *in vivo* and *in vitro* data, the NTP and IARC both concluded that there was limited evidence of the carcinogenicity of AITC in experimental animals. In the absence of epidemiological studies, confirmatory animal carcinogenicity studies, or mechanistic data, it would be premature to assess the carcinogenicity of dietary AITC in humans based on the limited *in vitro* and *in vivo* animal data available.

### 4 Bracken Fern Toxins

Bracken fern (BF) (*Pteridium aquilinum*) is sporadically distributed throughout the world, with its consumption, which occurs in many different forms due to varied preservation methods, limited mostly to Japan, New Zealand, Canada, and northeastern U.S. Despite evidence of carcinogenicity for nearly 40 years, consumption remains high. Attempts have been made to reduce its carcinogenicity by boiling, but data indicate this is only partially effective in reducing its carcinogenicity; however, complete reduction has been shown with intensive alkaline treatment or thorough washing in the preparation of the flour. Indirect consumption occurs via milk and dairy products in some countries, with experimental evidence demonstrating the transference of the carcinogenic factor(s) into cow’s milk.

The earliest evidence for the carcinogenicity of BF came via reports of urinary bladder neoplasia in cattle fed bracken fern for extended time periods. Subsequent controlled experiments with laboratory animals and cows fed bracken fern confirmed these findings. Initially, the small intestine (specifically, the ileum) and the urinary bladder were identified as the target organs, although additional sites of tumorigenicity have since been demonstrated (see Table 2). Attempts to isolate and characterize the carcinogenic agent(s) in bracken fern focused on three compounds: quercetin, shikimic acid, and ptaquiloside (Table 3). Upon dietary administration to rodents, each compound produced tumors in the
intestine and urinary bladder that were grossly and histomorphologically similar to those in BF-fed rats; however, the evidence for quercetin and shikimic acid is controversial since subsequent studies failed to confirm ileal and urinary bladder tumors in other rat strains or other species (see Section 6 for more on quercetin). On the other hand, the evidence for ptaquiloside as the principal carcinogenic of BF is strong but not conclusive, since confirmatory studies await to be done.

Detailed examination of BF isolates for mutagenicity using the Ames’ *Salmonella* assay and mammalian cells did not reveal any significant activity due to shikimic acid. In contrast, Van der Hoeven et al. determined that ptaquiloside was a potent *Salmonella* mutagen, accounting for over half of the mutagenic activity of methanol extracts of BF. Quercetin is also a very potent bacterial mutagen. Epidemiological evidence indicating that the high incidence of stomach cancer in Japan may be partially due to BF is confounded by poor exposure data and the presence of other environmental influences. The IARC has evaluated BF and determined the carcinogenicity evidence to be inadequate in humans and sufficient in animals (classified as a Group 2B carcinogen). Although the putative carcinogenic principle of BF has been identified as ptaquiloside, its possible role in the etiology of human cancer awaits further elucidation.

### TABLE 2

Carcinogenic Target Organs for Bracken Fern (BF), Ptaquiloside (PT), Quercetin (QT), and Shikimic Acid (SA)

<table>
<thead>
<tr>
<th>Species</th>
<th>Small Intestine</th>
<th>Cecum</th>
<th>Large Intestine</th>
<th>Urinary Bladder</th>
<th>Mammary Gland</th>
<th>Lung</th>
<th>Blood Cells</th>
<th>Liver/Bile Duct</th>
<th>Various Sites</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>BF, PT, QT</td>
<td>BF</td>
<td>BF, PT, QT</td>
<td>BF, PT</td>
<td>44–47,</td>
<td>60–61,</td>
<td>56–58</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>BF</td>
<td>BF</td>
<td>BF</td>
<td>BF</td>
<td>SA^a</td>
<td>50–52,</td>
<td>59</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hamster</td>
<td>BF</td>
<td>BF</td>
<td>BF</td>
<td>BF</td>
<td>51</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guinea pig</td>
<td>BF</td>
<td>BF</td>
<td>BF</td>
<td>BF</td>
<td>51</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quail</td>
<td>BF^a</td>
<td>BF^+</td>
<td>BF^a</td>
<td>BF^+</td>
<td>51</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toad</td>
<td>BF</td>
<td>BF</td>
<td>BF</td>
<td>BF</td>
<td>53</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cattle</td>
<td>BF</td>
<td>BF</td>
<td>BF</td>
<td>BF</td>
<td>43, 45,</td>
<td>48, 49</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>BF</td>
<td>BF</td>
<td>BF</td>
<td>BF</td>
<td>51, 55</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

^a Hot ethanol extract of BF.  
^b Six glandular stomach tumors, four leukemias, one lung tumor.

5 CINNAMIC ACID DERIVATIVES

Cinnamic acid derivatives are phenolic acids that usually occur in nature in the conjugated or esterified form, commonly with quinic acid or sugars. For example, chlorogenic acid, which is particularly abundant in coffee beans (up to 3.8%) is an ester of caffeic and quinic acid and is commonly found in several isomeric and derivatized forms (Figure 1). Coumarin shares the same three carbon side-chain as cinnamic acid, but with the chain formed into a oxygen heterocycle, or lactone (Figure 1). This section will discuss the available evidence of carcinogenicity for caffeic acid and coumarin.

Caffeic acid, chlorogenic acid, and closely related compounds are present in a variety of vegetables, fruits, and seasonings. Ester conjugates may be hydrolyzed upon ingestion, yielding a variable absorption of caffeic acid. Caffeic acid (free and conjugated) is abundant
<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Chemical Name</th>
<th>Level in BF (mg/kg)²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ptaquiloside</td>
<td><img src="image1.png" alt="Ptaquiloside Structure" /></td>
<td>7(^{a})-(β-D-Glucopyranosyloxy)-1(^{a}),3(^{a}),4(^{a}),7(^{a})-tetrahydro-4(^{-})-hydroxy-2(^{a}),4(^{a}),6(^{a})-trimethyspiro-[cyclopropane-1,5(^{a})-(5(^{H})-inden)]-3(^{a})-(2(^{a})(^{H}))-one</td>
<td>210–2400</td>
</tr>
<tr>
<td>Quercetin</td>
<td><img src="image2.png" alt="Quercetin Structure" /></td>
<td>2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4(^{H})-1-benzopyran-4-one</td>
<td>570</td>
</tr>
</tbody>
</table>
Shikimic Acid

3,4,5-Trihydroxy-1-cyclohexene-1-carboxylic acid

* Dry weight

(50 to 200 ppm) in apple, carrot, celery, cherry, eggplant, endive, grapes, lettuce, pear, plum, potato (peel), and in a number of herbs (e.g., thyme, basil, aniseed, caraway, rosemary, tarragon, marjoram, savory, sage, dill, and absinthe) at concentrations greater than 1000 ppm.75,76 Beverages, such as coffee, apple juice, and wine are other significant sources.75

Caffeic acid, or 3,4-dihydroxycinnamic acid, is a natural anti-oxidant, with antitumorigenic activity in a number of animal models;77,78 however, Hagiwara et al.79 found that the chronic feeding of 2% caffeic acid to rats and mice produced a significant increase in tumors of the forestomach, kidney, and lung (the latter in male mice only and within historical range). Earlier work by the same laboratory using the same model and protocol found only forestomach tumors in rats and mice.80 The authors concluded that caffeic acid is a weak, nongenotoxic carcinogen that appears to exert a tumorigenic response via secondary mechanisms, and the human carcinogenic risk may be negligible. In vitro studies determined that caffeic acid was not mutagenic in the Ames' Salmonella assay but did show some weak genotoxic activity in nonbacterial assays.81-83 There is no epidemiological data available. Despite these limited and tenuous findings of carcinogenicity, the IARC determined that there was sufficient evidence in laboratory animals for the carcinogenicity of caffeic acid, and the overall evaluation was that caffeic acid was "possibly carcinogenic to humans" (i.e., Group 2B).75 Until further evidence of carcinogenicity becomes available, concerns about the possibility of mechanisms and tumor manifestations unique to the rodent should prevent the consideration of caffeic as a human carcinogen.

Coumarin (2H-1-benzopyron-2-one) is present in a variety of plants (e.g., tonka beans, sweet clover, and woodruff) and essential oils (e.g., lavender).84 Although banned in the U.S. in 1954, coumarin is still used in Europe as a food additive in some alcoholic beverages and wines.85 Several carcinogenicity studies in rats,86,87 hamsters,88 and baboons89 have found contradictory results. Coumarin is either inactive or weakly active in the Ames' Salmonella mutagenic assay.90 In 1975, the IARC examined the available carcinogenicity evidence for coumarin and concluded that it was carcinogenic in rats;91 however, in 1990, the NTP felt that the evidence was less than satisfactory and examined the carcinogenicity of coumarin by gavage in mice and rats.92 Results in F334/N rats indicated that there was some evidence of carcinogenic activity in males and equivocal evidence in females, based on an increased incidences of renal tumors. In B6C3F1 mice, results indicated that there was some evidence of carcinogenicity in males based on lung tumors. Clear evidence of carcinogenicity was evident in female mice based on increased incidences of lung and liver tumors. Nonetheless, given the less than consistent results in the various carcinogenicity assays coupled with concerns about species differences in coumarin metabolism93 and the lack of epidemiological data, the role that coumarin may have in the etiology of human cancer is uncertain.

6 FLAVONOIDS

Flavonoids, and their glycosides, are a broad group of related polyphenolic compounds that occur widely in the plant kingdom. These hydrophilic substances have an important influence on the flavor and taste of many foods. They possess the fundamental chemical
structure of the parent compound, flavone (2-phenylbenzopyrone). Hydroxyl, methoxy, and saccharide moieties are attached at various locations. D-glucose is the most common sugar, but D-galactose, L-rhamnose, L-arabinose, D-xylose, and D-apiose, as well as some uronic acids, can also be found. The flavonoids can be subdivided as flavones, flavonols, flavanones, iso flavonones, and catechins. About 2000 individual members of the flavonoid class have been described,\textsuperscript{94} with most of the compounds occurring in the form of various glycosides. For example, quercetin, the most common flavonol, has over 70 glycosidic combinations that have been fully characterized, with many more partially identified. The flavonol glycosides, especially those of quercetin (e.g., rutin, isoquercitrin, and quercitrin), and kaempferol (e.g., astragalin and tiliroside), are found at significant concentrations in the edible portion of the majority of plant foods, e.g., fruits, berries, leaf and root vegetables, cereal grains, tea, coffee, and cocoa.\textsuperscript{95} For a good review of the levels of quercetin (see Table 3 for structure) and kaempferol in various edible plants see Reference 96. A rough estimate of the total daily intake of flavonoids in the average American diet is about 1 g, with daily intake of quercetin, and its related forms, of about 25 mg per person.\textsuperscript{97}

It was this large and widespread consumption of flavonoids that generated considerable interest when in 1977 it was reported that quercetin and kaempferol were mutagenic in the Ames’ \textit{Salmonella} assay.\textsuperscript{98-100} Nearly 75 aglycones of various flavonoids have been tested in the Ames’ \textit{Salmonella} assay, with about one third positive (for a general review of the mutagenicity of flavonoids, see Brown\textsuperscript{101} and Nagao et al.\textsuperscript{102}). Despite considerable investigation into the structural requirements for mutagenicity in many of these compounds,\textsuperscript{103} only a handful have been adequately tested for carcinogenicity. Quercetin and rutin (which is quercetin conjugated to rutinose) have been the focus of the majority of carcinogenicity studies, and aside from three studies by Pamukçu and colleagues,\textsuperscript{104-106} all results have been negative. Interestingly, the initial investigations by Pamukçu, Erturk, and coworkers found intestinal and urinary bladder tumors in Norwegian rats,\textsuperscript{104} but their later studies found that dietary quercetin significantly increased liver tumors in F344\textsuperscript{105,106} and Sprague-Dawley rats.\textsuperscript{106} Reasons for this discrepancy might be related to differences in the rat strains used and the dietary levels of quercetin; however, other long-term rat, mouse, and hamster feeding studies of quercetin (and rutin) have not confirmed this carcinogenic effect (see Table 4).

