Food Mutagens:

Mutagenic Activity, DNA Mechanisms, and Cancer Risk

Potent mutagens, called heterocyclic amines, are produced when foods derived from muscle and other protein sources are cooked. We have studied the metabolic pathways of these compounds and their interactions with DNA. This report, the second of two, focuses on the mechanisms by which food mutagens may lead to cancer and on the potential risks associated with their consumption.

OUR diets expose us to many substances that can be beneficial in maintaining health or harmful by causing disease. Of all the substances known to be produced during cooking, we now think that the most genetically toxic compounds are the heterocyclic amines.

The role of these potent food mutagens in the human diet has been the subject of ongoing research at LLNL for 17 years. A previous report in the July 1995 issue of *Science and Technology Review* provided an overview of the ways we identify and quantify mutagens in cooked food. Although isolating the toxic compounds and determining their amounts in various protein-containing foods are major efforts in themselves, they tell only part of our research. The other part concerns our efforts to understand how food mutagens can lead to genetic damage and, ultimately, to cancer—at least in laboratory animals that have received very high doses of mutagens.

Research on the genetic damage that can be caused by food mutagens begins with a paradox. Why does the human body make a cancer-causing substance out of certain trace compounds in food that, on ingestion, are virtually inert biologically?

Researchers have now identified more than a dozen heterocyclic amines in cooked foods commonly found in the Western diet. Five of these heterocyclic amines were first identified at LLNL. All are lipophilic (they have a strong affinity for fats). However, they are not, in themselves, either mutagenic or carcinogenic when eaten. Rather, the compounds become harmful only after they are chemically changed by metabolizing enzymes present in animal tissues, such as the liver. When the body encounters foreign substances with an affinity for fats (known as lipophilic xenobiotics), it tries to make them more soluble in water so they can be excreted. Most of the intermediate compounds that are formed in this process are further metabolized and harmlessly eliminated, primarily in the urine. However, some intermediate compounds, including those derived from cooked food, are highly reactive, binding to DNA (deoxyribonucleic acid) and potentially resulting in genetic damage.

The sequence of events leading from eating mutagenic precursors (promutagens) to DNA interactions and cancer is highly complex. The principal unknowns in the disease process are the reactions within cells and among molecules, and it is these events that drive our research efforts on food mutagens and the induction of cancer.

Major difficulties arise in our attempts to calculate the dose of food mutagens in the human diet and, therefore, to make realistic assessments of cancer risk to an individual. We are concerned with trace levels of exposure at the part-per-billion or even part-per-trillion level. The content of mutagen precursors even in one type of food, such as a hamburger, can vary widely depending on the details of cooking. In addition, some food mutagens are a thousand times more potent than others. Moreover, human dietary habits differ appreciably. As with most environmental carcinogens, the cancer risks posed to humans are a function of many variables, and estimating those risks entails making assumptions. Fortunately, LLNL investigators performing research on food mutagens have at their disposal several advanced techniques and tools with exquisite sensitivity, such as accelerator mass spectrometry, that are providing answers to daunting questions.

Facts and Basic Questions

Biologists use the term "mutagenic activity" to describe the potency of a mutagen known to cause structural damage to the molecular units that make up the genes. The mutagenic activity of the heterocyclic amines found in cooked food is strongly affected by several features of their molecular structure. Even small structural changes can have large effects on mutagenic activity.

The imidazole ring is a common feature in all of the heterocyclic amines (Figure 1). We know that mutagenic activity is increased when a methyl group (CH_3) is present on the imidazole ring. Both the position and number of methyl groups have an effect. Thus, as shown in the illustration, the mutagenic activity of one highly potent food mutagen, IQ, increases with the addition of a methyl group at the number-4 position of the molecule (to make 4-MeIQ). Conversely, mutagenic activity can be decreased by the addition of a methyl group to other positions, such as the number-5 position. The numbers and positions of double bonds and aromatic rings also have a large effect. However, the variations in mutagenic activity associated with changes in chemical structure are not always consistent in tests using different types of cells, tissues, or animals. That is, one species may be more susceptible to colon tumors for one mutagen, whereas a different species may be more susceptible to liver tumors for the same mutagen or a different one.

How does the potency of food mutagens generally compare with the potency of other biological toxins as measured by standard tests using bacteria? Benzo[*a*]pyrene is a widely studied carcinogen and common environmental pollutant that has been isolated in cigarette smoke, diesel

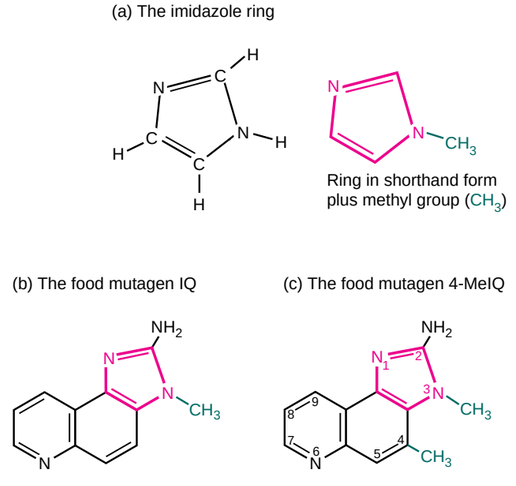

Figure 1. (a) The imidazole ring is a common feature in all of the heterocyclic amines. (b) IQ is a highly potent food mutagen that has been widely studied. (c) When a methyl group (CH_3) is added to the number-4 position of the molecule to make 4-MeIQ, the mutagenic activity is increased.

