

## Food Mutagens<sup>1</sup>

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**ABSTRACT** Several lines of evidence indicate that diet and dietary behaviors can contribute to human cancer risk. One way that this occurs is through the ingestion of food mutagens. Sporadic cancers result from gene-environment interactions where the environment includes endogenous and exogenous exposures. In this article, we define environment as dietary exposures in the context of gene-environment interactions. Food mutagens cause different types of DNA damage: nucleotide alterations and gross chromosomal aberrations. Most mutagens begin their action at the DNA level by forming carcinogen-DNA adducts, which result from the covalent binding of a carcinogen or part of a carcinogen to a nucleotide. However, the effect of food mutagens in carcinogenesis can be modified by heritable traits, namely, low-penetrant genes that affect mutagen exposure of DNA through metabolic activation and detoxification or cellular responses to DNA damage through DNA repair mechanisms or cell death. There are some clearly identified (e.g., aflatoxin) and suspected (e.g., *N*-nitrosamines, polycyclic aromatic hydrocarbons or heterocyclic amines) food mutagens. The target organs for these agents are numerous, but there is target-organ specificity for each. Mutagenesis, however, is not the only pathway that links dietary exposures and cancers. There is growing evidence that epigenetic factors, including changes in the DNA methylation pattern, are causing cancer and can be modified by dietary components. Also DNA damage may be indirect by triggering oxidative DNA damage. When considering the human diet, it should be recognized that foods contain both mutagens and components that decrease cancer risk such as antioxidants. Thus nutritionally related cancers ultimately develop from an imbalance of carcinogenesis and anticarcinogenesis. The best way to assess nutritional risks is through biomarkers, but there is no single biomarker that has been sufficiently validated. Although panels of biomarkers would be the most appropriate, their use as a reflection of target-organ risk remains to be determined. Also even when new biomarkers are developed, their application in target organs is problematic because tissues are not readily available. For now most biomarkers are used in surrogate tissues (e.g., blood, urine, oral cavity cells) that presumably reflect biological effects in target organs. This article reviews the role of food mutagens in mutagenesis and carcinogenesis and how their effects are modified by heritable traits and discusses how to identify and evaluate the effects of food mutagens. *J. Nutr.* 133: 965S–973S, 2003.

**KEY WORDS:** • *biomarkers* • *diet assessment* • *epidemiology* • *mutagens* • *nutrition*

Foods and dietary behaviors are thought to increase cancer risk, which is due in part to the consumption of food mutagens. These mutagens contribute to cancer along the route of exposure (oral cavity, esophagus, gastrointestinal tract) and in organs that are distant to the route of exposure (e.g., liver). Although there are some clearly identified food mutagens (e.g., aflatoxin) and other suspected mutagens (e.g., *N*-nitrosamines or heterocyclic amines), we do not know what if any mutagens cause cancers that have been associated with different types of

diet (e.g., high fat). On the other side, and not reviewed herein, are dietary constituents that reduce the risk of cancer, in some cases by decreasing the effects of food mutagens, and dietary compounds that might indirectly affect the cellular control of DNA regulation via methylation.

Sporadic cancers result from gene-environment interactions where the environment includes endogenous and exogenous exposures (1,2). In this article, we define environment to include dietary exposures and the exposures that occur at the cellular and macromolecular levels. Food mutagens cause different types of DNA damage, namely, nucleotide alterations and gross chromosomal aberrations. Most mutagens begin their action at the DNA level by forming carcinogen-DNA adducts (3–5), which result from the covalent binding of a carcinogen or part of a carcinogen to a nucleotide. However, the effects of food mutagens in carcinogenesis can be modified by heritable traits, namely, low-penetrant genes that affect mutagen exposure of DNA through metabolic activation and detoxification or cellular responses to DNA damage through DNA repair mechanisms or cell death.

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This article reviews the role of food mutagens in mutagenesis and carcinogenesis and how their effects are modified by heritable traits; it also discusses how to identify and evaluate the effects of food mutagens. There are data to indicate that food mutagens and carcinogens affect specific organs rather than affecting every organ in the body. Separately foods contain many substances that likely reduce cancer risk, such as antioxidants or some types of fibers. These are not reviewed herein, but it should be recognized that nutritionally related cancers occur through an imbalance of carcinogenesis and anticarcinogenesis.