Despite the preponderance of negative studies of carcinogenicity for quercetin, the NTP undertook in 1992 a 2-year carcinogenicity feeding study in rats.\textsuperscript{107} Evidence from this study found a dose-responsive increase in renal tumors in male rats only. Based on this, along with positive results from genotoxicity studies, the NTP determined there was some evidence of carcinogenicity. Similarly, the IARC concluded in 1983 there was limited evidence for the carcinogenicity of quercetin in animals, but without epidemiological data no determination of the carcinogenicity of quercetin to humans could be made.\textsuperscript{96}

In contrast to the multiple investigations into the carcinogenicity of quercetin, the investigation of kaempferol has been limited to one study, which showed no effect.\textsuperscript{108} It is mutagenic in the Ames’ \textit{Salmonella} assay, with other limited evidence of genotoxicity.\textsuperscript{101} Overall, the IARC determined there was inadequate evidence to determine the carcinogenicity of kaempferol.\textsuperscript{109} Epidemiological studies of the relationship between flavonoid intake and human cancer incidence have not been conducted, and hence the role that quercetin or kaempferol may have in the etiology of human cancer is uncertain.

\section{FUROCOUMARINS}

Furocoumarins encompass a group of secondary plant metabolites which surprisingly have a chemical relationship to aflatoxins by virtue of their common use of the coumarin ring structure. Furocoumarins can be divided into two classes, the larger of which are the linear furocoumarins that are derived from the most common member, psoralen. The second, smaller
group is called angular furocoumarins because the furan ring is attached at an angle to the coumarin ring. Angelicin, which is the unsubstituted ring analog, defines this group. The physical and chemical properties of a number of furocoumarins can be found in Reference 119. Of the nearly 30 different psoralen derivatives that have been isolated from natural sources, 10 have tested for carcinogenicity. Of these, only the three that have been adequately tested by the oral route will be discussed.

Occurrence of furocoumarins is common in a number of plant species, especially those in the Rutaceae and Umbelliferae families. Human exposure to furocoumarins is predominately from limes (upwards of 97% due to lemon-lime flavored beverages), with a secondary source being celery. Parsley, parsnip, carrots, and other citrus fruits contribute a small amount to the diet. Estimated total dietary furocoumarin exposure ranges from 10 to 35 mg/kg body weight (b.w.), depending on a person’s age, race, and gender (see Wagstaff120 for a good review of dietary exposure).

Psoralen is found at relatively low concentrations (1 to 10 ppm) in celery, parsley, and parsnip.120 5-Methoxypsoralen (5-MOP, or bergapten) is found in the oil of bergamot (a Mediterranean citrus fruit) and is used as a flavoring agent in foods and as a fragrance in perfumes and other consumer products.121 Other naturally occurring sources of 5-MOP are from the same Umbelliferae plants as psoralen, but in some cases at 2 to 3 times its concentrations.120 Dietary intake of 8-methoxypsoralen (8-MOP, or methoxsalen) occurs through the ingestion of parsley and parsnip, with concentrations in parsnip root reported to be 26 to 29 ppm,122 or up to 1100 ppm.123 (See Figure 2 for chemical structures.)

Since the psoralens are phototoxic agents, carcinogenicity studies have generally been limited to topical application in combination with ultraviolet radiation.124-126 Results of these studies clearly indicate that under these conditions psoralen and 5-MOP produced skin tumors. Mutagenicity and genotoxicity studies were also positive, but in most cases light activation was required.119 Based on the evidence, the IARC has determined that there was

### TABLE 4

<table>
<thead>
<tr>
<th>Compound Tested</th>
<th>Dietary Level</th>
<th>Species</th>
<th>Result</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rutin</td>
<td>1%</td>
<td>Rat</td>
<td>Neg.</td>
<td>110</td>
</tr>
<tr>
<td>Quercetin</td>
<td>0.25–1%</td>
<td>Rat</td>
<td>Neg.</td>
<td>111</td>
</tr>
<tr>
<td>Quercetin</td>
<td>0.25–1%</td>
<td>Rat</td>
<td>Neg.</td>
<td>111</td>
</tr>
<tr>
<td>Quercetin</td>
<td>0.1%</td>
<td>Rat</td>
<td>Intestinal and bladder tumors</td>
<td>104</td>
</tr>
<tr>
<td>Quercetin</td>
<td>2%</td>
<td>Mouse</td>
<td>Neg.</td>
<td>112</td>
</tr>
<tr>
<td>Quercetin</td>
<td>1, 5, 10%</td>
<td>Rat</td>
<td>Neg.</td>
<td>113</td>
</tr>
<tr>
<td>Rutin</td>
<td>5, 10%</td>
<td>Rat</td>
<td>Neg.</td>
<td>113</td>
</tr>
<tr>
<td>Quercetin</td>
<td>5%</td>
<td>Mouse</td>
<td>Neg.</td>
<td>114</td>
</tr>
<tr>
<td>Quercetin</td>
<td>1, 4, 10%</td>
<td>Hamster</td>
<td>Neg.</td>
<td>115</td>
</tr>
<tr>
<td>Rutin</td>
<td>10%</td>
<td>Hamster</td>
<td>Neg.</td>
<td>115</td>
</tr>
<tr>
<td>Quercetin</td>
<td>1, 2%</td>
<td>Rat</td>
<td>Hepatomas and bile duct tumors</td>
<td>105</td>
</tr>
<tr>
<td>Quercetin</td>
<td>5%</td>
<td>Rat</td>
<td>Neg.</td>
<td>116</td>
</tr>
<tr>
<td>Quercetin</td>
<td>0.1%</td>
<td>Rat</td>
<td>Neg.</td>
<td>108</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>0.04%</td>
<td>Rat</td>
<td>Neg.</td>
<td>108</td>
</tr>
<tr>
<td>Quercetin</td>
<td>1, 2%</td>
<td>Rat</td>
<td>Hepatic tumors and biliary adenomas</td>
<td>106</td>
</tr>
<tr>
<td>Quercetin</td>
<td>0.5%</td>
<td>Rat</td>
<td>Hepatomas</td>
<td>106</td>
</tr>
<tr>
<td>Rutin</td>
<td>2%</td>
<td>Rat</td>
<td>Hepatomas</td>
<td>106</td>
</tr>
<tr>
<td>Quercetin</td>
<td>1.25, 5%</td>
<td>Rat</td>
<td>Neg.</td>
<td>117</td>
</tr>
<tr>
<td>Quercetin</td>
<td>2%</td>
<td>Mouse</td>
<td>Neg.</td>
<td>118</td>
</tr>
<tr>
<td>Rutin</td>
<td>4%</td>
<td>Mouse</td>
<td>Neg.</td>
<td>118</td>
</tr>
</tbody>
</table>

---

*a Neg. = negative; no difference from control group.

*b Only lung tissue was examined.
sufficient evidence of carcinogenicity in laboratory animals in combination with ultraviolet or solar-stimulated radiation, but there was inadequate evidence of their carcinogenicity in humans. In addition, the IARC deemed the evidence was inadequate to evaluate their systemic carcinogenicity in animals or humans. The IARC has also determined that there was insufficient evidence of carcinogenicity for other psoralens and angelicins.\textsuperscript{119}

Three years after the IARC evaluation, the NTP completed a rat gavage study of 8-MOP (without light activation) and found renal and other tumors in male rats.\textsuperscript{127} In addition, 8-MOP was mutagenic without light activation (but with metabolic activation) in the Ames’ Salmonella assay and produced genotoxicity in other tests.\textsuperscript{126} The NTP concluded that there was clear evidence of carcinogenicity for 8-MOP; therefore, based on this evidence, structure-activity relationship data, and the significant human dietary exposure to furocoumarins, there is valid concern about the human carcinogenic risk posed by 8-MOP and possibly other psoralens, under certain conditions.

8 HETEROCYCLIC AMINES

The seminal work in 1977 by Sugimura and colleagues\textsuperscript{128,129} led to the realization that the burnt and browned material produced by the cooking of meat was highly mutagenic as measured by the Ames’ Salmonella assay. The reader is referred to Eisenbrand and Tang\textsuperscript{130} and Chen et al.\textsuperscript{131} for good overall literature reviews. Further studies by Sugimura and others\textsuperscript{132-136} showed that the pyrolysis products of certain amino acids and proteins, as well as the charred surface of meat, contained strongly mutagenic substances, which were eventually isolated and chemically defined.\textsuperscript{137-141} These pyrolytic mutagens were found to be formed most efficiently under extreme cooking conditions involving high temperatures (300°F or greater), and, except for charred surfaces, they are normally not found in cooked foods in significant quantities. All these compounds are heterocyclic amines (HAs) (except Lys-P-1, which is a heterocyclic imine), and most can be further subdivided into pyridoindoles and pyridoimidazoles. They require metabolic activation to exert mutagenic activity, and some have demonstrated carcinogenic activity (see Table 5). Additional investigations using commercial beef extract, cooked ground beef, and broiled sardines revealed the presence of a new class of HAs which were formed under more realistic cooking conditions\textsuperscript{142-151} (see Table 6). It became apparent that these HAs, which were further subdivided into three subgroups (i.e., imidazoquinolines, imidazoquinoxalines, and imidazopyridines),\textsuperscript{148} contribute most of the mutagenic activity of cooked meat. Formation has been hypothesized to occur as result of a browning, or Mallard reaction, and involves creatinine or creatine, free amino acids, and monosaccharides.\textsuperscript{152-156}

Heterocyclic amines have been found to be carcinogenic in various organs in mice, rats, and nonhuman primates in long-term feeding studies. Most of the research was done in Japan and generally involved dietary administration with one or two doses, usually at the MTD (maximum tolerated dose) and some large fraction of the MTD.\textsuperscript{130,131} The amino acid
<table>
<thead>
<tr>
<th>Structure</th>
<th>Scientific Name</th>
<th>Abbreviation</th>
<th>1° Carcinogenicity Target Site(s)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Food Sources&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Carcinogenicity Ref.</th>
</tr>
</thead>
</table>
| CH₃\(\text{N}^\text{NH}_2\)CH₃  
\(\text{N}\) \(\text{H}\)  
\(\text{CH}_3\) | 3-Amino-1,4-dimethyl-5\(\text{H}\)-pyrido[4,3-\text{b}]indole | Trp-P-1 | M — liver; R — liver | Broiled, grilled sardines; broiled chicken; broiled fried beef | 191, 192 |
| CH₃\(\text{N}^\text{NH}_2\)CH₃  
\(\text{N}\) \(\text{H}\) \(\text{CH}_3\)  
\(\text{N}\) | 3-Amino-1-methyl-5\(\text{H}\)-pyrido[4,3-\text{b}]indole | Trp-P-2 | M — liver; R — liver, clitoral gland, urinary bladder, mammary gland, hematopoietic system | Broiled, grilled sardines; broiled fried beef; boiled beef extract; broiled chicken; broiled mutton | 193, 194 |
| CH₃\(\text{N}^\text{NH}_2\)CH₃  
\(\text{N}\) \(\text{H}\)  
\(\text{CH}_3\) \(\text{N}\) | 2-Amino-6-methyldipyrido[1,2-\text{α}:3',2'-\text{d}]imidazole | Glu-P-1 | M — liver, blood vessels; R — liver, small and large intestine, brain, clitoral gland, Zymbal gland | Broiled fish | 195, 196 |
<table>
<thead>
<tr>
<th>Compound</th>
<th>Glu-P</th>
<th>M → liver, blood vessels; R → liver, small and large intestine, brain, clitoral gland, Zymbal gland</th>
<th>Broiled, grilled fish</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Aminodipyrido[1,2-α;3',2'-df]-imidazole</td>
<td>Glu-P</td>
<td>M → liver, blood vessels; R → liver, small and large intestine, brain, clitoral gland, Zymbal gland</td>
<td>Broiled, grilled fish</td>
<td>195, 196</td>
</tr>
<tr>
<td>2-Amino-9H-pyrido[2,3-b]indole</td>
<td>AαC</td>
<td>M → liver, blood vessels</td>
<td>Grilled beef; broiled chicken; broiled mutton; baked, broiled, barbecued salmon; fried fish</td>
<td>195</td>
</tr>
<tr>
<td>2-Amino-3-methyl-9H-pyrido[2,3-b]indole</td>
<td>MeAαC</td>
<td>M → liver, blood vessels</td>
<td>Grilled beef; broiled chicken; broiled mutton</td>
<td>195</td>
</tr>
</tbody>
</table>