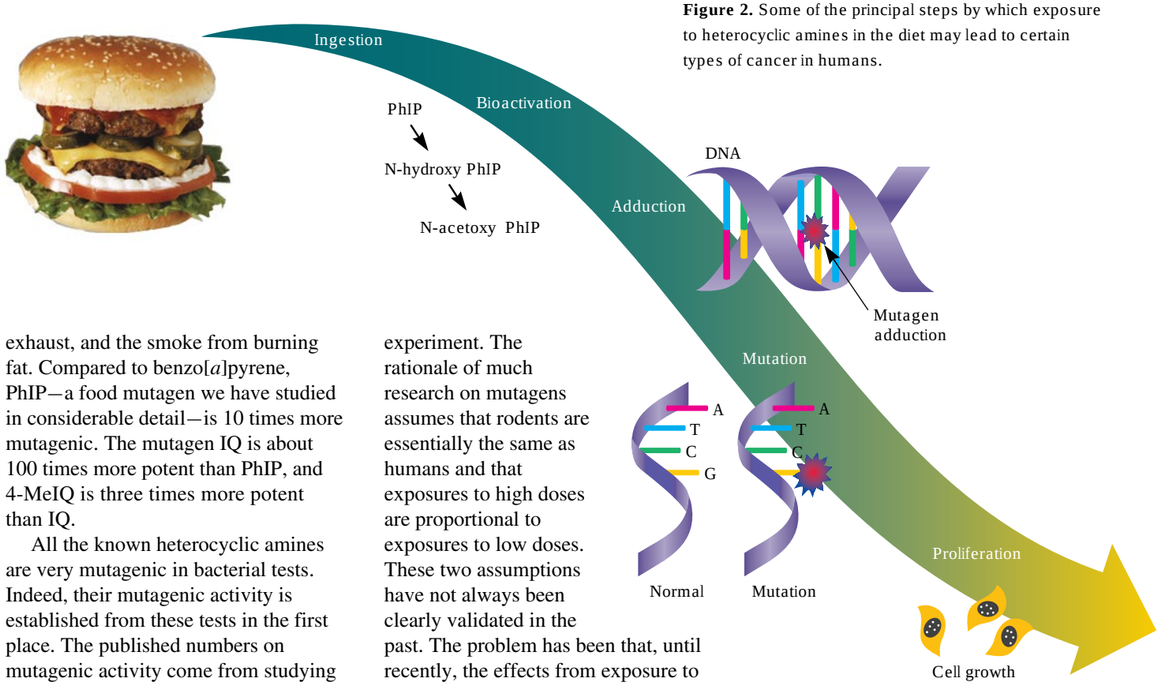

Figure 2. Some of the principal steps by which exposure to heterocyclic amines in the diet may lead to certain types of cancer in humans.

exhaust, and the smoke from burning fat. Compared to benzo[*a*]pyrene, PhIP—a food mutagen we have studied in considerable detail—is 10 times more mutagenic. The mutagen IQ is about 100 times more potent than PhIP, and 4-MeIQ is three times more potent than IQ.

All the known heterocyclic amines are very mutagenic in bacterial tests. Indeed, their mutagenic activity is established from these tests in the first place. The published numbers on mutagenic activity come from studying specific strains of the bacterium *Salmonella typhimurium* used in the Ames mutation assay. (See the July 1995 issue of *Science and Technology Review* for a detailed description of this test.) Beyond the bacterial tests, at least 11 heterocyclic amines have been shown to be carcinogenic in rodents, and at least one, IQ, is a potent inducer of liver tumors (carcinomas) in monkeys.[1]*

It is important to remember that the studies establishing mutagenic activity are done in bacteria, and other studies prompting further questions about diet-related carcinogenic compounds are done in animals, usually rodents. The studies on rodents involve very high doses of mutagens, in part because such research can be quite costly, and most studies are limited to about 30 to 50 animals per experiment. The rationale of much research on mutagens assumes that rodents are essentially the same as humans and that exposures to high doses are proportional to exposures to low doses. These two assumptions have not always been clearly validated in the past. The problem has been that, until recently, the effects from exposure to very low doses of mutagens were impossible to test empirically because our measuring instruments were not sensitive enough to detect them.

One of our recent studies is a good example of how we are addressing the problem of low-dose exposure. This work makes use of instruments that were not previously available in biomedical research. It was inspired by other researchers who raised the possibility that mothers eating well-done meat could pass on heterocyclic amines to their babies through breast milk. Concern about this route for transmitting mutagens is based on an experiment involving nursing pups when the maternal rats are given 10 mg of the mutagen PhIP per kilogram of body weight. Humans eating typical amounts of well-done meats consume 10,000 to 100,000 times less of the mutagenic material daily per kilogram of body weight than do the rats in such experiments. What is the plausibility, then, of extrapolating from high-dose experiments to the low doses experienced in actual human exposures?

If we are to make realistic estimates of risk, we need to understand the specific effects of chemicals at the relatively low levels that are characteristic of human exposures. To do so, we conducted a study in which we gave PhIP to rodents at doses spanning many orders of magnitude. We found that even at extremely low doses—down to the level of mutagens found in a single hamburger—the effects of PhIP were still visible in six types of tissue, notably the colon, breast, and pancreas.

Whereas this type of research answers some questions, it raises others. How much confidence can we place in extrapolations made from rats (and other animals) to humans? After all, mammals differ from one another in many ways, including the expression of various enzymes. Thus, even a species related more closely to humans than the rat may pose problems when we study animals to make human assessments. To address, in part, these differences and to evaluate the relevance of such information in human disease, we use many different kinds of model systems to estimate risk, including whole animals, human and animal tissue fractions, and bacterial assays, coupled with state-of-the-art research techniques. We have made remarkable discoveries at the molecular level on specific mechanisms by which food mutagens can lead to adverse health consequences.

Steps Leading to Cancer

Figure 2 shows some of the main steps by which exposure to heterocyclic amines may lead to certain types of cancer in humans. Of necessity, this scheme is highly simplified. In reality, many different kinds of chemical reactions, enzymes, intermediate metabolites, inhibitors, tissues, and genes—including tumor-suppressor and DNA-repair genes—play a role in whether or not humans will develop cancer after being exposed to dietary carcinogens.

Nevertheless, for convenience, we can break down the complex process into the following steps:

1. *Ingestion*. Humans eat foods, such as fried meat, containing promutagens that can become highly mutagenic when acted upon by enzymes.
2. *Bioactivation*. The body attempts to excrete the ingested toxins. Naturally occurring intracellular enzymes catalyze the formation of intermediate metabolites, which have the potential to react strongly with DNA.
3. *Adduction*. Certain intermediate food-mutagen molecules bind covalently to specific atoms in the DNA macromolecule and form bulky lesions called adducts.
4. *Mutation*. Structural changes in the molecular units that make up the genes can cause DNA replication errors, preventing the gene from functioning properly in daughter cells. DNA repair mechanisms may determine whether the structural changes are fixed or not.
5. *Proliferation*. In some cases, the mutations occur in genes controlling cell proliferation and replication, leading to tumors. Oncogenes or tumor-suppressor genes are specific examples of such genes.

Steps 1 and 5 are generally related to our research efforts in dose and risk assessment, respectively. But before one can understand how we assess risk, one must understand how food mutagens are biologically transformed into highly reactive intermediate molecules that are capable of linking up with and damaging the genetic material.

Bioactivation Is Key

Once the promutagens in cooked food are ingested, even in doses that are one-millionth those used in many animal tests, studies have shown that they survive the acid in the stomach. After they pass through the stomach and enter the intestine, the compounds are taken up by the bloodstream and are metabolized by the liver. Located within the cells of the liver and other organs is a family of enzymes called cytochrome P450s essential for many functions, including the metabolism of hormones and defense against harmful environmental chemicals.