Mutagenesis is not the only pathway that links dietary exposures and cancers. There is growing evidence that epigenetic factors including changes in DNA methylation patterns are causing cancer (6) and can be modified by dietary components (7). Also DNA damage may be indirect by triggering oxidative DNA damage (8,9).

### *Mutagenesis and carcinogenesis*

Upon entering the body, food mutagens typically undergo metabolic activation and detoxification by endogenous enzymes (10) whose roles are to rid the body of foreign compounds. Sometimes the chemically modified mutagens that are more reactive (electrophilic) bind to DNA rather than the excretory carrier molecules. This binding can then cause coding errors at the time of DNA replication. However, redundant DNA repair mechanisms exist that can repair DNA adducts (excision repair), which is aided by cellular processes that trigger cell-cycle arrest and thereby allow for more time to repair DNA. These cell-cycle processes are triggered by DNA damage. If the DNA adducts however are not repaired, they can cause point mutations, deletions, insertions or gross chromosomal abnormalities. Not all adducts are promutagenic, and some sequences are more prone to allowing adduct formation or mutation. If gross chromosomal abnormalities occur, then there are DNA repair enzymes (recombination repair), which are aided by other proteins, that delay the cell cycle. If the DNA damage persists, the cells undergo cell death (oncosis or apoptosis) unless there is a selective clonal advantage.

Some dietary exposures can plausibly modify the effects of food mutagens. For example, alcoholic beverages induce an isoform of cytochrome P450 (CYP)2E1,<sup>3</sup> which metabolically activates *N*-nitrosamines, whereas the ingestion of vitamin C prevents the formation of *N*-nitrosamines. Exposure to certain food mutagens such as polycyclic aromatic hydrocarbons (PAH) can induce CYP1A1, which then causes increased metabolic activation of these compounds.

Cancer-related genes can be classified as protooncogenes and tumor-suppressor genes. The former are normally functioning genes that regulate normal cell growth, replication and differentiation but contribute to carcinogenesis when they are mutated in such a way that leads to uncontrolled gene expression and cellular proliferation. Tumor-suppressor genes also regulate normal cell growth, replication and differentiation but contribute to carcinogenesis when a mutation leads to loss of function. More recently cancer-related genes have been classified as caretaker, gatekeeper and landscaper genes (11,12). Caretaker genes are responsible for maintaining genomic integrity (e.g., DNA repair, metabolic activation and

detoxification), and when mutated they increase the probability of mutations in other genes. The gatekeeper genes are responsible for cell-cycle control, signal transduction and replication. When mutated, these genes allow for selective clonal expansion. Landscaper genes are responsible for providing signals to adjacent cells. Although the classification of these genes was originally proposed in the context of high-penetrant genes and familial cancer syndromes, the concept is also applicable to low-penetrant genes and gene-environment interactions (2). Consideration of the specific genetic functions and how they are mutated should lead to different paradigms in cancer risk and statistical models for approaching gene-gene interactions.

The effects of food mutagens on caretaker and gatekeeper genes can theoretically be modulated by interindividual variation in function of any enzyme involved in DNA damage and response (metabolic activation, detoxification, DNA repair, cell-cycle control, apoptosis, etc.). For food mutagens that lead to cancer, interindividual variation is governed by genetic polymorphisms where the frequency of the genetic variant is > 1% in the population of interest. Sporadic cancer risk is usually modified by genetic polymorphisms in low-penetrant genes (risk of the genetic trait is > 1 but < 2), and the risks of these genes are more often identified in the context of exposure and not as a main effect. It is important to realize that although the increase in cancer risk associated with polymorphisms in low-penetrant genes is small, the attributable risk in the population is large due to the high frequency of the variants (2). Most research for genetic susceptibilities has focused on carcinogen metabolism and detoxification (13–15), and more recently there has been a focus on DNA repair (16). Clearly research efforts need to expand toward the study of gatekeeper and other caretaker genes.

### *Methods to study food mutagens*

This section addresses methods for identifying food mutagens that might cause cancer in experimental studies and the use of biomarkers to assess human effects. Establishing the role of food mutagens in carcinogenesis requires the availability and interpretation of different experimental and human experiential evidence, and approaches to assess causality have been proposed (17). The study of food mutagens includes complementary approaches such as chemical model systems that identify the structure of carcinogens and their mode of action (18,19), animal and human cell-culture models that verify the applicability of the chemistry to an *in vivo* system (20), animal *in vivo* models of carcinogenesis (21,22) and additional epidemiological studies that incorporate biomarkers identified by using the other methods (1,15). Each of these has limitations and advantages (Table 1).