*a M = mouse; R = rat.
b Representative food sources.
<table>
<thead>
<tr>
<th>Structure</th>
<th>Scientific Name</th>
<th>Abbrev.</th>
<th>&quot;°&quot; Carcinogenicity Target Site(s)( ^a )</th>
<th>Food Sources( ^b )</th>
<th>Carcinogenicity Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>2-Amino-3-methyl-imidazo[4,5-f]quinoline</td>
<td>IQ</td>
<td>M — liver, forestomach, lung; R — liver, small and large intestine, mammary gland, Zymbal gland, clitoral gland, skin; P — liver</td>
<td>Broiled, fried beef; boiled beef extract; fried pork; broiled sardines; broiled salmon; fried fish; fried egg</td>
<td>161, 197–199</td>
</tr>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>2-Amino-3,4-dimethyl-imidazo[4,5-f]quinoline</td>
<td>MeIQ</td>
<td>M — liver, forestomach; R — Zymbal gland, oral cavity, colon, skin, mammary gland</td>
<td>Fried beef; boiled beef extract; fried pork; broiled sardine; broiled salmon</td>
<td>200, 201</td>
</tr>
<tr>
<td><img src="image" alt="Structure" /></td>
<td>2-Amino-3,4-dimethyl-imidazo[4,5-f]quinoline</td>
<td>4-MeIQx</td>
<td>M — liver, lung, hematopoietic system</td>
<td>Broiled, fried beef; broiled, barbecued chicken; broiled mutton; boiled beef extract; fried pork; bacon; smoked, dried tuna; fried fish; baked, broiled, barbecued salmon</td>
<td>202, 203</td>
</tr>
</tbody>
</table>
2-Amino-3,8-dimethylimidazo[4,5-f]quinoxaline

\[ \begin{array}{c}
\text{H}_3\text{C} \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N\textsubscript{1}} \end{array} \]

\[ \begin{array}{c}
\text{N} \\
\text{N} \\
\text{N} \\
\text{CH}_3 \\
\text{NH}_2 \end{array} \]

R — liver, Zymbal gland, skin

Broiled, fried beef; broiled, barbecued chicken; broiled mutton; boiled beef extract; fried pork; bacon; smoked, dried tuna; fried fish; baked, broiled, barbecued salmon

202, 203

2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine

\[ \begin{array}{c}
\text{CH}_3 \\
\text{N} \\
\text{N} \\
\text{N} \\
\text{N} \end{array} \]

\[ \begin{array}{c}
\text{N} \\
\text{NH}_2 \end{array} \]

M — lymph nodes, lung, spleen;
R — colon, mammary gland

Fried beef; broiled chicken; broiled mutton; fried, barbecued pork; bacon; baked, broiled, barbecued salmon; fried fish

204–206

\(^a\) M = mouse; R = rat, P = primate.

\(^b\) Representative food sources.
pyrolysates (Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2, MeAαC, and AαC) have primarily produced
tumors of the liver and blood vessels in mice and the liver and intestines in rats (see Table 5). The murine carcinogenicity target organ for the low-temperature-forming HAs (i.e., IQ, MeIQ, MeIQx, and PhIP) was mainly the liver, but other sites included the forestomach, lung, and hematopoietic system (see Table 6). Rats developed tumors in the liver, intestines, mammary gland, skin, and other organs. It appeared from these studies that females were more susceptible than males and that PhIP was uniquely nonhepatocarcinogenic in both rodent species (see reviews by Sugimura and Sato,157 Ohgaki et al.,158 Wakabayashi et al.,159 and Munro et al.160). IQ was tested by gavage in macaque monkeys and produced hepatocellular carcinoma at doses much less than the MTD.161 Based on tumor potency estimates (i.e., TD50 values), the carcinogenic potency of some HAs appears to be comparable to other carcinogens such as N-nitrosodimethylamine and dibenzo[a,h]anthracene. In evaluating the carcinogenicity of IQ, MeIQ, MeIQx, and PhIP, the IARC determined that there was inadequate evidence in humans but sufficient evidence in experimental animals.162 The overall evaluation by IARC classified these compounds as being “probably carcinogenic to humans” (Group 2A).

The importance of cooking method, time, and temperature in the formation of HAs has
been well demonstrated.133,163-168 In general, frying or flame-broiling meats produced the
greatest mutagenic activity and heterocyclic amine formation.169,171 Deep frying, roasting,
and baking produced a lesser response, and stewing, steaming, poaching, and microwave
cooking showed little mutagenic activity.172 Pariza and coworkers135 studied the mutagenic
activity of hamburgers fried at 143, 191, and 210°C and found activity remained low at all
cooking times (4 to 20 min) at the lowest temperature; however, frying at 191 or 210°C for
up to 10 min resulted in a considerably greater response. Other researchers have also con-
firmed the importance of cooking temperature and have generally found a sharp rise in the
rate of mutagen formation from 140 to 180°C.173 In general, frying or broiling of meats
results in a 10- to 50-fold increase in the mutagenic activity as opposed to other methods of
cooking.171 For a summary of HA ranges in various cooked meats, see Table 7.

In 1982, Bjeldanes and colleagues169,170 conducted an extensive survey of mutagen
formation in the cooking of various sources of protein in the U.S. diet and found significant
amounts in fried ground beef, broiled beef steak, ham, pork chops, bacon, and baked and
broiled chicken. Other sources of protein, such as milk, cheese, tofu, and organ meats,
produced insignificant amounts of mutagens upon cooking. Analysis of canned food indicated
beef- and seafood-containing products exhibited mutagenic activity,174 while crackers, corn
flakes, rice cereal, bread crust, and toast exhibited low activity.135 Cooking method has only
a slight effect on the mutagenic activity for eggs, vegetables, and predominately carbohydrate-
Based on the totality of evidence, it is clear that certain HAs (IQ, MeIQ, MeIQx, and PhIP) are animal carcinogens and should be considered as presumptive human carcinogens, too. It should be realized that normal consumption levels of HAs are minute in comparison to their TD50 values (i.e., dose at which 50% of the animals develop tumors). Hence, it is
### TABLE 7

Range of Heterocyclic Amine (HA) Concentrations in Cooked Meats and Fish

<table>
<thead>
<tr>
<th>Food</th>
<th>Cooking Method</th>
<th>Temp. (°C)</th>
<th>Time (min)</th>
<th>IQ</th>
<th>8-MeIQx (ng/g)</th>
<th>DiMeIQx (ng/g)</th>
<th>PhIP (ng/g)</th>
<th>AoC (ng/g)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground beef</td>
<td>Fried</td>
<td>150–300</td>
<td>2–12</td>
<td>0–1.8 (0.1)</td>
<td>0–10.8 (0.8)</td>
<td>0–9.35 (0.25)</td>
<td>0–21.8 (1.2)</td>
<td>—</td>
<td>146, 148, 159, 178, 179, 180, 181, 183, 184, 186, 188</td>
</tr>
<tr>
<td>Beef steak</td>
<td>Broiled or fried</td>
<td>190–225</td>
<td>3–6.5</td>
<td>—</td>
<td>0.5–8.3 (5.1)</td>
<td>0.1–2 (1.3)</td>
<td>0.6–48.5 (23.5)</td>
<td>1.2–8.9 (3.2)</td>
<td>159, 184, 187, 190</td>
</tr>
<tr>
<td>Ground pork</td>
<td>Fried</td>
<td>180–250</td>
<td>5</td>
<td>0.01–0.04</td>
<td>0.4–1.4</td>
<td>0.24–0.6</td>
<td>1.7–4.5</td>
<td>—</td>
<td>182, 189</td>
</tr>
<tr>
<td>Salmon</td>
<td>Fried</td>
<td>200</td>
<td>3–12</td>
<td>—</td>
<td>1.4–4.7 (3.7)</td>
<td>—</td>
<td>1.7–17 (14)</td>
<td>0–9 (4.6)</td>
<td>185</td>
</tr>
<tr>
<td>Salmon</td>
<td>Baked</td>
<td>200</td>
<td>20–40</td>
<td>—</td>
<td>0–4.6 (3.1)</td>
<td>—</td>
<td>0–18 (5.9)</td>
<td>0</td>
<td>185</td>
</tr>
<tr>
<td>Salmon</td>
<td>Broiled or barbecued</td>
<td>270</td>
<td>4–12</td>
<td>—</td>
<td>0</td>
<td>2–73 (69)</td>
<td>2.8–109 (73)</td>
<td>185</td>
<td></td>
</tr>
</tbody>
</table>

*Heterocyclic amine range concentrations are included contingent only on two or more independent determinations (most ranges include four determinations and some as many as 20).*
likely that only an unusual diet that involved the large consumption of fried and/or broiled meat might pose a significant human carcinogenic risk.

9 HYDRAZINES AND THEIR DERIVATIVES

Hydrazines have long been known to organic chemists due to their high reactivity, but it was not until 1951 that the first example of a naturally occurring nitrogen-nitrogen double bond was reported. Since then, more than 40 compounds have been reported in bacteria, fungi, and higher plants. The most common human dietary exposure to these compounds probably occurs through the ingestion of mushrooms and cycad seeds.

In the early 1960s, agaritine \((\beta-N-[\gamma-L(+)]\text{glutamyl}][4\text{-hydroxy-methylphenylhydrazine})\) was identified and characterized as a naturally occurring hydrazine obtained from the commonly eaten cultivated mushroom, \textit{Agaricus bisporus}, along with other \textit{Agaricus} spp. Subsequent experiments indicated that \(p\)-aminobenzoic acid and glutamic acid may be the precursors of agaritine.

The carcinogenic potential of hydrazines was elegantly demonstrated in a series of experiments by Toth using nearly forty substituted hydrazine analogs. These studies clearly demonstrated the tumorigenic potential of these compounds in mice, rats, and hamsters, with neoplasia developing in intestines, brain, lungs, blood vessels, liver, mammary gland, and kidneys, among other sites. Carcinogenicity of agaritine was found to be negative in mice; however, three breakdown compounds (4-hydroxymethylphenylhydrazine; \(N^2-[\gamma-L(+)]\text{-glutamyl}\)-4-carboxyphenylhydrazine; and 4-[hydroxymethyl] benzenediazonium ion) were found positive. Another closely related nitrogen-nitrogen bond-containing compound (\(p\)-hydrazinobenzoic acid) was also shown to be carcinogenic. It should be noted that these studies generally administered the metabolites as various salts, often by gavage or via the drinking water. Finally, chronic feeding of uncooked \textit{A. bisporus} to rats produced tumors in several sites. These results can be explained by the instability of agaritine and its likely near complete destruction through storage and cooking. Unfortunately, this does not hold true for its metabolites, which on average are reduced only 25% by baking. Analysis of \textit{A. bisporus} revealed the presence of agaritine at 360 to 700 ppm, while the breakdown products were found at much lower levels (0.6 to 42 ppm).

A weak mutagenic effect of agaritine in the Ames’ \textit{Salmonella} assay was reported. No epidemiological data is available. Based on the totality of evidence, the IARC determined there was insufficient evidence of carcinogenicity for agaritine, but there was limited evidence for the two metabolites of agaritine (or more precisely, their derivatives). Lacking dose-response tumorigenicity studies and epidemiological studies of any sort, it is extremely difficult to assess the role that \textit{A. bisporus} (or its active ingredients) may have in the etiology of human cancer.

Another example of a hydrazine-containing mushroom comes from the false morel (\textit{Gyromitra esculenta}), which is widely eaten in northern Europe. From this mushroom, gyromitrin (acetaldheyde-\(N\)-formyl-\(N\)-methyl-hydrazone) was isolated, and, similar to agaritine, the carcinogenicity of its metabolites was demonstrated in a series of experiments by Toth. However, unlike agaritine, gyromitrin administered in 52 weekly intragastric doses was found be tumorigenic in mice. The gyromitrin content of dried false morel is between 0.05 and 0.3%, and \(N\)-methyl-\(N\)-formylhydrazine, a metabolite, is present at a concentration of 0.06%. Gyromitrin, but not its metabolites, is destroyed by cooking or drying. Gyromitrin was not mutagenic in bacteria, but a metabolite gave positive results. No epidemiological data is available. Based on the totality of the evidence, the IARC determined that there was insufficient evidence for the carcinogenicity of gyromitrin in experimental animals.