In the liver, the heterocyclic amines interact with cytochrome P450 enzymes that are involved in defensive reactions. Biologists use the terms metabolic activation, or bioactivation, to describe the kinds of chemical changes that take place when cytochrome P450s act on foreign chemicals to convert them into chemically more polar and, consequently, biologically more reactive forms.

Researchers at LLNL and elsewhere have shown that the mutagenic activity of PhIP, for example, clearly depends on the reactive intermediates that form after it is acted upon by cytochrome P450 enzymes. As shown in Figure 3, one of the cytochrome P450 enzymes (in particular, P450IA2) converts PhIP into an intermediate molecule containing the hydroxy (OH^-) group. This polar intermediate, N-hydroxy PhIP, can bind to DNA, but it does so with low affinity.

We have shown that N-hydroxy PhIP is transformed into still more biologically active intermediates, which appear to be necessary for the stronger binding with DNA in the living body. The intermediate molecules include acetates (the acetate, N-acetoxy PhIP, is shown in Figure 3), sulfates, and other forms.

We have investigated many metabolic pathways that lead to the genetic toxicity of food mutagens like PhIP and MeIQx. In our investigations, we have used cells from animals and humans, enzyme extracts, bacterial cell cultures, and radioactive labeled isotopes. We have studied whether certain enzyme inhibitors could, in effect, block the binding of suspected intermediates to the DNA molecule, and whether other agents could increase the levels of P450 enzymes and the rate of DNA binding. This type of research

*All references are on p. 23.

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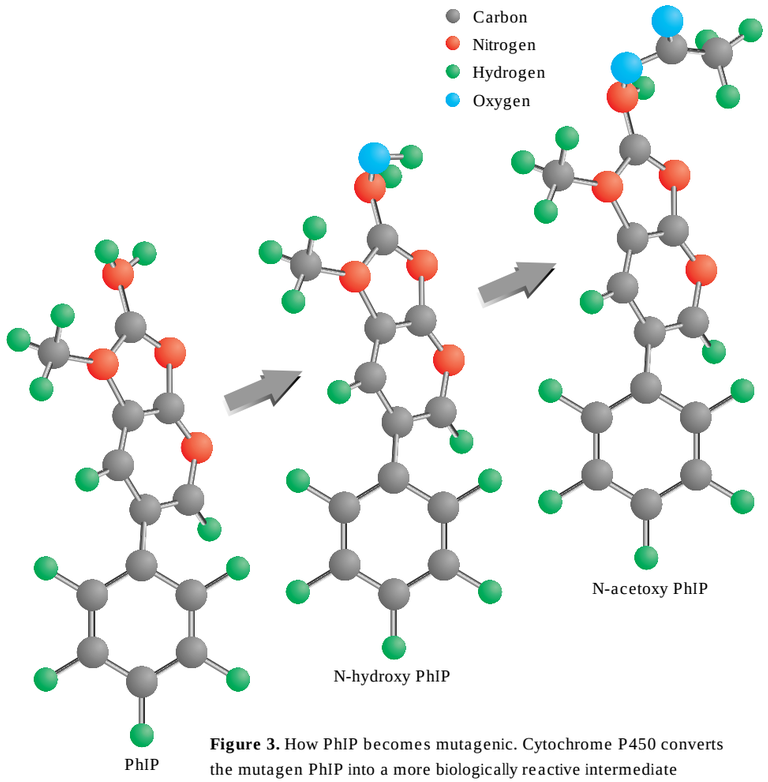


Figure 3. How PhIP becomes mutagenic. Cytochrome P450 converts the mutagen PhIP into a more biologically reactive intermediate molecule containing the hydroxy (OH^-) group. N-hydroxy PhIP is transformed into an acetate, called N-acetoxy PhIP, which is highly reactive with DNA.

helps us to better understand the mechanisms of toxicity and how compounds like PhIP and MeIQx can pose human health risks.

Our research suggests that the rules that apply to the metabolism of one food mutagen may not apply to another, tissue differences are important, and so are species differences. However, we now believe that the primary bioactivation of food mutagens reacting with P450 enzymes takes place mostly in the liver in both rodents and monkeys. Such activation occurs after the administration of either high experimental doses or very low doses of the sort typically found in human diets. Furthermore, bioactivation probably occurs in other tissues that are the targets of tumors, such as the breast and colon. Following the transport of the first intermediate (such as N-hydroxy PhIP) in the blood from the liver, bioactivation in these target tissues provides the acetates and sulfates that are close to the unknown, very reactive molecular species (possibly the nitrinium ion) that binds strongly with DNA.

Food Mutagens:

DNA Mechanisms

DNA is the spiral, double-stranded macromolecule that contains the genetic blueprint. As shown in Figure 4, DNA encodes the genetic information in the sequence of four different nucleotide bases: adenine (A), thymine (T), cytosine (C), and guanine (G). In DNA, the nucleotide base A on one strand of DNA always pairs with T on the other strand, and C always pairs with G. A specific string or sequence of the base pairs, which can typically range from about one thousand to two million pairs long, makes up a gene.

Adduction is the covalent binding of chemicals with large molecules, such as DNA or protein. Most often, adduction occurs after the chemicals are metabolized into reactive intermediates through the process of bioactivation. Adduction is of great interest to researchers for several reasons. It can serve as an integrated indicator of exposure to carcinogens and the bioactivation of promutagens, thus revealing individual susceptibility to cancer. Furthermore, DNA adduction could be an important indicator (biologists use the term "marker") as we look for ways to intervene and reduce individual cancer risk.

Figure 4 shows an artist's interpretation of DNA adduction. The adduct is the large molecule—in our case, a mutagen derived from cooked food—that can chemically bond to one (or possibly more) of the bases of the DNA sequence. The heterocyclic amines from cooked food are relatively bulky molecules. When reactive intermediates (such as N-hydroxy PhIP) chemically bond to DNA, they distort the normal DNA helix. The adduction can cause errors (mutations) to occur when the DNA replicates, or it may even block the ability to replicate at all. In either case, the normal function of the affected stretch of DNA will be impaired.

One of the problems for researchers is that it is difficult to detect adducts at the low exposure levels that are relevant to humans. To address this key problem, researchers at Livermore have assessed DNA damage at very low doses using two techniques: traditional ^{32}P-postlabeling and accelerator mass spectrometry (AMS).