The different types of methods for studying food mutagens must be interpreted in the contexts of one another (23), the doses used (24) and the target organs (25). For example, the predictability for human carcinogenesis of any single method (e.g., *in vitro* cell culture or experimental animal study) is low, and the concordance among different experimental systems is variable. There are significant differences in susceptibility among species; some are more sensitive than others, so that extrapolation from animal to human experience can be difficult (25–28). Importantly, although a study might indicate the mutagenic potential of a food mutagen, whether it actually causes mutations and cancer in humans can only be conclusively demonstrated in humans. Genetically altered cells and animals might increase the accuracy of predictive laboratory tests (29).

<sup>3</sup> Abbreviations used: ADH, alcohol dehydrogenase; BaP, benzo(a)pyrene; CYP, cytochrome P450; PAH, polycyclic aromatic hydrocarbon

TABLE 1

*Methods to assess food mutagens*

Method	Example	Advantage	Limitation
In vitro DNA assays	Chemical modeling, DNA binding	Rapid, inexpensive	Provides information only about chemical structures
In vitro cell-culture testing	<i>Salmonella typhimurium</i> (Ames) mutation assay, HGPRT <sup>1</sup> forward mutation assays, chromosomal aberrations, unscheduled DNA synthesis, cell transformation assays, microarray expression analysis	Rapid results, human cells can be used, use of genetically engineered cells provides functional information, provides specific information about site-directed mutagenesis, best control of experimental conditions, economical	Uncertain in vitro-to-in vivo extrapolations, frequent false positives and negatives, mutagenicity is not the same as carcinogenicity, substantial interlaboratory variation, complex mixtures difficult to evaluate
Animal bioassay	National Toxicology Program rodent bioassays, genetically altered animals	More predictive of human experience than short-term tests, elucidates species differences, can provide functional relationships to carcinogenesis	Expensive, doses are higher than those experienced by humans, uncertain animal-to-human extrapolation
Epidemiology	Prospective cohort studies, case-control studies, case-case comparisons, human experimental exposure studies	Direct measurement of human experience, covariates examined, dose-response data, now incorporating genetic and other biomarkers	Insensitive, does not prove causation, unknown confounding variables, biomarker data studies in early stage of development and validation

<sup>1</sup> HGPRT, Hypoxanthine-guanine phosphoribosyltransferase.

High-throughput screening methodologies for the mechanistic and toxicological endpoints are being developed for use in experimental systems and epidemiological studies (30–32). High throughput can refer to the ability to identify a large number of cellular effects in a single experiment or the application of an assay in a large number of subjects. The new methods include large-scale genomic screens based on microarrays (33,34) and proteomic screens based on 2-D gel electrophoresis with mass spectrometric detection (35,36) or surface-enhanced laser desorption/ionization (37). The genomics and proteomic methods are plausibly useful to screen for toxicological effects and earlier carcinogenic events in vitro and in in vivo experimental studies (38,39). Some methods can be applied to large numbers of epidemiological subjects (40). However, the interpretation of data when there are a large number of data points for both known and unknown genes or proteins challenges our current statistical models and requires novel approaches to the interpretation of data. For example, the best methods for determining unique patterns of expression that are representative of a particular toxic effect or clustering responses that predict a combination of effects are only now being tested. These methods use either cluster data based on statistical relationships or biological pathways. Other new advances are providing researchers with powerful tools for analysis of diet-related carcinogenic pathways. The combinatorial synthesis of chemical compounds has tremendously increased the production of novel chemicals and drugs including dietary supplements (41–43). These provide new opportunities to expand cancer research as well as the understanding of carcinogenesis.

Biomarker assays are frequently used to assess exposure to food mutagens and how the body responds to those exposures. Any assay that is performed on a biological fluid or tissue can be considered a biomarker assay. Importantly exposure in this context refers to exposure at the cellular and macromolecular levels rather than what might be in foodstuffs or reflected in dietary behaviors. An important conceptual approach is to consider the biologically effective dose of a mutagen (44),

which is a measure of the effect of the mutagen in DNA (i.e., carcinogen-DNA adducts, nucleotide alterations, chromosomal aberrations) or its surrogate. The biologically effective dose is a phenotype of a person's response to the exposure; namely, the net result of metabolic activation and detoxification, a lack of DNA repair and a lack of triggered cell death. Because epidemiological studies that necessarily rely on a subject's recall of dietary behavior present some challenges to identifying food-mutagen intake, the use of internal dosimeters and biomarkers can reduce this limitation by improving exposure classification at the macromolecular level and ultimately improving risk assessments (45,46). Currently questionnaires and biomarker assays are mostly complementary and are most powerful when used together.