The Cycad family is composed of the surviving members of an ancient line of palm-like plants found throughout the tropical and subtropical regions of the world. Of the nine genera,
Cycas are the mostly widely distributed, with a region that includes the south Pacific centered around Indonesia and also Indochina and the west coasts of Africa and Australia. The important species are *Cycas revoluta* and *C. circinalis*, with cycad meal used as a source of food in the form of a starch and sometimes in the bean paste, miso; however, in order to avoid toxicity, its preparation requires great care (for a review of the utilization of cycads, see Whiting). Toxicity is caused by either or both of the glycosides, cycasin or macrozamin. These two compounds differ only in carbohydrate moiety, with cycasin containing glucose and macrozamin containing primeverose. Both contain the active component, methylazoxymethanol (MAM) as the aglycone (for a good review of the chemistry and biological effects of cycasin and related compounds see Zedeck). Cycasin is a major constituent (ranging from 0.5 to 3.6%) of the seeds, husks, trunk, and leaves of the cycad plant.

Interestingly, cycasin is toxic and carcinogenic only when given orally and after passage through the gastrointestinal tract (thus cleaving the glucosidic moiety; see Figure 3), whereas MAM is active irrespective of the route of administration and in germ-free rats. This finding prompted the investigation of dialkylhydrazines, azoalkanes, and azoxyalkanes, which led to the realization that these compounds are potent and selective colon carcinogens. Use of these compounds in experimental models has proven to be of great benefit in investigations of colon carcinogenesis.

Laqueur and his colleagues were the first to reveal that rats fed crude cycad meal developed tumors of the liver and kidney and, rarely, the intestine and lung. Subsequent work identified the carcinogen as cycasin and ultimately led to the conclusion that MAM is the proximate carcinogen. Carcinogenicity of cycasin has been demonstrated in fish, guinea pigs, hamsters, mice, monkeys, rabbits, and rats (see Table 8). Primary sites of tumor initiation included the kidney and intestine for the rat and the liver for mice and hamsters. Effects observed are dependent on species, sex, age, route of administration, and dosing regimen. MAM was mutagenic in the Ames' *Salmonella* assay, but cycasin was inactive. Cycasin, and MAM, are clearly potent animal carcinogens based on tumor development in single-dose experiments; however, without epidemiological data and sound exposure data, the role that cycasin and MAM may have in the etiology of human cancers is uncertain.

**10 MYCOTOXINS**

Although there are many hundreds of naturally occurring, mold-produced entities given the moniker “mycotoxin”, there are only slightly more than a dozen that have sufficient evidence of carcinogenicity to warrant discussion. These include the aflatoxins (B₁, B₂, G₁, G₂, M₁, and aflatoxicol), sterigmatocystin, ochratoxin A, fumonisin B₁, fusarin C, T-2 toxin,
10.1 *Aspergillus* Toxins

A tremendous research effort has gone into the study of a particular group of mycotoxins produced by *Aspergillus* spp., such that more is known about the occurrence and toxicity of this group of toxins than any other natural substance in the food supply. This group of mycotoxins, called aflatoxins, is a collection of more than a dozen closely related compounds that are produced by the mold species, *A. flavus* and *A. parasiticus*. For a good overall review of aflatoxin chemistry, biology, and occurrence in the food supply, see Busby and Wogan.260

Although some contamination happens prior to crop harvesting, the principle outgrowth of these molds occurs under poor storage conditions. Factors that affect preharvest fungal infection are stress related and include drought and insect damage. Mycotoxin production during storage is related to environmental conditions such as humidity and temperature (see review by Sauer and Tuite261).

In the U.S., aflatoxin (AF) contamination is often found in farm products destined for animal feed, such as peanuts, corn, cottonseed, and occasionally in some grains such as rye, sorghum, and wheat.259,262,263 Contamination of agricultural products intended for human consumption include peanuts, corn, and to a much lesser extent certain tree nuts and, in rare cases, dairy and meat products from animals fed highly contaminated feeds.259,263,264 There is a strong geographical bias in the U.S., with corn from the southeast having a higher level of aflatoxin contamination (41% incidence) than corn from the midwest (2.5%).265 Some caution is needed in evaluating evidence of contamination, since laboratory data on mold growth and AF production in certain commodities does not necessarily hold true under natural conditions in the field. Evidence seems to indicate that significant human exposure in the U.S. is limited to corn and peanuts.266 For recent data on aflatoxin presence in corn and peanut in the U.S., see Tables 9 and 10. Worldwide considerations indicate a wider and more extensive occurrence of AF contamination of foodstuff (upwards of 97% of the corn in some severely affected countries)267 and consequently greater implications for human health effects.

Aflatoxins toxicity was first realized in 1960 when moldy peanuts were identified as the cause of a disease, subsequently called aflatoxicosis, which killed turkeys, ducks, and pheasants.268,269

In the decades that followed, aflatoxin isolation, purification, characterization, and assessment of biological activity has resulted in literally thousands of publications. Aflatoxins constitute a unique group of heterocyclic, oxygen-containing compounds that possess a bis-difurano ring...
system. Four major aflatoxins (B₁, B₂, G₁, G₂) have been isolated and well characterized, with most measurements in foods generally expressed as the sum of these, or alternatively as aflatoxin B₁ (AFB₁), since it is by far the most prevalent congener. Aflatoxins B₂ and G₂ are dihydro derivatives of B₁ and G₁, respectively. Aflatoxin M₁ (AFM₁) and aflatoxin M₂ (AFM₂), hydroxilated derivatives of B₁ and B₂, respectively, are principally found in milk from cows fed contaminated fodder. Results from controlled feeding studies indicated that 0.4 to 4% of AFB₁ ingested by cows as contaminated feed was converted to and excreted as AFM₁ in the milk. Surveys conducted in the 1960s, 1970s, and 1980s of the occurrence of AFM₁ in milk from countries from around the world indicate a significant presence, but at levels usually less than 0.05 ppb. Another important metabolite, aflatoxicol, is mutagenic (about 66% of the potency of AFB₁) and possesses approximately 50 to 100% of the carcinogenicity of AFB₁ in trout. Of all the aflatoxin metabolites found in food, it is generally believed that only these three (AFB₁, AFM₁, and aflatoxicol) have a significant role in the etiology of human carcinogenesis.

Food processing methods that involve thermal treatment have been shown to have a variable and largely incomplete detoxification effect. Substantial reductions have been achieved for oil- and dry-roasting of peanuts under laboratory conditions that simulate commercial practices. Alkaline treatment of corn (e.g., in the preparation of masa used for tortillas) and the brewing process are other food processes that reduce aflatoxin levels. In contrast, significant detoxification of AFM₁ during the pasteurization, storage and processing of milk has not been found without destroying the milk. Under normal cooking conditions, aflatoxins are not readily degraded. See Scott for a good reference on the effect of food processing on aflatoxins and other mycotoxins.

In addition to being the most prevalent congener, AFB₁ is by far the most biologically active (see IARC). Aside from its extremely potent hepatotoxic effects, AFB₁ is one of the most potent hepatocarcinogens ever found, inducing liver tumors in a broad array of laboratory

<table>
<thead>
<tr>
<th>Year</th>
<th>Total No. Tested</th>
<th>Ave. Conc. (ppb)</th>
<th>Incidence of Detection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1991</td>
<td>219</td>
<td>32.2</td>
<td>26</td>
</tr>
<tr>
<td>1992</td>
<td>239</td>
<td>15.6</td>
<td>17</td>
</tr>
<tr>
<td>1993</td>
<td>248</td>
<td>29.2</td>
<td>11</td>
</tr>
<tr>
<td>1994</td>
<td>236</td>
<td>15.3</td>
<td>16</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Year</th>
<th>Total No. Tested</th>
<th>Ave. Conc. (ppb)</th>
<th>Incidence of Detection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1991</td>
<td>188</td>
<td>16.3</td>
<td>10</td>
</tr>
<tr>
<td>1992</td>
<td>158</td>
<td>17.6</td>
<td>14</td>
</tr>
<tr>
<td>1993</td>
<td>133</td>
<td>58.0</td>
<td>5</td>
</tr>
<tr>
<td>1994</td>
<td>168</td>
<td>12.6</td>
<td>5</td>
</tr>
</tbody>
</table>

a “Aflatoxin” refers to the sum of aflatoxins B₁, B₂, G₁, and G₂.
b Data from U.S. FDA Compliance Program, kindly supplied by Dr. G. E. Wood.
c Level of detection is 1 ppb.
animals, including rodents (mice, rats, hamsters, and tree shrews), fish (rainbow trout, sockeye salmon, and guppy), birds (ducks), carnivores (marmosets and ferrets), and subhuman primates (rhesus, cynomolgus, African green, and squirrel) by several routes of administration. In most species, tumors were largely found in the liver, but kidney, lung, and colon neoplasia have also been found. Laboratory experiments have examined a wide variety of parameters that influence aflatoxin carcinogenesis, such as: dose response; route of administration; sex, age, and strain of test animal; diet; hormonal status, liver injury, and enzyme induction; and concurrent administration of other carcinogens and pharmacologically active substances. AFB1 is one of the most potent mutagens ever tested in the Ames’ Salmonella assay and has produced positive results in all in vitro and in vivo genotoxicity tests.

AFM1, which is the hydroxylated derivative of AFB1, has been isolated and purified from fungal cultures in sufficient quantities to be used in carcinogenicity rodent feeding studies. Results from these studies revealed the hepatocarcinogenicity of AFM1, to be similar to AFB1, but with only 2 to 10% the potency. Corroborating evidence indicated genotoxicity in the Ames’ Salmonella assay (with 1.6 to 3.0% the potency of AFB1) and in mammalian liver cells as well as carcinogenicity in the trout (about 30% the potency of AFB1).

Epidemiological studies in Uganda, Thailand, Kenya, and Mozambique revealed a strong positive correlation between ingested aflatoxins and incidence of liver cancer, thus providing further evidence of carcinogenicity. There is some concern that many of these studies may be limited due to confounding factors such as hepatitis B virus (HBV) infection. Data from China by Yeh and coworkers suggested that HBV infection may be a necessary co-factor. Based on the totality of scientific evidence, the IARC evaluated AFB1 and concluded

---

**TABLE 10**

<table>
<thead>
<tr>
<th>Year</th>
<th>Total No. Lots Tested</th>
<th>Ave. Conc. (ppb)</th>
<th>Incidence of Detection (%)</th>
</tr>
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<tr>
<td>1987</td>
<td>37,889</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>1988</td>
<td>40,225</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>1989</td>
<td>41,311</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>1990</td>
<td>33,269</td>
<td>15.0</td>
<td></td>
</tr>
<tr>
<td>1991</td>
<td>45,343</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>1992</td>
<td>35,501</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>1991</td>
<td>116</td>
<td>5</td>
<td>34</td>
</tr>
<tr>
<td>1992</td>
<td>82</td>
<td>3</td>
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</tr>
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<td>1993</td>
<td>64</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>1994</td>
<td>77</td>
<td>5</td>
<td>30</td>
</tr>
</tbody>
</table>

a “Aflatoxin” refers to the sum of aflatoxins B1, B2, G1, and G2.

b Data from National Peanut Council; kindly supplied by Mr. R. Henning.

c Data from U.S. FDA Compliance Program; kindly supplied by Dr. G. E. Wood.

d Level of detection is 1 ppb.
that it is a Group 1 human carcinogen. There was inadequate evidence of human carcinogenicity for AFM1.