Detecting DNA Adducts

The technique of ^{32}P-postlabeling involves tagging a chemical adduct of interest with the a radioactive isotope of phosphorus, ^{32}P. Researchers use ^{32}P because it is relatively easy to detect and has low natural abundance in biological material. As an assay for detecting DNA adducts, ^{32}P-postlabeling does not require prior knowledge about the type of exposure or the adduct structure, and it does not require prior radioactive labeling of the chemical of interest (thus, the name postlabeling). First, DNA is broken down into its component units (the nucleotide bases). Next, ^{32}P is added to the bases. The specific radioactively labeled adducts are separated from the nonadducted, nonlabeled DNA using inexpensive, thin-layer chromatography.

In practice, the method of postlabeling is sensitive enough to qualitatively detect

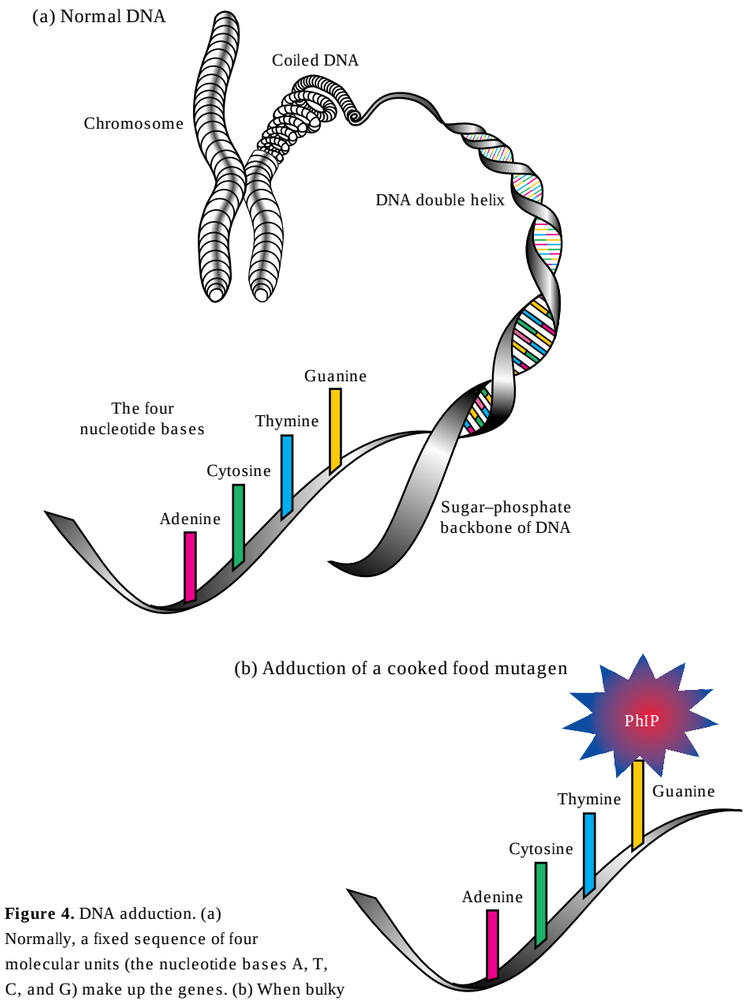


Figure 4. DNA adduction. (a) Normally, a fixed sequence of four molecular units (the nucleotide bases A, T, C, and G) make up the genes. (b) When bulky molecules (adducts) chemically bond to DNA, they distort the helix and can block the ability of DNA to replicate or can cause errors in replication.

roughly one adduct in about 10 cells (about one adduct per 10 billion nucleotides). However, it can semiquantitatively measure one adduct in one million to one billion nucleotides. The assay's principal use is in analyzing a group of structurally different adducts. Postlabeling is highly useful in studying potential human exposures to mutagens as well as carcinogens and the mechanisms and levels of DNA binding.

AMS is a nuclear physics technique for measuring radioisotopes (see Figure 5). Its use as an extremely precise, sensitive, and versatile tool in the field of biomedical research is relatively recent.[2] LLNL researchers Ken Turteltaub and John Vogel are responsible for much of the AMS work described in this article. The instrument consists of several mass spectrometers, separated by an electrostatic accelerator, and a detector for counting rare isotopes. (The box on p. 13 describes the set-up and applications of AMS in more detail.)

Rather than measuring atomic decay, as in liquid scintillation counting, AMS isolates and counts specific nuclei, particle by particle. Depending on which isotope is used to tag the molecules of interest, AMS can be up to a million times more efficient than decay counting in detecting specific, tagged molecules. When we tag mutagens with one or more radiocarbon (^{14}C) atoms, we can quantitatively measure as little as one DNA adduct in about a thousand cells (roughly one adduct per trillion nucleotides). This sensitivity and accurate quantification make it possible for us to study DNA adduction at doses as low as 5 nanograms per kilogram of body weight—close to actual human exposures from the environment.

The Effect of Low Doses

In one series of studies, we followed the DNA–food mutagen interactions that occur when rodents are given low doses of MeIQx. This mutagen is sometimes present in cooked meat products typical of the American diet. Animals were fed daily an amount of radioactively labeled MeIQx equivalent to what a human would receive eating about two hamburgers a day (200 g).

We found that concentrations of MeIQx stabilize in the tissues in about 7 days. We could detect DNA adducts 24 hours after ingestion, but it took about 40 days for the number of adducts to reach maximum concentration in the liver and kidney.

In related studies, we gave rodents ^{14}C-labeled MeIQx for 7 days. The doses ranged from levels below those that may be typical of human exposure to very high levels used in cancer assays. We then