The use of biomarkers within epidemiology improves exposure assessments (e.g., characterizing low-dose exposures or low-risk populations), provides a relative contribution of individual chemical carcinogens from complex mixtures (e.g., *N*-nitrosamines) and estimates the total burden of a particular exposure when there are numerous sources [e.g., benzo(a)pyrene (BaP) from diet, air, tobacco and occupation] (47). In general, biomarkers are intuitively more informative and should be better disease risk markers. However, often these assays are technically limited and target tissue is difficult to obtain. Although surrogate markers and the use of surrogate tissues are popular because they reduce technical challenges, only a small effort has been undertaken to ensure that the surrogates actually reflect the effects of interest. Correlative studies are needed, and therefore assays that directly measure the effects of interest in target organs also are needed even though these might be technically limited, labor intensive or used in small numbers of subjects. As an example, the relationship of surrogate markers such as carcinogen-DNA adducts in blood to the target organ has been partially established (48,49). Recently the emphasis on methodology and quality control in research laboratories that focus on mutagen-related biomarkers has been improving.

A variety of assays are available for identifying carcinogen-macromolecular adducts in human tissues (50–56). These

include the  $^{32}\text{P}$ -postlabeling assay/nucleotide chromatography, immunoassays such as immunohistochemistry, fluorescence spectroscopy, gas chromatography/mass spectroscopy and electrical chemical detection. Recent methods take advantage of new methodologies including radiolabels detectable at very low doses (56) and fluorescent detectors. Each of these adduct assays has its usefulness and limitations, and all are challenged by sensitivity and/or specificity.

Other biomarker assays have been used to assess the biological consequences of mutagen exposure. A nonspecific assay is to measure urine mutagenicity, where extracts of human urine are used in the *Salmonella* mutation assay. Also sister chromatid exchanges and chromosomal aberrations can be measured in cultured lymphocytes, but these are probably too nonspecific to be useful in assessing dietary effects.

The study of mutations in the *p53* tumor-suppressor gene is uniquely suited for the study of cancer etiology, exposure and susceptibility (57), because *p53* is involved in many cellular processes including maintenance of genomic stability, programmed cell death (apoptosis), DNA repair and cell-cycle control (58,59). The *p53* mutation frequency in cancer varies by organ site and histological subtype (60), which indicates that cancers occur through different pathways and exposures at the cellular level. There are several examples of specific carcinogenic exposures that are linked to cancers via a *p53* mutational mechanism particularly for dietary aflatoxin B<sub>1</sub> exposure and a consistent finding of mutations in the third nucleotide pair of codon 249 of liver cancers in regions with endemic exposure to aflatoxin B<sub>1</sub> (61,62). Combinations of exposures also can lead to different outcomes in the same organ site. An interactive effect of hepatitis B exposure and aflatoxins increase the risk of mutations (63).

Biomarker assays can be useful in confirming a hypothesis regarding etiological relationships. For example, although alcoholic beverages are associated with oral cavity cancers and weakly with breast cancers (64–66), the carcinogenic agent remains unknown. Ethanol is oxidized to acetaldehyde, which is weakly mutagenic. This oxidation is governed by alcohol dehydrogenases (ADH) and the ADH3 gene is polymorphic. In different studies, the allele corresponding to increased oxidation capacity was associated with both oral cavity (67) and breast (68) cancers. Separately a role for oxidative damage in breast cancer is supported by studies of the manganese

superoxide dismutase gene; polymorphic variants increased breast cancer risk, which was greatest in persons with low antioxidant food intake (69).

Examples of biomarkers associated with specific exposures and target organs are shown in **Table 2**.