Aflatoxins are not the only carcinogenic toxins produced by Aspergillus. Sterigmatocystin (ST) is produced by several Aspergillus spp., especially A. versicolor, as well as by Penicillium luteum and unidentified species of Bipolaris, Chaetomium, and Emericella. ST has been found to contaminate cereals, country hams, salami, green coffee beans, cheese, and wheat. Resembling aflatoxin, ST is chemically characterized by a bis-dihydrofuran ring fused to a substituted anthraquinone. It is mutagenic and clastogenic and has between one tenth and one hundredth the hepatocarcinogenic potency of aflatoxin B1 (see review by Terao). Retaining the key structural elements of aflatoxin which allow epoxide formation and hence DNA binding, it would be reasonable to expect a similar mechanism of action. Analysis of more than 500 samples from 1974 to 1975 by the FDA failed to detect ST contamination; however, occurrence in Japan and elsewhere is greater. Unfortunately, there are no epidemiological studies which have attempted to link ST exposure with any carcinogenic outcome. Hence, the role of ST exposure in human cancer remains speculative at this time.

Ochratoxin A (OT-A) and other related isocoumarin derivatives were first isolated from Aspergillus ochraceus and later from other Aspergillus spp. and one species of the genus Penicillium, namely P. verrucosum. Chemically, OT-A is a dihydroisocoumarin linked to L-beta-phenylalanine. Worldwide contamination of food with OT-A includes cereals (wheat, rye, barley, and oats), corn, sorghum, groundnuts, green coffee beans, wine products, and animals feeds, with the latter containing the highest levels. With the exception of northern Europe, surveys have not revealed any significant contamination of human foods. A potent nephrotoxin, OT-A has demonstrated carcinogenicity via long-term feeding studies that have resulted in tumors in mice liver and kidneys and rat kidneys. It should be noted that the mouse studies suffered from one or more of the following: poor survivability, use of only one sex, high dose in excess of the MTD, presence of impurities with the OT-A (including benzene), and significant increases in tumor incidence still within historical control range (see review by Kuiper-Goodman and Scott). OT-A has tested nonmutagenic in several bacterial and mammalian gene mutation assays (see reviews by Kuiper-Goodman and Scott and Dirheimer and Creppy). The IARC concluded that there was sufficient evidence of carcinogenicity in experimental animals for OT-A. Based on the totality of the evidence, including the investigations that have showed an association between ochratoxin and human urinary tract cancers, the IARC concluded that OT-A was a “possible human carcinogen” (Group 2B). Further studies are needed to more fully address the possible genotoxicity of OT-A and its endocrine disruptive effects, prior to reaching any definite conclusions on the human carcinogenic risk.

10.2 Fusarium Toxins

The fungus Fusarium moniliforme is a ubiquitous contaminant of corn and sorghum, with lesser occurrence for wheat and barley, and is most prevalent in warm, dry years in the presence of insect damage. Several mycotoxins are produced by F. moniliforme or a related Fusarium species, of which two classes contain putative carcinogens. Fumonisins B1 is the major toxin of at least seven other fumonisins (B2, B3, A1, A2, C, and D) which are known to be produced by F. moniliforme-contaminated corn and culture material. However, only fumonisins B1, B2, and B3 have been detected in milled corn intended for human consumption; of these three, only fumonisin B1 has been implicated as a carcinogen. Fumonisins appear to be poor mutagens but good cancer promoters. Human exposure to F. moniliforme-contaminated maize has been associated with elevated rates of esophageal cancer in S. Africa, allegedly due to fumonisin intake.
Fusarins are another important class of <em>Fusarium</em> mycotoxins, and there are at least five produced by <em>F. moniliforme</em>. Within this class, only fusarin A, C, and F are found in unprocessed contaminated corn, with only fusarin C considered as a presumptive carcinogen. Measurements of fusarin C in food are limited as a result of its poor stability.

Evidence for the carcinogenicity of fumonisin B<sub>1</sub> and fusarin C have been evaluated by the IARC, and their conclusion was that there was sufficient evidence to classify “toxins derived from <em>Fusarium moniliforme</em>” as possible human carcinogens (the exact chemical identity was unspecified). It is important to note that the IARC found the evidence for the carcinogenicity of the specific toxins fumonisin B<sub>1</sub> and fusarin C to be limited, and for fumonisin B<sub>3</sub> the evidence was found inadequate.

Fumonisin B<sub>1</sub> content of corn and corn-based products in the U.S. was determined to range up to 330 ppm, with the greatest levels being reported for animal feed. Analysis of corn and corn-based human foods indicate fumonisin B<sub>1</sub> levels in the range of 200 to 800 ppb, with processed foods generally having the lowest amounts. In general, food processing methods do not destroy fumonisins; however, calcium-hydroxide treatment (nixtamalization) of corn used to make tortilla flour reduces fumonisin levels through hydrolysis, resulting in uncertain effects on its subsequent carcinogenicity. Although studies of fumonisin residues in meat, milk, and eggs are limited, evidence seems to indicate a low or nonexistent occurrence. Quantification of fusarins in corn and corn-based products has been hampered by the instability of these compounds.

T-2 toxin, which rarely occurs in cereals (e.g., wheat and maize) as a toxic metabolite of several <em>Fusarium</em> spp. but principally <em>F. sporotrichioides</em>, has been found worldwide. T-2 toxin (3α-hydroxy-4ß,15-diaacetoxyl-8α-[3-methylbutyryloxy]-12,13-epoxy-trichothec-9-ene) belongs to a large class of tetracyclic sesquiterpenoid compounds known as trichothecenes, with their carcinogenicity presumably due to an epoxy group and/or an olefinic bond. Cancer feeding studies in mice revealed tumors in the liver, lung, and forestomach; however, no evidence of neoplasia was found in treated trout. Studies in rats are inadequate for evaluation. Evidence of genotoxicity, which for the most part has been limited and not compelling, and has been reviewed by Haschek. The IARC determined there was “limited evidence” of carcinogenicity in experimental animals for T-2 toxin, without any evidence in humans. Although the chemistry of T-2 toxin is suggestive of carcinogenic activity, the limited evidence in animal studies and the absence of any human data make drawing any definite conclusions premature.

Three other well-known <em>Fusarium</em> mycotoxins are zearalenone (ZEN), deoxynivalenol (DON), and nivalenol (NIV). Post-harvest contamination of cereals with these toxins is known to occur worldwide as the result of several <em>Fusarium</em> spp., with surveys indicating a significant level and frequency of contamination of wheat, barley, oat, rye, corn, and rice. ZEN, chemically described as 6-(10-hydroxy-6-oxo-trans-1-undecenyl)-ß-resorcylic acid lactone, was first isolated from fungal cultures in 1962. The major ZEN-producing <em>Fusarium</em> spp. are <em>F. graminearum</em> and <em>F. culmorum</em>, with their natural occurrence in agricultural commodities being the subject of many studies. Surveys done in the U.S. in the 1960s and 1970s indicate appreciable levels of ZEN in corn and wheat (including corn products intended for food use), with variable effects of food processing. Carcinogenicity studies in rats showed no neoplastic effects; however, studies in mice produced liver and pituitary tumors. The IARC classified ZEN with “limited evidence” of carcinogenicity since positive findings were exclusive to only one species. Results in the Ames’ <em>Salmonella</em> assay were conclusively negative. Overall, the evidence of carcinogenicity for ZEN is weak (with the absence of epidemiological data), and until the ZEN-induced tumorigenesis can be confirmed in another species, it would be premature to make a definite conclusion about its possible carcinogenic activity in humans. Concerning the mycotoxins DON and NIV, the IARC has evaluated the carcinogenicity evidence and deemed them to be of no special
concern, although co-occurrence with aflatoxins may lead to an interaction that produces increased carcinogenicity.352

10.3 *Penicillium* Toxins

Lesser known carcinogenic mycotoxins are: (–)luteoskyrin, cyclochlorotine, (+)rugulosin, and citrinin. The first two were initially isolated from the growth of *Penicillium islandicum* on rice and were later determined to be hepatocarcinogenic in mice in several studies.353,354 (+)Rugulosin, first isolated from *P. rugulosum*, is very similar to (–)luteoskyrin, lacking only two hydroxy groups, but is much less potent in producing murine liver tumors.353,355 Citrinin, isolated from *P. citrinum* (and other *Penicillium* and *Aspergillus* spp.), has produced liver tumors in long-term feeding studies with rats356 but was found negative in two other carcinogenicity studies,357,358 as well as several independent Ames’ *Salmonella* assays.359 See the review by Enomoto and Saito360 for a fuller description of the fungal producers of these compounds. Occurrence of all these compounds in the U.S. food supply is scarce. Based on the limited available data, including the lack of epidemiological studies, any definitive judgment on the role these compounds may play in the etiology of human cancer is untenable.

11 N-NITROSO COMPOUNDS

The current interest in the carcinogenic activity of N-nitroso compounds (NOCs) stems from the original reports by Magee and Barnes361,362 in which they demonstrated that the chronic dietary administration of N-nitrosodimethylamine (NDMA) produced liver and kidney tumors in rats. Following this report, numerous carcinogenicity studies on a variety of NOCs have been reported (for an in-depth review, see Preussman and Stewart363). To date, of the approximately 300 NOCs that have been evaluated for carcinogenicity, about 90% were positive in one or more of 44 different laboratory animal species, including nonhuman primates.363 The most widely tested NOC, N-nitrosodiethylamine (NDEA) has been shown to be carcinogenic in 40 species.364 No species tested to date have been found to be resistant to their carcinogenic action.

NOCs can be divided into two chemical classes: nitrosamines and nitrosamides (and related compounds). Nitrosamines are N-nitroso derivatives of secondary amines, whereas nitrosamides are N-nitroso derivatives of substituted ureas, amides, amino acids, and other nitrogen-containing compounds.365 There is a more important distinction than the chemical classification between these two classes. Nitrosamines must be activated by enzyme systems and can exert their carcinogenic effects at remote sites in the body. Conversely, nitrosamides are direct-acting carcinogens, do not require enzyme activation, and can cause tumor development at their site of application. In practice, NOCs are broadly divided into two groups based on analytical methods, namely, volatile nitrosamines (VNAs) and nonvolatile nitrosamines (NVNAs). The four VNAs most commonly found in food are N-nitrosodimethylamine (NDMA), N-nitrosopyrrolidine (NPyR), N-nitrosopiperidine (NPIP), and N-nitrosomorpholine (NMOR).366 Some NVNAs commonly found in food, or formed *in vivo* from foods due to intragastric nitrosation, are N-nitrosothiazolidine (NTHZ), N-nitrosothiazolidine-4-carboxylic acid (NTCA), 2-hydroxymethyl-N-nitrosothiazolidine-4-carboxylic acid (HMNTCA), N-nitrosodibenzylamine (NDBzA), and N-nitroso-N-methylurea (NMU).367

The occurrence of NOCs (principally VNAs) in foods and beverages has led to a worldwide sampling effort, especially for those products preserved with nitrate and/or nitrite (the reader is referred to the following excellent reviews: Gray,368 Pensabene and Fiddler,369

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Data on the occurrence of nonvolatile nitrosamines in foodstuff are sparse due to the lack, until recently, of adequate and reliable analytical methods. In regard to nitrosamides, the instability of these compounds makes it unlikely that significant amounts would accumulate and persist in the food supply. NOCs can occur in foods and beverages in three different ways: (1) formation as a result of the use of curing agents, such as nitrate and nitrite, (2) formation during processing, and (3) via migration from secondary sources such as packaging or other materials. Most Western-style foods have been analyzed for volatile nitrosamines, with results indicating that only bacon and beer consistently showing levels greater than 1 ppb. Nitrosamines are formed in bacon (by a process known as N-nitrosation) as an indirect result of the addition of nitrite. A detailed study in 1986 of nitrosamine formation and occurrence in bacon found total nitrosamine (based on the analysis of four nitrosamines) content in 39 samples of cooked bacon to be, on average, about 30 ppb. These levels in bacon are approximately tenfold less than the initial determinations made in the early 1970s and are a consequence of food processing changes, principally via a reduction in added nitrite. In addition, humans are exposed to other dietary sources of nitrite and nitrate (which can be enzymatically converted to nitrite) through the consumption of vegetables and via drinking water contaminated with nitrogen-containing fertilizer.