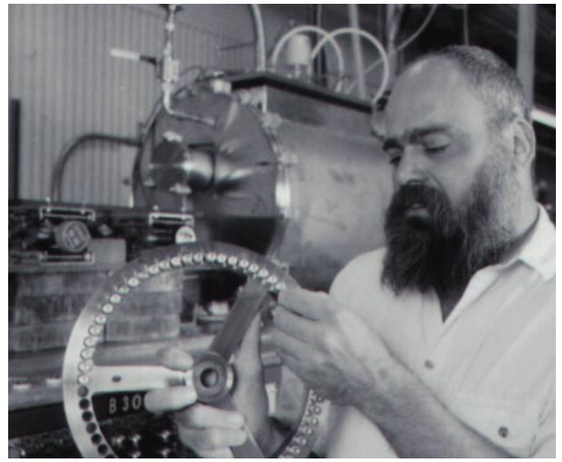


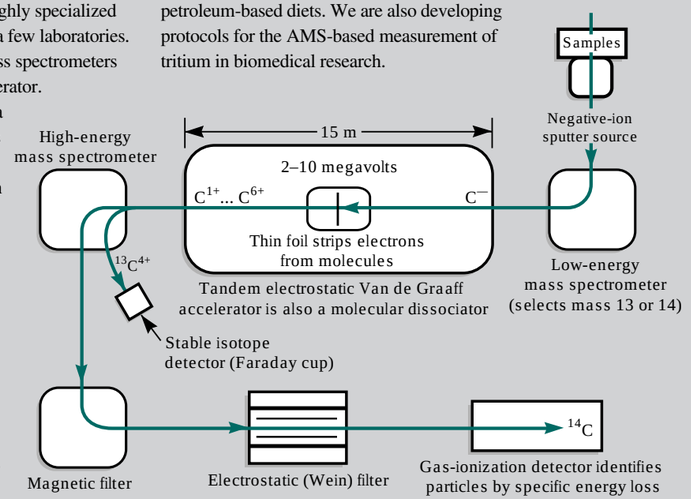
Figure 5. LLNL researcher John Vogel loads samples for analysis by accelerator mass spectrometry (AMS). Originally designed for measuring radioisotopes in nuclear physics experiments, AMS has proven to be precise and versatile in tracking very low doses of radioisotope-tagged food mutagens in laboratory animals.

Accelerator Mass Spectrometry as a Biological Tool

Accelerator mass spectrometry, or AMS, is a nuclear physics technique that has been developed over the past 15 years primarily for detecting long-lived isotopes for the earth and space sciences. Originally, it was a tool for carbon dating geological events, but its capabilities and applications are now far-ranging. In biomedical research, it is a new technology that is still developing. AMS was first applied to LLNL research on DNA adducts in 1990. The main advantage is its precision and sensitivity for quantifying radionuclides, especally ^{14}C. Because it requires highly specialized equipment and expertise, its use today is limited to a few laboratories.

As shown in the illustration, AMS uses two mass spectrometers separated by an electrostatic (Van de Graaff) accelerator. Negative ions from samples are initially sorted by a low-energy mass spectrometer (top right). The ions are then accelerated to much higher energy in the Van de Graaff accelerator, where they pass through a thin foil that dissociates (essentially destroys) any remaining molecules. Rare ions (^{14}C)—those tagging the molecules of interest—are separated from the more abundant, naturally occurring isotope (^{13}C) in a high-energy mass spectrometer (top left) and are subjected to other selection techniques. Finally, the rare ions are counted in a gas-ionization detector and reported relative to the abundant ions measured in a Faraday cup.

The important points are that this instrument allows for specific counting of radionuclei particle-by-particle, that all interference from molecules is destroyed by the foil, and that the acceleration of ions to high energy allows the rare ions to be transmitted to the detector with very high efficiency. For most of our current biomedical applications, we tag molecules of interest with the isotope ^{14}C because of its low natural abundance in biological material, thus giving a reduced background. We are developing animals depleted to 1% in ^{14}C, which will increase our sensitivity a hundredfold or more. We can achieve low levels of ^{14}C in mouse tissue by feeding them petroleum-based diets. We are also developing protocols for the AMS-based measurement of tritium in biomedical research.



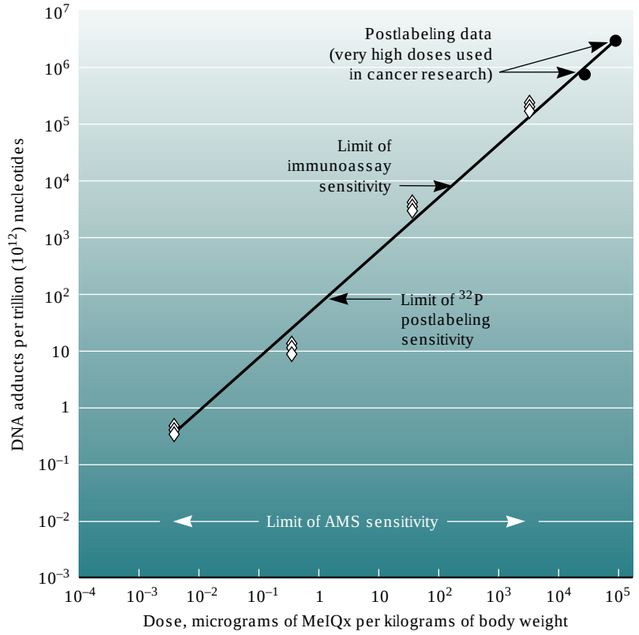


Figure 6. Using accelerator mass spectrometry (AMS), we analyzed the levels of DNA adducts in the livers of rodents that had been exposed to varying doses of the food mutagen MeIQx for seven days. Doses ranged from the amount in about one bite of a hamburger to the very large amounts used in cancer studies. On the *y* axis, one adduct per trillion nucleotides is roughly one adduct per 1000 cells. The level of sensitivity was not possible before the advent of AMS. The level of adduct formation is a linear function of dose. Data like these tell us that DNA adducts can form at the doses equivalent to human dietary exposure.

used AMS to analyze adduct levels in DNA from the liver and other tissues.

Our results in Figure 6 are expressed in a standard type of graph used in biomedical research, which plots dose on one axis and response on the other. This dose–response graph shows a linear relation over many orders of magnitude between the administered dose of MeIQx and the response, namely, adduct levels in the liver. Seven days after the beginning of the study, the levels of DNA adducts in rodents fed a low dose were proportional to those for rodents fed one million times more MeIQx for the same length of time. These data tell us that adducts can form at human exposure levels and that DNA adducts can indicate the amount of exposure for this carcinogen. In addition, the data tell us that the bioactivation processes and the DNA repair mechanisms function *at the same relative rates at high and low doses*. Next, we need to study what happens after continuous exposure to this heterocyclic amine.

In other studies, we assessed DNA adducts in a variety of tissues after giving rodents varying doses of the food mutagens PhIP, MeIQx, or IQ. For this research, we analyzed the DNA adducts using the technique of ^{32}P-postlabeling. By varying both dose and type of mutagen, we can see whether different amounts of mutagens are handled differently in different types of tissues. We found that the response to varying doses depends on the tissue type and the mutagen type. As shown in Figure 7, the pancreas of mice had the greatest level of adducts with PhIP. In contrast, we found high levels of adducts in the liver with MeIQx and IQ. Other studies have shown that PhIP does not cause liver tumors, whereas MeIQx and IQ do. Such results clearly show a correlation between DNA adducts and tumors in specific tissues. However, even though PhIP generates high levels of adducts in the pancreas, it does not appear to cause pancreatic tumors in rodents.