### Aflatoxin B<sub>1</sub>

Studies of aflatoxin B<sub>1</sub> exposure have provided a clear paradigm for a food mutagen related to cancer risk. Although hepatitis B and C infections carry a higher risk than aflatoxin B<sub>1</sub> exposure, both laboratory and epidemiological data exist that causally establish aflatoxin's role in liver carcinogenesis (46). Aflatoxin B<sub>1</sub> exposure occurs through the consumption of mold-contaminated corn and animal feed (70), which can be transmitted transplacentally (71) and to newborns via breastfeeding (72). Exposures are low in the U.S.A. but can be high in China and parts of Africa. Laboratory animal models indicate that aflatoxins are mutagenic and carcinogenic (46). Epidemiological studies in Africa and Asia, where high levels of exposure occur, link aflatoxin B<sub>1</sub> exposure and hepatocellular carcinomas (63,73–76), and the risk is synergistic with hepatitis viral infections (63). The mechanism of action for aflatoxin B<sub>1</sub> mutagenicity begins with metabolic activation by CYP3A4, CYP3A5 and/or CYP1A2 (77–81) that forms an *exo*-8,9-epoxide and subsequent adduct formation and DNA damage (82,83). This damage has been shown *in vitro* to cause guanine nucleotide substitutions (84) specifically to codon 249 of the *p53* gene (85). Liver cancers from areas with high levels of aflatoxin contamination almost always have codon-249 *p53* mutations, whereas the frequency of this mutation is low in areas of low contamination and intermediate in areas of intermediate contamination (60,86,87). Interestingly, there seems to be an interactive effect for increasing *p53* mutations in persons with hepatitis B and coexposure to aflatoxin (74).

Several biomarker assays for aflatoxin exposure have been developed (46,88). Although it would be preferable to measure the biologically effective dose in the liver, because this is the target organ, it is not practical. Thus methods have focused on blood and urine markers. Levels of aflatoxin adducts vary among areas of low and high contamination (89). Measurements of the adduct or its metabolite in the urine indicate

**TABLE 2**

*Examples of biomarkers of exposure and susceptibility*

Carcinogen	Target organ	Biomarker			
		Genetic susceptibility		Phenotypic biomarker	
		Activating enzyme with genetic polymorphisms	Detoxifying enzyme with genetic polymorphisms	Adduct	<i>p53</i> Mutation
Aflatoxin B <sub>1</sub>	Liver	CYP3A4, <sup>1</sup> CYP3A5, CYP1A2	Glutathione transferases, glucuronyl transferases	Adducts in DNA, albumin, hemoglobin and urine; urinary metabolites	Codon 249 AGG to AGT
Polycyclic aromatic hydrocarbons	Lung, oropharyngeal, breast, gastrointestinal and genitourinary tracts	CYP1A1, CYP1B1, epoxide hydroxylase	Glutathione transferases, glucuronyl transferases	Adducts in DNA, albumin, hemoglobin; urinary metabolites	Increased G-to-T mutations at codons 157, 248, 273
<i>N</i> -nitrosamines	Gastrointestinal, lung, oropharyngeal	CYP2A6, CYP2E1	—	<i>O</i> <sup>6</sup> -methylguanine, 7-methylguanine	—
Heterocyclic amines	Colon, breast	CYP1A2, NAT1 <sup>2</sup>	NAT2	Adducts in albumin, and DNA adducts	—

<sup>1</sup> CYP, cytochrome P450.

<sup>2</sup> NAT, *N*-acetyltransferase.

a dose-response effect for aflatoxin B<sub>1</sub> exposure and liver cancer (46,63). The effect is multiplicative in persons infected with hepatitis B or C (90,91).

The detoxification of aflatoxin *exo*-8,9-epoxides by conjugation to glutathione has prompted studies of oltipraz as a chemoprotective agent, because it is an inducer of glutathione transferase M1 enzyme (92). The preliminary results from a phase II clinical trial show a significant reduction in the biomarkers of aflatoxin damage in the oltipraz-treated group (93).

### Polycyclic aromatic hydrocarbons

PAH compounds are formed during the incomplete combustion of organic matter. Eleven PAH compounds have been classified as carcinogenic to laboratory animals (94), and PAH compounds are human carcinogens (95). It is estimated that our diet provides 3  $\mu\text{g}$  PAH/d (96,97), which compares to an exposure of 2–5  $\mu\text{g}$  PAH/d per pack of cigarettes in a regular smoker. Thus dietary exposure can be significant in non-smokers and even exceed the level of regular smokers. Corroborative data that indicate that PAH exposure from diet is important are the findings that the intake of charcoal-broiled meat is more correlated to blood PAH DNA adducts than smoking (98,99).