Among those foods that undergo processing conditions that favor nitrosamine formation, those that are directly dried (e.g., malt used in beer manufacture) are the most susceptible. In response to these findings in the late 1970s, the malting and brewing industries altered their processes to reduce the nitrosamine content in beer. An extensive survey of imported and domestic beer in 1990 revealed generally very low levels (less than 0.1 ppb), which were approximately 20-fold less than determinations made in the prior decade. Similar efforts were undertaken with other dried foods, such that a recent survey of 57 samples of dried milk averaged less than 1 ppb. Indirect contributions via migration into foods have been reduced to negligible levels.

There are several lines of evidence that nitrosamines and nitrosamides are endogenously formed in humans, in large part due to the ingestion of nitrate (principally from vegetables) and nitrite (from cured meat, vegetables, and cereals). Indeed, preliminary data seem to indicate that the in vivo formation of nitrosamines may actually represent the largest source of exposure to NOCs; however, other contradictory evidence suggests that much further research is needed to gain a clear understanding of the quantitative impact of endogenous nitrosation under the realistic conditions found in humans.

It is important to note that human exposure to other nondietary sources of NOCs occurs through products such as cosmetics, vulcanized rubber products (e.g., nursing nipples, tires, gloves, etc.), new automobile interiors, and other consumer products. In fact, exposure to NOCs from foods represents a much smaller potential exposure than from some of these consumer products.

NOCs are a unique group of carcinogens in that they can induce tumor formation in so many organs of so many animal species. Target organ selectivity depends strongly on structure-activity relationships and the particular animal species, but other factors such as nutritional status, age, sex, route of administration, dosing schedule, and the presence of modifying agents also play a role. The large interspecies differences in target organs affected by a particular NOC make predictions for humans problematic (for a review of the structure-activity relationships, see Lijinsky). Various dose-response studies have been carried out that show carcinogenic effects even at fairly low doses. Extensive studies conducted by Druckery et al. and Peto et al. have provided relatively reliable data on tumorigenesis for use by regulators in estimating carcinogenic risk to humans. Many NOCs are potent mutagens. In general, there is a reasonably good qualitative, but not quantitative, correlation between NOC
mutagenicity in the modified Ames’ *Salmonella* assay and the carcinogenicity results. Combined with data of carcinogenicity in single-dose, transplacental, and co-administration studies of NOCs, there is sufficient evidence from *in vivo* and *in vitro* studies to support the conclusion that the human exposure to nitrosamines could have a causal relationship in the development of certain cancers.

Numerous epidemiological investigators have tried to establish a relationship between NOC exposure and the development of cancer. Many studies have used the ingestion of NOC precursors (e.g., nitrite, nitrate, etc.) as a surrogate exposure estimate. Many have focused on gastric cancer because it is the primary site of nitrosation. Two independent reviews by the U.S. Assembly of Life Sciences and by the World Health Organization concluded that the epidemiological studies have failed to provide convincing evidence of a link to human cancer.

The clear evidence of carcinogenicity from well-conducted animal experiments should provide a reasonable basis to conclude that NOCs have some level of carcinogenic risk to humans. The magnitude of this risk is related to the exposure to the causative agent. Unfortunately, the state of the science is imperfect in providing accurate determinations of the human carcinogenic risk based on animal studies, and useful epidemiological studies incorporating sound NOC exposure data have been lacking.

## 12 POLYCYCLIC AROMATIC HYDROCARBONS

Polycyclic aromatic hydrocarbons (PAHs) are relatively simple organic compounds composed of two or more fused aromatic rings, which may or may not have substituted groups. Although hundreds of compounds have been identified as belonging to this class, about 20 compounds have been found to occur in food, of which half have been determined to be carcinogenic in laboratory animals by skin application and/or by injection; however, carcinogenicity experiments using oral administration have identified only three, specifically benzo[a]pyrene (BaP), benz[a]anthracene (BaA), and dibenz[a,h]anthracene (DBahA) (Table 11). Two other compounds shown to be carcinogenic by the oral route (3-methylcholanthrene and 7,12-dimethylbenz[a]anthracene) are not normally found in food.

PAHs are ubiquitous environmental pollutants, having been found in air, water, soil, and food. Although this class of compounds is composed of many members, the majority of the toxicological research has focused on BaP, one of the most prevalent of the carcinogenic PAHs. Analytical determinations of PAHs in food are often expressed as either BaP or total PAHs, the latter of which includes many noncarcinogenic compounds. Hence, the BaP content is a more valuable expression of the carcinogenic potential of a particular PAH-containing food, although it may account only for up to 10% of its noncarcinogenic toxicity. Since many of its members are relatively potent carcinogens and due to their extensive, and in some cases heavy, occurrence in a variety of foods, PAHs constitute one of the more important naturally occurring classes of carcinogens. Although anthropogenic contributions of PAHs to the environment should not be discounted, natural processes play a significant role in the presence of PAHs in the diet. In fresh vegetables, PAHs are thought to be end-products of one or more biosynthetic pathways. The sharp increase in the PAH content of decaying vegetables has been used as evidence that they result from catabolic processes. This accumulation of PAHs in decaying vegetables accounts to a large degree for the PAHs in soil and water and, in turn, back into fresh vegetables. This cycle makes vegetables and their oils, cereals, and fruits significant contributors of PAHs to the diet (Table 12).

The other major source of PAHs in the diet is a result of the formation and deposition of PAHs on foods via thermal processes, such as grilling, roasting, and smoking. Formation of PAHs at temperatures less than 400°C is limited, while the amount formed increases linearly in the range of 400 to 1000°C. Hence, “endogenous” formation on the surface...
### TABLE 11
Orally Active Carcinogenic Polycyclic Aromatic Hydrocarbons (PAHs) Found in Food

<table>
<thead>
<tr>
<th>Structure</th>
<th>Name</th>
<th>Abbreviation</th>
<th>Mutagenicity&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Carcinogenicity&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Carcinogenicity Target Organs</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benz[a]anthracene</td>
<td>BaA</td>
<td>+</td>
<td>++</td>
<td></td>
<td>Liver, lung</td>
<td>411</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>BaP</td>
<td>+++</td>
<td>++</td>
<td></td>
<td>Forestomach, mammary gland, lung, hematopoietic system</td>
<td>402–405</td>
</tr>
<tr>
<td>Dibenz[a,h]anthracene</td>
<td>DBaA</td>
<td>+</td>
<td>+</td>
<td></td>
<td>Lung, mammary gland, forestomach</td>
<td>412</td>
</tr>
</tbody>
</table>

<sup>a</sup> Relative to PAHs; +, ++, +++ indicate increasing activity.
of food occurs only under certain limited conditions. The conduction of heat in frying and radiation in the electrical broiling of meats do not result in sufficient temperatures under normal cooking conditions to generate significant amounts of PAH. Only when meat was placed in direct contact with open flames did significant amounts (6 to 212 mg BaP/kg) of PAHs form. PAH occurrence in food can also be the result of deposition as a consequence of the fuel combustion. In general, this is nearly insignificant since the amount yielded by charcoal briquettes commonly used in grilling is only zero to 1.0 mg BaP/kg. Somewhat higher levels can result from the embers of a log fire (1 to 25 mg BaP/kg). Lastly, and perhaps

### TABLE 12
Benzo[a]pyrene Content in Some Foods

<table>
<thead>
<tr>
<th>Fooda</th>
<th>Level (ppb)b</th>
<th>Year</th>
<th>Remarks</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetables</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cereals</td>
<td>0.2–4.1</td>
<td>1984</td>
<td>—</td>
<td>413</td>
</tr>
<tr>
<td>Kale</td>
<td>12.6–48.1; 0.6–4.5c</td>
<td>1988; 1984</td>
<td>Surface contamination likely</td>
<td>400; 413</td>
</tr>
<tr>
<td>Salad</td>
<td>2.8–5.3</td>
<td>1984</td>
<td>Surface contamination likely</td>
<td>413</td>
</tr>
<tr>
<td>Spinach</td>
<td>7.4; 0.09–0.5c</td>
<td>1988; 1984</td>
<td>Surface contamination likely</td>
<td>400; 413</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>0.2</td>
<td>1984</td>
<td>—</td>
<td>413</td>
</tr>
<tr>
<td>Fats and Oils</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn oil</td>
<td>ND; 0.7</td>
<td>1966; 1979</td>
<td>Retail products</td>
<td>414; 415</td>
</tr>
<tr>
<td>Olive oil</td>
<td>ND; 0.5</td>
<td>1966; 1979</td>
<td>Retail products</td>
<td>414; 415</td>
</tr>
<tr>
<td>Peanut oil</td>
<td>ND; 0.6</td>
<td>1966; 1979</td>
<td>Retail products</td>
<td>414; 415</td>
</tr>
<tr>
<td>Shortening</td>
<td>ND</td>
<td>1979</td>
<td>Retail products</td>
<td>415</td>
</tr>
<tr>
<td>Vegetable oil</td>
<td>ND</td>
<td>1979</td>
<td>Retail products</td>
<td>415</td>
</tr>
<tr>
<td>Meats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacon, smoked</td>
<td>ND; 0.42; 0.59</td>
<td>1968; 1970</td>
<td>10 nondetects</td>
<td>397; 416</td>
</tr>
<tr>
<td>Beef patty, grilled</td>
<td>18.8–24.1</td>
<td>1979</td>
<td>6 determinations</td>
<td>396</td>
</tr>
<tr>
<td>Bologna</td>
<td>ND</td>
<td>1979</td>
<td>—</td>
<td>415</td>
</tr>
<tr>
<td>Chicken, grilled</td>
<td>3.7</td>
<td>1967</td>
<td>—</td>
<td>417</td>
</tr>
<tr>
<td>Chicken, smoked</td>
<td>TR, 0.5, 0.7; ND</td>
<td>1968; 1979</td>
<td>—</td>
<td>397; 415</td>
</tr>
<tr>
<td>Frankfurters, smoked</td>
<td>ND, TR, 0.8; ND</td>
<td>1968; 1979</td>
<td>9 nondetects (beef and pork)</td>
<td>397; 415</td>
</tr>
<tr>
<td>Ham, smoked</td>
<td>ND, TR, 0.5–1.5</td>
<td>1968</td>
<td>—</td>
<td>397</td>
</tr>
<tr>
<td>Lamb patty, grilled</td>
<td>8.8–12.3</td>
<td>1979</td>
<td>6 determinations</td>
<td>396</td>
</tr>
<tr>
<td>Pork chop, grilled</td>
<td>7.9</td>
<td>1967</td>
<td>—</td>
<td>417</td>
</tr>
<tr>
<td>Pork patty grilled</td>
<td>25.8–31.6</td>
<td>1979</td>
<td>6 determinations</td>
<td>396</td>
</tr>
<tr>
<td>Ribs, barbecued</td>
<td>10.5</td>
<td>1965</td>
<td>—</td>
<td>418</td>
</tr>
<tr>
<td>Steak, grilled</td>
<td>5.8, 8.0; 11.1, 50.4</td>
<td>1965; 1967</td>
<td>—</td>
<td>417; 418</td>
</tr>
<tr>
<td>Turkey, smoked</td>
<td>TR</td>
<td>1968</td>
<td>—</td>
<td>397</td>
</tr>
<tr>
<td>Turkey patty, grilled</td>
<td>ND</td>
<td>1979</td>
<td>6 determinations</td>
<td>396</td>
</tr>
<tr>
<td>Misc.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheese, smoked</td>
<td>ND</td>
<td>1968</td>
<td>—</td>
<td>397</td>
</tr>
<tr>
<td>Coffee, whole, roasted</td>
<td>ND</td>
<td>1968</td>
<td>—</td>
<td>397</td>
</tr>
<tr>
<td>Liquid smoke</td>
<td>ND</td>
<td>1968</td>
<td>—</td>
<td>397</td>
</tr>
</tbody>
</table>

a Vegetable analysis from samples collected in the Netherlands; all other samples from U.S.
b ND = not detected; TR = trace.
c Level of detection > 0.03 ppb.