In general, data like these suggest that levels of DNA adducts correlate with exposure, but not necessarily with the development of tumors in specific tissues. The fact that metabolism of a particular food mutagen may be high in the liver, for example, but adduct levels can be low and liver tumors infrequent, suggests that food mutagen metabolites might circulate throughout an organism. We believe that PhIP is an example of this type of mutagen. It is also likely that other factors, such as DNA repair in the tissues, influence the persistence of adducts and the development of tumors.

Picture of an Adduct

Our studies with bacterial and animal models indicate that only three or four principal kinds of DNA adducts are formed by most food mutagen intermediates. In bacterial studies, we developed a DNA cloning method to analyze the mutated DNA sequences in special strains of bacteria. Bacterial strains exposed to food mutagens showed that these compounds have a high affinity for DNA segments containing the nucleotide bases cytosine and guanine in an alternating sequence—a DNA "hotspot."

We used ^{32}P-postlabeling to look for individual "fingerprints" that show up when a food mutagen intermediate binds with DNA. Most of the adducts of IQ, MeIQx, and PhIP prefer not only the guanine in DNA, but also one particular carbon atom position (C-8) on that base (Figure 8). Our animal studies with the other three nucleotide bases—adenine, thymine, and cytosine—show no adducts.

We currently are using a highly detailed, quantum-chemistry approach to study the mechanism by which PhIP interacts with DNA.[3] This research is a collaborative effort among scientists in LLNL's Biology and Biotechnology Research and Chemistry and Materials Science programs and computational engineers at Sandia National Laboratories, Livermore. As part of the analysis, we take into consideration the fact that, unlike most of the other heterocyclic amines, the PhIP molecule is not a flat structure. Rather, PhIP has a phenyl ring structure that is 40 to 45 degrees out of plane with the rest of the molecule. To study the possible modes of binding of a bulky molecule with complex twists and angles (see Figure 3), the rates of reaction change, and the specificity of binding to various

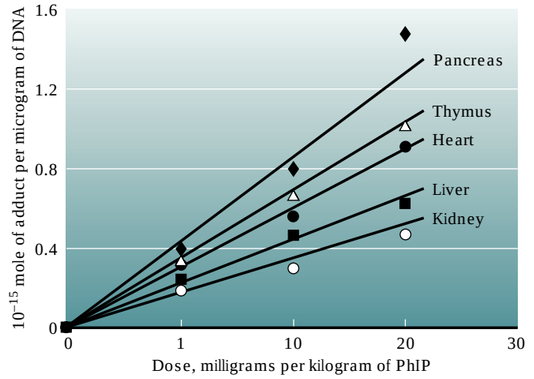


Figure 7. The response to varying doses of the mutagen PhIP depends on the tissue type and species. For mice, the pancreas had the greatest level of adducts with PhIP, followed by the thymus, heart, liver, and kidney. Rats show a different profile.

locations in DNA, we are using ultraviolet, fluorescence, and nuclear magnetic resonance spectroscopies together with quantum mechanical calculations.

We know that PhIP bonds covalently with guanine, and we now believe that such bonding follows the physical (noncovalent) association of that carcinogen with a groove of DNA (specifically, the minor groove). Such physical association, or groove binding, may be the initial event in the adduction of DNA by PhIP.

DNA Undergoes Repair

New data suggest that humans can differ substantially in their susceptibility to chemically induced cancer. People can inherit genetically based traits that may make them more or less prone to developing tumors. The inherited differences may be governed by how much bioactivation or deactivation takes place when foreign substances are taken into the body, the likelihood that DNA will undergo repair when molecules are adducted to the genetic material, and the rates at which tissue cells replicate.

The fact that DNA can encode its own repair was first demonstrated in bacteria. For more than a decade, LLNL has had a program investigating how DNA repair works and what genes and repair proteins are involved. The box on p. 17 gives background information on this important field of investigation. An article in the April 1993 issue of *Energy and Technology Review* contains more detail on this subject.

Cells from the ovary of the Chinese hamster offer a unique research tool for studying DNA repair processes. One major attribute is that they are fast-growing in culture. For several years, LLNL biologists have been using special lines of these ovary cells that either do, or do not, show a biochemical deficiency in DNA repair because of mutations. The cells that are "repair-deficient mutants" (repair incompetent) are highly useful in evaluating what happens when food mutagens are administered and the DNA repair process is essentially turned off.

In essence, we have found that if the DNA repair gene *ERCC2* is present and functioning in a repair-competent cell, then DNA repair takes place after administering a food mutagen, and we observe less DNA damage. On the other hand, if that gene is absent or not functioning in a repair-incompetent cell,