PAH food exposure and cancer risk have received little attention. The effects of PAH exposure, which are measured mostly in other settings (e.g., tobacco smoking and the workplace), indicate that the target organs for PAH compounds are the lung, oropharynx, breast and genitourinary and gastrointestinal tracts. Thus biomarkers for the assessment of PAH would focus on studies in these organs or surrogate tissues for these organs.

In laboratory animal studies, diets with PAH consistently induce foregut tumors and also can induce lung tumors (100–105). In humans there is some evidence for association of dietary PAH exposure with colon cancer (106,107). Animal (108,109) and human studies (110) suggest that dietary PAH is distributed to other organs besides the locally exposed tissues, so it is plausible to consider that dietary PAH might contribute to lung or breast cancer risk, for example. Although the dietary contribution of PAH to the total body burden may be sizeable (111,112), the ubiquitous presence of PAH in the environment (113) and the presence of other carcinogens in the same foods (114,115) makes the interpretation of epidemiological studies of cancer risk due to dietary PAH difficult. It may be possible to distinguish PAH exposures in diet from smoking by measuring biomarkers specific to each [e.g., simultaneous measurement of 1-hydroxypyrene to evaluate total PAH intake (116) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) to evaluate the contribution due to smoking (51)]. In addition, dietary PAH is invariably a complex mixture of compounds with hard-to-predict metabolic and carcinogenic consequences.

Benzo(a)pyrene is the best-characterized PAH compound available from the diet. The bay-region diol epoxide binds to DNA mostly as the N<sup>2</sup>-deoxyguanosine adduct (117). The metabolic activation by the CYP1A (118) and CYP1B (119,120) classes of enzymes are required for adduct formation. BaP adducts can be quantified by several sensitive methods (56,121,122). Other methods exist for detecting PAH metabolites [e.g., urinary BaP-tetrol (123) and 3-hydroxybenzo(a)pyrene (124)]. BaP adducts estimate the biologically effective dose and suggest a link to cancer risk in the lung (125,126); they are also associated with site-specific hotspot mutations in the *p53* tumor-suppressor gene (60,127) and mutations observed in lung cancer of smokers (128). Similar

evidence for dietary PAH-associated cancer should be sought, for example, in gastrointestinal cancers.

### N-Nitrosamines

Humans are exposed to N-nitroso compounds in diet from a variety of cured meats and fish products (129,130). N-nitrosamines can be formed *in vivo* during simultaneous ingestion of nitrite or nitrogen oxides and a nitrosatable substrate such as a secondary amine (131). Dietary N-nitrosamines have been linked to esophageal and other gastrointestinal cancers (130,132); for example, N-nitrosamines are considered an important carcinogen in parts of China and Japan. Biomarker studies show that N-nitrosamine adducts are higher in these parts of the world compared to low-N-nitrosamine areas (133, 134). Although tobacco smoke and tobacco-specific nitrosamines cause lung cancer (51,135), dietary N-nitrosamines might also contribute to lung cancer (136–138).

Experimental animal models strongly support the carcinogenic properties of dietary N-nitrosamines (130). In fact, there is a large concordance between animal species and strains albeit with different organ specificity, type of compound and dose (139). Cancer of the lung, liver, kidney, mammary gland, stomach, pancreas, bladder or esophagus has been observed (140). These sites also are considered to be the target organs in humans, and so biomarker assays are best suited for them.

The N-nitrosamines are a large group of compounds with a common carcinogenic mechanism. N-nitrosodimethylamine frequently is formed as a result of dietary exposures. N-nitrosodimethylamine undergoes enzymatic hydroxylation and subsequent hydrolysis to an aldehyde and a monoalkylnitrosamine that rearranges and releases a carbocation that is reactive toward DNA bases (141,142). The hydroxylation is catalyzed mainly by CYP2E1 (143,144), but other cytochrome P450 isoforms including CYP2A6 have been implicated (14,145).