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most importantly, the occurrence of PAHs can occur when fat drips on the heat source, gets pyrolyzed into the air, and is subsequently deposited on the food.\(^{395}\) This was clearly illustrated in an experiment by Doremire and coworkers,\(^ {396}\) in which ground-beef patties containing from 15 to 40% added fat were charcoal-grilled under identical conditions. Analysis of cooked patties indicated BaP levels from 16 to 121 \(\mu\)g/kg, which were directly related to the fat content.

Smoking is a traditional method used to preserve meats and fish, although currently its main purpose is to give certain foods desirable organoleptic traits. Exogenous contamination can occur through absorption of PAHs during the smoking process. Analysis of curing smoke has identified more than 300 compounds, including many PAHs. In general, low-molecular-weight PAHs (e.g., phenanthrene, anthracene, and pyrene) are more frequently found on smoked foods, at total PAH levels of 10 \(\mu\)g/kg. Higher-molecular-weight compounds, including the carcinogens BaP, BaA, and DBahA, are found at much lower concentrations in smoked foods (total PAHs < 1 \(\mu\)g/kg).\(^ {388}\)

The processes used for some distilled spirits can result in the contamination of PAHs, although analysis of bourbon, whisky, and Scotch has not found any\(^ {397}\) or has found very low levels (from 0.03 to 0.08 ppb).\(^ {398}\) Roasting of coffee does not increase the BaP content.\(^ {399}\)

Estimates of dietary intake in the Netherlands, based on duplicate analysis of 50 24-hour diets, revealed total PAH intake of from 1.1 to 22.5 \(\mu\)g per capita.\(^ {400}\) Results also indicated that about 30% of the total ingested PAHs are carcinogens. Another survey of BaA, BaP, and DBahA individual intakes ranged from nondetectable to about 0.5 \(\mu\)g per capita per day.\(^ {391}\) The EPA estimated a daily BaP intake from food of 50 ng.\(^ {401}\) Total exposure to BaP, as well other PAHs, can be substantially elevated depending on environment, habits, and occupation. Overall, consumption of charcoal-broiled and smoked meat and fish does represent a significant exposure medium, but certain atmospheric conditions in urban environments and other nondietary exposures can also serve as substantial sources of BaP and other PAHs.\(^ {401}\)

Among all the PAHs, BaP has received the most biological testing; however, few studies have adequately examined the carcinogenic effect of orally administered BaP. Available studies (which for the most part are from the 1960s) have several shortcomings, such as the less than life-time exposure, small number of test animals, inappropriate controls, and tumors generally limited to the site of application and to an organ that has no structural analogue in humans (i.e., the rodent forestomach). Incidence data from perhaps the best study indicated BaP to be a potent and robust forestomach carcinogen in the mouse;\(^ {402}\) however, other studies by the same researchers also revealed systemic carcinogenicity (lung adenomas and leukemia) in mouse species prone to these particular spontaneous neoplasia.\(^ {403,404}\) Likewise, Huggins and Yang\(^ {405}\) found an increase in spontaneous mammary cancer in rats by a single intragastric dose of BaP (however, poor reporting of this study makes it inadequate for evaluation). Other studies have repeatedly found induction of forestomach cancer by oral administration of BaP.\(^ {406-410}\) Repeated gavage administration of BaA produced murine liver and lung tumors and, to a slight extent, forestomach tumors.\(^ {411}\) Dietary administration of DBahA to mice produced carcinoma of the lung, mammary gland, and forestomach.\(^ {412}\) Mutagenicity results are somewhat varied, with strong positive activity in nearly every test conducted for BaP but mixed and generally weak responses for DBahA. BaA has tested mutagenic in the Ames’ \textit{Salmonella} assay, and in \textit{vivo} tests of genotoxicity are also generally but not uniformly positive (see EPA\(^ {401}\) document for summary of genotoxicity testing). Epidemiological data on the carcinogenic effect of PAHs are available from both occupational and community air pollution studies; however, neither addresses oral exposure. Given this lack of human data on oral exposure, one can only speculate on the role that PAHs, individually or in mixtures, may have on human gastrointestinal or other site tumors. Nonetheless, the animal carcinogenicity evidence for BaP, BaA, and DBahA is sufficiently strong enough to warrant serious consideration of their potential risk to human health depending on their intake level.
PYRROLIZIDINE ALKALOIDS

About 250 pyrrolizidine alkaloids (PAs) have been isolated from more than a dozen unrelated plant families (principally Compositae, Boraginaceae, and Leguminosae), encompassing more than 60 genera. Although many of these plants have limited or nonexistent consumption by humans, plants such as coltsfoot (*Tussilago farfara*), comfrey (*Symphytum officinale*), and petasites (*Petasites japonicus*) have found fairly widespread use as herbal remedies, dietary supplements, or even as foods. Recent analysis of a number of commercial comfrey products (*Symphytum* spp.) obtained at health food stores in the Washington, D.C., area revealed PA content generally under 10 ppm, although bulk root and leaf levels were at several hundred ppm. As an example, a cup (250 ml) of comfrey tea prepared from a root infusion contained from 8.5 to 26 mg of PAs, depending on the preparation. A more detailed review of PA content in samples of *Symphytum* spp. can be found in Mattocks.

The fundamental structure of PAs is composed of two parts, necine and necic acid, combined by an ester link (Figure 4). Necine consists of a basic structure of various hydroxylated congeners of pyrrolizidine, which can be further subdivided into four groups: (1) a trachelanthamidine group of monohydroxylated derivatives, (2) a retronecine group of dihydroxylated derivatives, (3) a rosmarincine group of trihydroxylated derivatives, and (4) an otonecine group. In most alkaloids, the various necines combine with the various 5 to 10 carbon branched-chain necic acids to form an ester structure. These combinations can also be divided into four groups: (1) nonesters, (2) monoesters, (3) acyclic diesters, and (4) macrocyclic diesters (see Mattocks for a comprehensive review of the chemistry).

PAs were among the first natural products to be proven as animal hepatocarcinogens. Despite this, the total number of PAs tested for carcinogenicity is quite small. Aside from the limited availability of purified material, the pharmacological activity of these compounds has hampered efforts to select the appropriate dosing regimens that would permit sufficient animals to survive long enough for tumor development. Eight PAs have received carcinogenicity testing that yielded evidence of treatment-related tumor development: clivorine, isatidine, lasiocarpine, monocrotaline, monocrotaline, petasitenine, retrosine, and symphytine (see Table 13). Except for senkirkine and symphytine, the remaining compounds were the subject of at least one study employing some type of oral administration (diet, drinking water, or gastric intubation). All were found to be exclusive liver carcinogens except for lasiocarpine, which produced additional tumors of the skin, lung, ileum, and hematopoietic system in rats (see Mattocks for a detailed review of the carcinogenicity data). It should be noted that although the results of these studies are highly suggestive of carcinogenicity, limitations in animal number, survivability, variable dosing regimens (including parenteral administrations), possible mycotoxin contamination of feed, and, in general, poor reporting make drawing definitive conclusions difficult. Bacterial mutagenic activity has not been consistently found for the PAs tested so far (see Woo et al. for a good...
<table>
<thead>
<tr>
<th>Common or Trivial Name</th>
<th>Chemical Structure</th>
<th>Route of Administration</th>
<th>Carcinogenic Target Organ</th>
<th>Example Source</th>
<th>Carcinogenicity</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clivorine</td>
<td><img src="image1" alt="Clivorine Structure" /></td>
<td>Drinking water^b^</td>
<td>Liver^a^</td>
<td>Ligularia dentata</td>
<td></td>
<td>438</td>
</tr>
<tr>
<td>Isatidine</td>
<td><img src="image2" alt="Isatidine Structure" /></td>
<td>Drinking water^c^</td>
<td>Liver^a^</td>
<td>Senecio jacobea (common ragwort)</td>
<td></td>
<td>439</td>
</tr>
</tbody>
</table>
Lasiocarpine

Parenteral\(^a\) Liver, skin, lung, ileum Heliotropium europeum 440
Dietary\(^b\) Liver 441
Dietary\(^f\) Liver, hematopoietic system 442

Monocrotaline

Gastric intubation\(^e\) Liver Crotalaria refusa 443
Parenteral\(^h\) Liver, lung, and other sites 444
Parenteral\(^i\) Pancreas 445

Petasitenine

Drinking water\(^j\) Liver\(^a\) Petasites japonicus 446
<table>
<thead>
<tr>
<th>Common or Trivial Name</th>
<th>Chemical Structure</th>
<th>Route of Administration</th>
<th>Carcinogenic Target Organ</th>
<th>Example Source</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retrorsine</td>
<td></td>
<td>Drinking water*</td>
<td>Liver*</td>
<td>Senecio jacobaea (common ragwort)</td>
<td>439</td>
</tr>
<tr>
<td>Senkirkine</td>
<td></td>
<td>Parenteral†</td>
<td>Liver</td>
<td>Tussilago farfara (coltsfoot)</td>
<td>447</td>
</tr>
<tr>
<td>Symphytine Parenteral&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Liver</td>
<td>Symphytum officinale (comfrey)</td>
<td>447</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------------------</td>
<td>------</td>
<td>-------------------------------</td>
<td>-----</td>
<td></td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="Chemical Structure" /></td>
<td><img src="image" alt="Chemical Structure" /></td>
<td><img src="image" alt="Chemical Structure" /></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Study limited by small number of animals in treated and control groups.
- 0.005% solution for 340 days (experiment terminated at 480 days).
- 0.03 mg/ml solution 3 days per week until death (10–23 months).
- Intraperitoneal injections of 7.8 mg/kg b.w. twice per week for 4 weeks and then once per week for 52 weeks (majority of treated animals killed between 60 and 76 weeks).
- 50 ppm in diet for 55 weeks (majority of treated animals killed between 48 and 59 weeks).
- 7, 15, or 30 ppm in diet until study termination (104 weeks).
- Weekly gastric intubations of 25 mg/kg b.w. for 4 weeks and then 8 mg/kg b.w. for 38 weeks.
- Subcutaneous injections of 5 mg/kg b.w. biweekly for 12 months (experiment terminated at 24 months).
- Single subcutaneous injection of 40 mg/kg b.w. (experiment terminated at 500 days).
- 0.01% solution until study termination at 16 months.
- Intraperitoneal injections of either 22 mg/kg b.w. (senkirkine) or 13 mg/kg b.w. (symphytine) twice per week for 4 weeks and then once per week for 52 weeks (experiment terminated at 650 days).
summary), and no clear structure activity pattern has emerged.\cite{420} The IARC\cite{436,437} has reviewed the available data for each of the above-mentioned seven PAs (clivorine was not evaluated), and determined that in each case there was limited evidence of carcinogenicity in experimental animals, but without epidemiological studies no evaluation of the carcinogenicity to humans could be made. Clearly, these compounds are weak rodent hepatocarcinogens, with evidence that suggests that their carcinogenicity is due to the formation of ultimate carcinogens in the liver; however, to reiterate the IARC evaluation, the possible human carcinogenic hazard posed by the PAs is uncertain due to limited long-term animal studies (which have been exclusive to the rat) and the absolute lack of epidemiological data incorporating sound exposure estimates.