(a) Normal double strand of DNA

(b) The food mutagen PhIP adducted to guanine

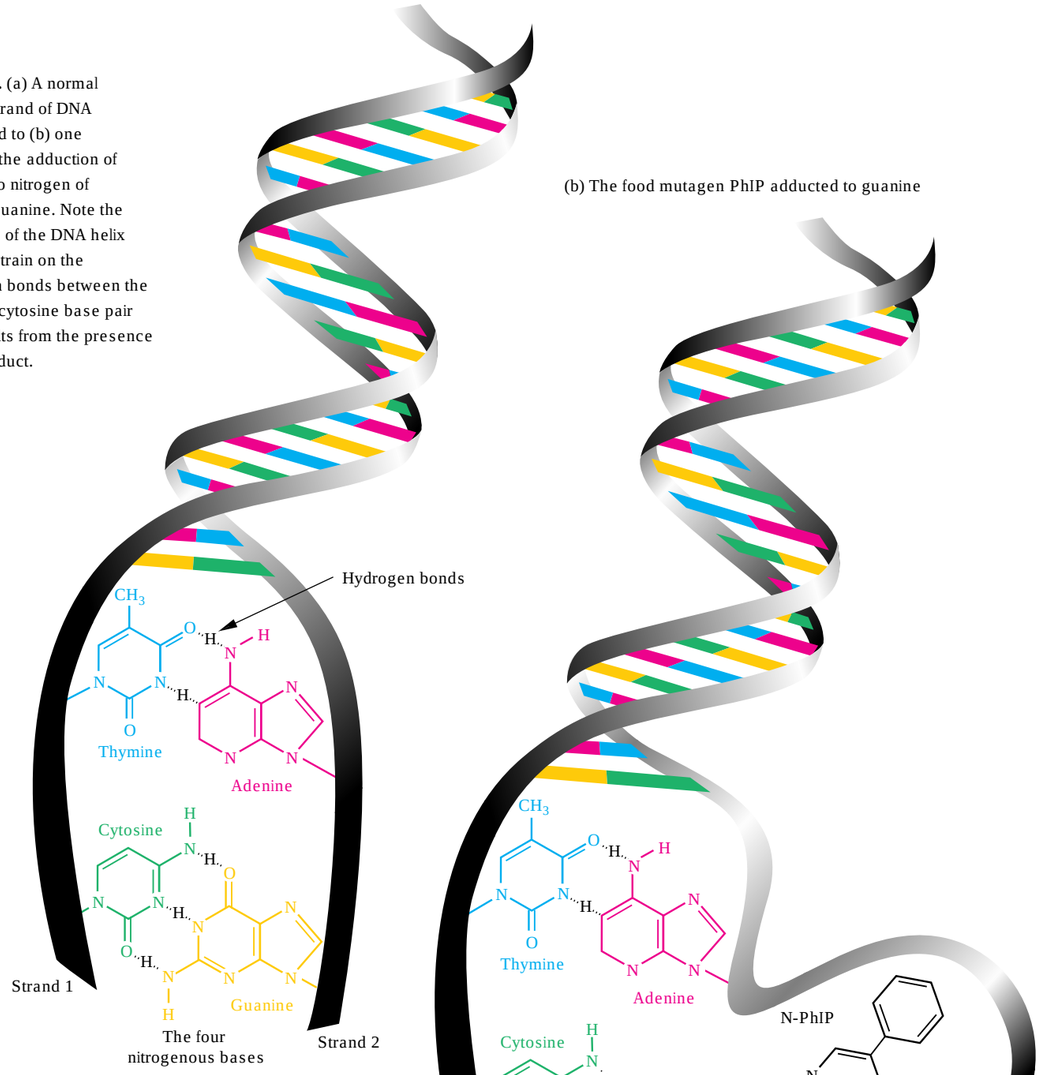


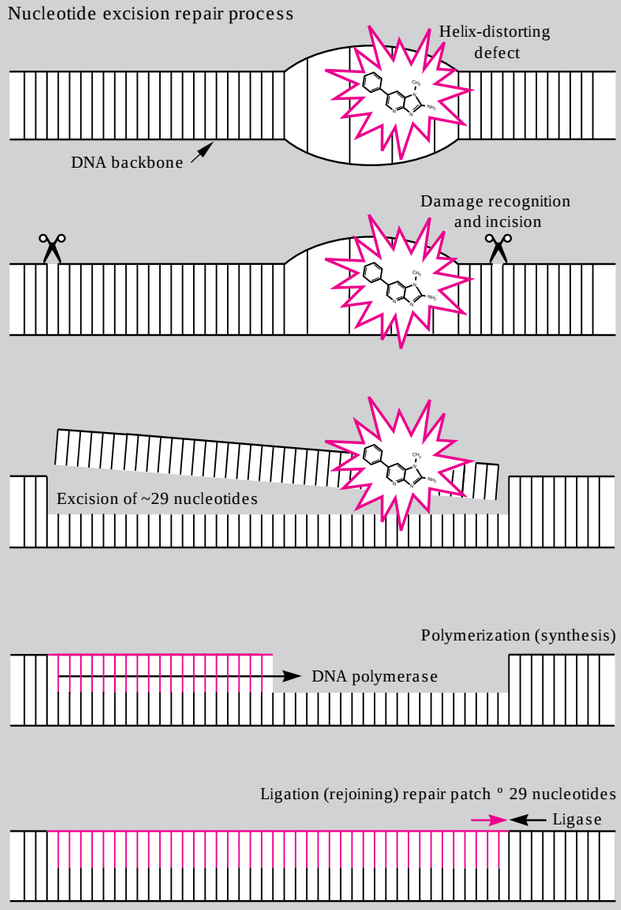
Figure 8. (a) A normal double strand of DNA compared to (b) one showing the adduction of the amino nitrogen of PhIP to guanine. Note the distortion of the DNA helix and the strain on the hydrogen bonds between the guanine-cytosine base pair that results from the presence of the adduct.

then we observe more DNA damage. Moreover, we are learning that the repair that is done seems to favor certain types of DNA sequences—in other words, *ERCC2* removes specific types of damage.

We have also manipulated other genes in Chinese hamster ovary cells. For example, in very recent work, we have taken the genes responsible for manufacturing enzymes that bioactivate (acetylate) food mutagens and placed them in cells to mimic human metabolism. The result, as expected, is a dramatic increase in the amount of mutagens caused by heterocyclic amines.

The lesson from this type of research is that the ability to repair DNA damage has a major impact on how harmful food mutagens will be after they are eaten. If humans vary in their ability to repair DNA, as we suspect, then they will also vary in susceptibility to heterocyclic amines and in the amount of genetic damage that occurs. To date, no one has quantified this type of human variability. Therefore, the issue of differences in human susceptibility to food mutagens represents a major new direction for our research.

DNA Repair



DNA makes up chromosomes and genes, and it is the genetic blueprint for life. The idea that DNA includes genes that could encode its own repair was first demonstrated in bacteria about three decades ago. We now know that virtually all living organisms, from bacteria and yeast to humans, have acquired some remarkable biochemical machinery for preserving the genetic material.

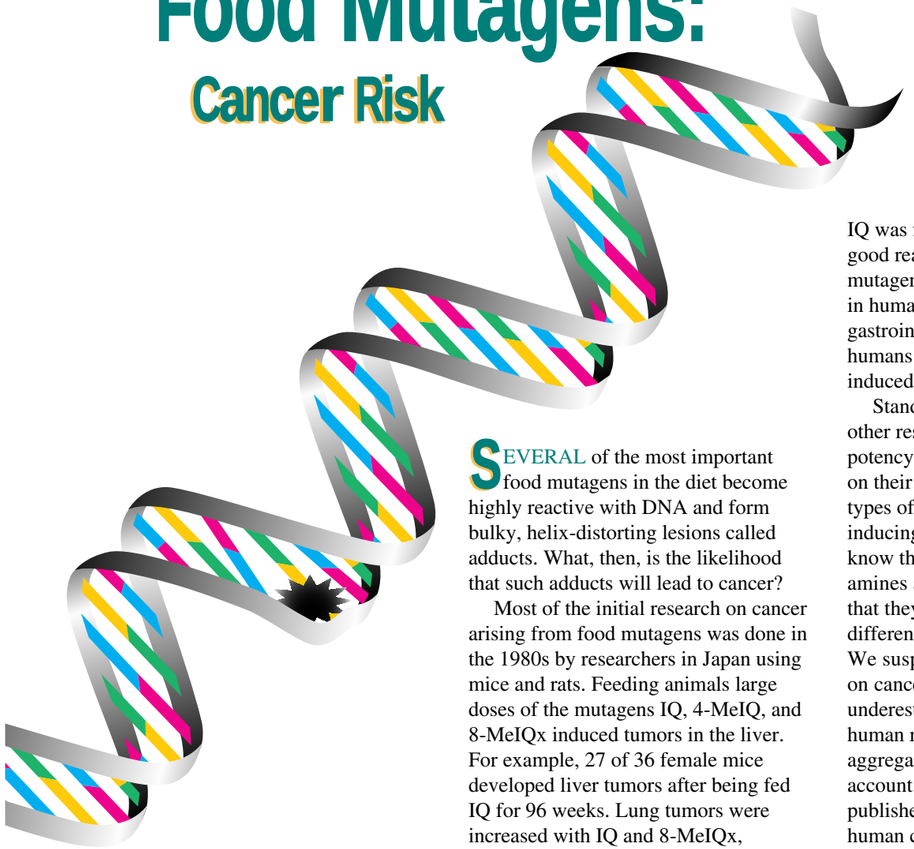
After being subjected to certain types of "insult," the usual double-helix structure of DNA can be changed. In the case of many environmental pollutants and food mutagens, bulky molecules (adducts) become attached to DNA and distort the phosphate backbone of this macromolecule. However, DNA has the ability to essentially fix itself by the action of DNA repair proteins. LLNL researchers have been studying the DNA repair process for more than a decade.