The O<sup>6</sup>-methylguanine is mostly responsible for the mutagenicity and carcinogenicity of alkylating agents (146,147). The O<sup>6</sup>-methylguanine leads to GC→AT transitions in cell-culture (148) and animal (149) models if not repaired by the O<sup>6</sup>-methylguanine methyltransferase (150, 151). A specific mutation was observed in codon 12/13 of *ras* oncogene in animals exposed to alkylating agents (152) [later evidence suggests that this may have been due to clonal selection rather than mutation (153)] and in human gastrointestinal tumors of unknown etiology (154). Although the O<sup>6</sup>-methylguanine is a promutagenic lesion, it is technically easier to measure the 7-methylguanine, which is not promutagenic, as a surrogate marker for exposure and genetic susceptibility, because the 7-methylguanine occurs at levels ~10-fold higher. Human studies of N-nitrosamine adducts in different tissues and the use of susceptibility markers should help elucidate the risks of N-nitrosamine exposures.

### Heterocyclic amines

Heterocyclic amines are formed during high-temperature cooking by pyrolysis of proteins, amino acids or creatine (155) and can be present in human diet in substantial concentrations depending on cooking habits (156,157). Heterocyclic amines are clearly bioavailable from normal human diet (158). The proposed bioactivation pathway consists of N-hydroxylation by CYP1A2 (159) and subsequent esterification (160,161). The nitrenium ion is the likely ultimate carcinogen binding to the DNA bases (162). Metabolic activation by CYP1A2 was documented in people after extensive characterization *in vitro* and in animal models as is reviewed elsewhere (163,164). The

activation by CYP1A2 can be induced in humans fed a diet rich in heterocyclic amines (114) and is affected by polymorphisms of phase II activating enzymes (161,165–168). The most active area of research for heterocyclic amines focuses on colon and breast cancer.

The parent heterocyclic compounds, their metabolites and biologically effective doses determined by DNA and protein adducts have been measured in human studies using accelerator mass spectrometry (55,169–171) and a variety of other very sensitive analytical methods (55,172,173). There is also good epidemiological evidence correlating the consumption of foods containing a high content of heterocyclic amines with colon cancer (174–176) although this correlation is not consistent (177). It appears that the mutations in the APC and *p53* tumor-suppressor genes suggest a connection to the exposure to heterocyclic amines (178), but further research is needed. Although the elimination of heterocyclic amines from the diet seems impractical, exposures can be reduced by cooking foods at lower temperatures (156,179). Possible chemopreventive interventions based on current understanding of the carcinogenic mechanism have been also proposed (180,181).

### Perspectives

New tools are allowing researchers to address many questions regarding food mutagens. These studies are being performed in the context of biologically based hypotheses. The biologically effective dose is an important measurement for assessing subsequent cellular outcomes including cancer. Currently assays that measure biologically effective doses are not sufficiently developed, but it is possible to investigate questions such as the measurement of dietary exposures in relation to nondietary exposures. Newer methods are now more sensitive and specific, so that they are becoming technically simpler and allowing for the use of smaller amounts of target tissue. In this way, the relationship between surrogate and target-organ effects can be addressed. But to date there is no single biomarker that has been sufficiently validated for the assessment of nutritional risk. A panel of biomarkers that reflects several gene-environment interactions would likely be more predictive of risk, but this needs to be determined. When possible, target-organ studies will provide the most direct evidence for the role of food mutagens in carcinogenesis. However, this is problematic, because tissues are usually not available from living people.

Newer technologies also will allow investigators to measure many types of effects simultaneously. Although the costs of these methods (such as microarray expression approaches) are prohibitive for large numbers of assays, the ability to observe many changes in a single experiment can be very powerful. Similarly some array methods can be applied to a large number of subjects, and once established (e.g., a tumor array), the results can be rapidly determined for a large number of markers. The current challenges include the lack of approaches to make meaningful interpretations of the data, and the varying of the data-interpretation methods depending on whether the data was collected with a priori hypotheses or through screening methods. Although these newer methods may be more powerful than other methods, the need for careful validation of the biomarker is still great.

This article has focused on the most commonly studied food mutagens, but many others exist in food as do agents that reduce cancer risk. The integrated consideration of all of these remains problematic because of the complex nature of the exposure and the documentation of dietary habits, and each of our research methods has strengths and limitations. The new methods for assessing biomarkers will not replace our estab-

lished epidemiological methods such as questionnaires and measurements of mutagens in foodstuffs, but they will be complementary.

Biomarkers that assess the effects of food mutagens range from markers of susceptibility to phenotypic markers of effect (*p53* mutations, overexpression of enzymes, etc.). Employing multiple biomarkers within well-designed epidemiological studies can be useful in identifying new food mutagens or the role of previously established mutagens.

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