14 TERPENES

Terpenes constitute a class of compounds that includes side chain substitution of cyclohexene with a methyl, isopropenyl, ethenyl, or other group. Terpenes and their derivatives owe their metabolic origin to the five carbon molecule isoprene. Terpenes are the chief constituents of essential oils, which are the volatile oils obtained from plant materials through steam distillation, solvent extraction, or physical expression. Limonene (with the possible exception of \(\alpha\)-pinene) is the most frequently naturally occurring monoterpane (or diisoprine) (for structure, see Figure 5), and certainly is the most well-studied. It is a major constituent (in some cases composing over 90%) of the terpenoid fraction of a wide variety of plant materials, especially the oils of citrus fruit peel, such as oranges and lemons.\cite{448} It occurs naturally in both the \(\delta\) and \(\I\) forms, with \(\delta\)-limonene predominate in the citrus peel oils of orange, grapefruit, and lemon.\cite{449} Commercial technical-grade sources are limited to \(\delta\)-limonene\cite{450} and have been used widely as flavor and fragrance additives in food, beverage, perfume, soap, etc., for 50 years. The consumption of \(\delta\)-limonene has been estimated to be in the range of 0.2 to 2 mg/kg b.w. per day.\cite{451}

Results of carcinogenicity testing conducted under the auspices of the NTP were negative in mice, while renal neoplasm were found in the male but not female rat.\cite{452} Extensive studies of the mechanism of the carcinogenic action of \(\delta\)-limonene revealed a characteristic nephrotoxicity, a key aspect of which is the dose-related accumulation of a protein (\(\alpha_{2u}\) globulin) which binds \(\delta\)-limonene and its metabolite. This phenomena is largely limited to the male rat and is generally believed to have no relevance to humans. Mutagenic activity of \(\delta\)-limonene was negative in the Ames' \textit{Salmonella} assay and in other tests of genotoxicity.\cite{448} The IARC concluded that there was limited evidence of carcinogenicity in animals, and that \(\delta\)-limonene was not classifiable as to its carcinogenicity to humans (i.e., Group 3).\cite{448} Unless other evidence surfaces, \(\delta\)-limonene should not be regarded as a human carcinogen.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure5.png}
\caption{Some terpenes: limonene and pinene.}
\end{figure}
URETHANE

Urethane, also known as ethyl carbamate, is a naturally occurring contaminant found in alcoholic beverages and other fermented products such as brandy, wine, beer, ale, sake, distilled spirits, liqueurs, soy sauce, bread, yogurt, and olives. Synthetic urethane is used as a chemical intermediate in the manufacture of pesticides, fumigants, and cosmetics. Chemically, it is the ethyl ester of carbamic acid. It should not be confused with the high-molecular-weight polyurethanes used as foams, elastomers, and coatings which are sometimes called urethanes. Such products are not made from, nor do they degrade to, urethane.

Increased levels of urethane in alcoholic beverages have been found to be the result of the use of urea as a yeast nutrient and the use of the food preservative, diethylpyrocarbonate. The latter was banned by the FDA in the early 1970s, and according to the Bureau of Alcohol, Tobacco, and Firearms, urea is no longer used by U.S. producers of alcoholic beverages; however, urethane is a natural byproduct of the fermentation process and can be detected in fermented beverages and foods at generally low but measurable levels.

Ough investigated the occurrence of urethane in fermented foods and reported detectable levels in soy sauce, yogurt, bread, and olives. Concentrations in most samples were between 2 and 5 ppb, or just above the detection limit of 2 ppb. Later analysis by the FDA confirmed these findings, except for soy sauce which had a range of urethane of 0 to 84 ppb (with a mean of 7 ppb). Substantially higher levels have been found for various categories of alcoholic beverages, domestic and imported. Highest levels of urethane were found for fruit brandies, whiskies, liqueurs, and distilled spirits (e.g., vodka, gin, tequila, and rum). Generally, these average urethane levels were below 200 ppb, but a few samples were as high as 2000 to 12,000 ppb. Wines generally had urethane levels under 100 ppb, and most malt beverages had undetectable amounts.

The carcinogenicity of urethane has been suspected since the initial observations by Nettleship et al. Since this observation, extensive carcinogenicity testing in several species by several routes has been carried out by many laboratories. Urethane has been shown to be carcinogenic in rats, mice, hamsters, and monkeys when administered orally, by parenteral injection, and by dermal application. It has produced multiple tumors at both local and systemic sites. These tumors were in the lungs, liver, forestomach, hematopoietic system, mammary gland, and skin, among other organs. Table 14 has a summary of the studies employing the oral route of exposure. Results from mutagenicity studies are either negative or inconclusive, for both bacterial and mammalian cells (for an extensive review of the genotoxicity of urethane see Bateman and Allen et al.). No studies on exposed humans are available. Based on the totality of evidence, the IARC concluded that there is sufficient evidence for the carcinogenicity of urethane in animals, and that it is “possibly carcinogenic to humans” (i.e., Group 2B). The EPA classifies urethane as a “probable human carcinogen” (i.e., a B2 carcinogen). Given the preponderance of evidence, there is sufficient justification to consider urethane as a carcinogenic risk to humans.

CONCLUSION

The food supply in the U.S. is the most wholesome, abundant, varied, low cost, and safest in the history of mankind. That is not to say that the food supply is devoid of all harmful compounds. It is a fundamental toxicological tenet that all compounds are toxic to some degree, and it is the amount ingested that will determine whether a dietary compound is harmful. The public concern with the development of cancer as a major food hazard may be a bit misguided, but it has some basis. It is known from the elegant epidemiological analysis conducted by Doll and Peto in 1981 that 20 to 50% of all human cancer is associated
<table>
<thead>
<tr>
<th>Species</th>
<th>Dose</th>
<th>Dosing Duration</th>
<th>Carcinogenic Target Organ(s)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>0.15% diet</td>
<td>15 months</td>
<td>Lung</td>
<td>461</td>
</tr>
<tr>
<td>Mouse</td>
<td>15 mg/wk i.g.</td>
<td>45 wk</td>
<td>No tumors without co-administered croton oil</td>
<td>462</td>
</tr>
<tr>
<td>Mouse</td>
<td>60–900 mg i.g. (total dose)</td>
<td>3–45 wk</td>
<td>Forestomach</td>
<td>463</td>
</tr>
<tr>
<td>Mouse$^b$</td>
<td>15 mg i.g., or 0.1% diet</td>
<td>1–10 wk, or 6 months</td>
<td>Lung</td>
<td>464</td>
</tr>
<tr>
<td>Hamster</td>
<td>0.2% d.w.</td>
<td>55–76 wk</td>
<td>Skin, forestomach</td>
<td>465</td>
</tr>
<tr>
<td>Hamster</td>
<td>0.2–0.4% d.w.</td>
<td>42 wk</td>
<td>Skin, forestomach, lung, mammary gland, liver</td>
<td>466</td>
</tr>
<tr>
<td>Mouse</td>
<td>0.4% d.w.</td>
<td>42 wk</td>
<td>Hematopoietic system</td>
<td>467</td>
</tr>
<tr>
<td>Mouse</td>
<td>0.1–0.3% d.w.</td>
<td>13–31 wk</td>
<td>Lung, hematopoietic system, mammary gland</td>
<td>468</td>
</tr>
<tr>
<td>Rat</td>
<td>0.1% d.w.</td>
<td>14–24 wk</td>
<td>Zymbal gland</td>
<td>469</td>
</tr>
<tr>
<td>Mouse</td>
<td>2.8 or 5.5 mg i.g.</td>
<td>3 times per wk for 5 wk</td>
<td>Hematopoietic system, liver, lung</td>
<td>470</td>
</tr>
<tr>
<td>Mouse</td>
<td>0.4% d.w.</td>
<td>5–30 days</td>
<td>Hematopoietic system, lung, liver, Harderian gland</td>
<td>471</td>
</tr>
<tr>
<td>Mouse</td>
<td>0.4% d.w.</td>
<td>10–20 days</td>
<td>Hematopoietic system, mammary gland</td>
<td>472</td>
</tr>
<tr>
<td>Rat</td>
<td>0.2% diet</td>
<td>15–20 days</td>
<td>Thymus, lung, Harderian gland, mammary gland</td>
<td>473</td>
</tr>
<tr>
<td>Rat</td>
<td>0.1% d.w.</td>
<td>Lifetime (ave. = 54 wk)</td>
<td>Cannot be determined due to no control group</td>
<td>474</td>
</tr>
<tr>
<td>Mouse</td>
<td>0.1% d.w.</td>
<td>10 wk</td>
<td>Tumor incidence not reported</td>
<td>475</td>
</tr>
<tr>
<td>Hamster</td>
<td>0.1% d.w.</td>
<td>Lifetime</td>
<td>Skin, forestomach, cecum, liver, adrenal, gall bladder</td>
<td>476</td>
</tr>
<tr>
<td>Mouse</td>
<td>158 mg/kg b.w. i.g. then 600 ppm diet</td>
<td>69–74 wk</td>
<td>Lung, liver, Harderian gland, hematopoietic system</td>
<td>477</td>
</tr>
<tr>
<td>Mouse</td>
<td>0.01% d.w.</td>
<td>Lifetime$^d$</td>
<td>Lung, bone, hematopoietic system, urinary bladder, mammary gland</td>
<td>478</td>
</tr>
<tr>
<td>Rat</td>
<td>0–12.5 mg/kg b.w. in d.w.</td>
<td>Lifetime</td>
<td>Mammary gland</td>
<td>479</td>
</tr>
<tr>
<td>Mouse</td>
<td>0–12.5 mg/kg b.w. in d.w.</td>
<td>Lifetime</td>
<td>Lung</td>
<td>480</td>
</tr>
<tr>
<td>Monkey$^b$</td>
<td>250 mg/kg b.w. i.g.</td>
<td>5 days/wk for ≤ 5 yr</td>
<td>Mammary gland</td>
<td>480</td>
</tr>
<tr>
<td>Mouse</td>
<td>0–42 mg/kg b.w. i.g.</td>
<td>3 times per wk for 8 wk</td>
<td>Liver, small intestine</td>
<td>481</td>
</tr>
</tbody>
</table>

$^a$ i.g. = intragastric; d.w. = drinking water; b.w. = body weight.

$^b$ Poor reporting of details limits evaluation of this study.

$^c$ Small group size.

$^d$ Six-generation study.
with dietary factors. Keeping this in mind, along with a substantial scientific database which includes actual morbidity and mortality data, the FDA has developed a list of food safety priorities. Topping the list are foodborne disease (e.g., pathogenic microbiological contamination) and nutritional imbalances, including overnutrition. Much less of a significant cause of human illness are environmental contaminants and naturally occurring toxicants, including carcinogens. This low-level occurrence of natural “carcinogenic” compounds present in the food supply for thousands of years does not pose a new safety risk. What is relatively new is that we can now crudely determine or estimate this low level of risk, but the fact remains that humans have lived with essentially the same food supply for many centuries without significant harm. Our body’s metabolic and other defense systems are well suited to detoxify the low-level exposure to natural carcinogens. Furthermore, the identification and evaluation of nearly all carcinogens have relied on experimental investigation using laboratory animals. This model has several limitations; chief among these is the use of “maximum tolerated dose” (i.e., the MTD). Use of the MTD ensures that even weak carcinogens are not missed; however, its use has the potential to exceed the test organism’s natural defense systems and other underlying biological processes and perhaps to produce tumors that are not relevant under conditions normal to humans. Finally, it should not be forgotten that the food supply contains many anticarcinogenic substances as demonstrated by data from hundreds of experimental laboratory studies. Further evidence of the efficacy of these anticarcinogenic substances can be found through the many epidemiological studies showing the clear benefit of consuming a diet high in fruits and vegetables in the prevention of cancer, despite the presence of potentially carcinogenic compounds.

A perusal of this chapter reveals several classes of compounds that have sufficiently potent carcinogenic activity and occur at significantly high enough levels in various foods to warrant attention. These compounds are for the most part limited to mycotoxins, nitrosamines, and compounds produced by cooking or other thermal processes. All of these compounds either receive scrutiny and control by governmental regulatory bodies or are subject to a large number of safeguards, controls, and tests utilized by the food industry. A second tier of compounds includes those that are plant derived but pose uncertainty in relating the animal carcinogenicity evidence to humans. Apart from the uncertainty in species differences in metabolism and sensitivity, the levels of exposures used in the animal studies are many orders of magnitude greater than the levels ingested by humans. In addition, the near total absence of epidemiological studies makes it extremely difficult to assess the human carcinogenic risk. The remaining group of compounds belong to foods that have limited geographical ingestion, typically are tested at unrealistically high levels, and possess evidence of carcinogenicity that may be weak and limited to a few isolated studies. Clearly, the difficulties in determining the human carcinogenic risk posed by the compounds belonging to the latter two groups (and to some extent for the first group of compounds, too) will not be resolved until experimental models are developed to assess carcinogenicity at low exposure levels and greater emphasis is placed on understanding the metabolic pathways of carcinogens and how they may differ among species. Finally, assessing the carcinogenic risk of a particular compound would be greatly enhanced by the inclusion of well-designed epidemiological studies incorporating sound exposure data.

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PART 3

Inhalation Toxicology

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