Several types of DNA damage-containment systems are known today. We have focused on the details of the most important repair system in human cells, one that corrects DNA damage produced by nearly all bulky chemicals, such as food mutagens, and the photoproducts of ultraviolet radiation. This precise, multistep process is called nucleotide excision repair (see the illustration). In essence, a certain fixed length of the DNA backbone on both sides of the damaged site is cut, the segment containing the damage is removed, and new material is synthesized in a predetermined direction to produce a repair patch. Finally, the repair patch is tied or rejoined to the parental DNA strand (a process called ligation). Our recent evidence suggests that 30 to 50 different proteins play a role in the damage-recognition, cutting, and mending process.

If DNA repair does not take place or if it is done incorrectly, mutations can occur. The consequences for the affected individual can be profound, including the triggering of tumors and cancer.

Food Mutagens:

Cancer Risk



SEVERAL of the most important food mutagens in the diet become highly reactive with DNA and form bulky, helix-distorting lesions called adducts. What, then, is the likelihood that such adducts will lead to cancer?

Most of the initial research on cancer arising from food mutagens was done in the 1980s by researchers in Japan using mice and rats. Feeding animals large doses of the mutagens IQ, 4-MeIQ, and 8-MeIQx induced tumors in the liver. For example, 27 of 36 female mice developed liver tumors after being fed IQ for 96 weeks. Lung tumors were increased with IQ and 8-MeIQx, stomach tumors with IQ and 4-MeIQ, and intestinal tumors with 8-MeIQx. Other studies have found an increased incidence of skin, colon, small intestine, clitoral, mammary gland, and ear duct tumors in animals fed IQ.

Recent studies show that most of the heterocyclic amines induce tumors at multiple sites at least in some species or sexes of rodents tested experimentally. For example, PhIP, which is highly mutagenic in mammalian cells, induces both breast and colon cancers in rats and lymphomas in mice. Tumors of the liver have also been demonstrated after IQ was fed to monkeys. Thus, we have good reason to think that the food mutagens might be potent carcinogens in humans. Researchers believe that the gastrointestinal tract and breast of humans may be targets for tumors induced by PhIP.

Standard methods previously used by other researchers to estimate the cancer potency of food mutagens were based on their potency in inducing specific types of tumors, not on the total tumor-inducing potency. But, in fact, we now know that most of the heterocyclic amines are multipotent. This means that they can induce tumors in many different and distinct types of tissues. We suspected that the earlier studies on cancer potency might have underestimated the actual potential human risk of cancer because the aggregate potency was not taken into account. Thus, within the last year, we published new estimates of the potential human cancer potency for ten heterocyclic amines.[1]

Our new estimates are derived from experimental data on 36 different species, strains, or sexes of animals—mostly mice and rats—that were fed heterocyclic amines and subsequently shown to develop one or more malignant or potentially malignant tumors. We considered 82 different types of cancer potency associated with specific tumors plus 24 additional estimates of aggregate potency.

We found that the carcinogenic potencies of the ten heterocyclic amines we investigated can be ranked approximately in the following order from most to least potent: MeIQ, Trp-P-1, IQ, MeIQx, Glu-P-1, Glu-P-2, Trp-P-2, MeAαC, PhIP, and AαC. The potency values we obtained were slightly higher than those suggested in previous studies. We estimate that DiMeIQx could be among the most carcinogenic of all the heterocyclic amines identified to date on the basis of its mutagenic activity, although no tumor data are available yet. These new estimates of potency serve as an important piece of information in determining the ultimate cancer risk to humans from eating food mutagens.

Risk and Dose

Before we can say anything about a connection between food mutagens and cancer risk in humans, we must first establish what doses or intake levels are realistic. The difficulty of this task arises from the many sources of possible error. People eat a variety of foods, and they prepare foods in different ways. Cooking descriptions are often not well defined, and cooking times and temperatures have large effects on the formation of promutagens.

Although the relative amounts of the heterocyclic amines found in foods are generally consistent among different studies and laboratories, the precise amount of one particular mutagen per gram of a given cooked food can span a tenfold range. A recent study of commercially cooked meat showed that products cooked differently had a 400-fold range of mutagenic activity. There are several explanations for the lack of concordance, the most important having to do with the time and temperature of cooking.

Thus, estimating the risk from exposure to carcinogens depends on making several generalizations and assumptions. Some of the important variables for estimating the cancer risk associated with food mutagens include:

- What type of risk to estimate; for example, the maximum (upper-bound) credible risk or some more conservative estimate.
- Which population to use; for example, worldwide, the U.S., or a high-exposure subgroup in the U.S.
- Which of the more than dozen known food mutagens are most prevalent in the diet of the chosen population.
- The level of chronic dietary exposure.
- An estimated human lifetime dose for the chosen mutagens.
- Data on the cancer potency of heterocyclic amines prevalent in the diet.

Assumptions must also be made, each adding uncertainty to the estimates. The most unavoidable are that:

- The relation between dose and response (for example, tumors in animal studies) is essentially linear.
- In extrapolating from one species to another, a given dose has equal toxicity. As with other assumptions, this one may be reasonable, but it also entails some uncertainty because repair processes and metabolism do differ from rodents to man.

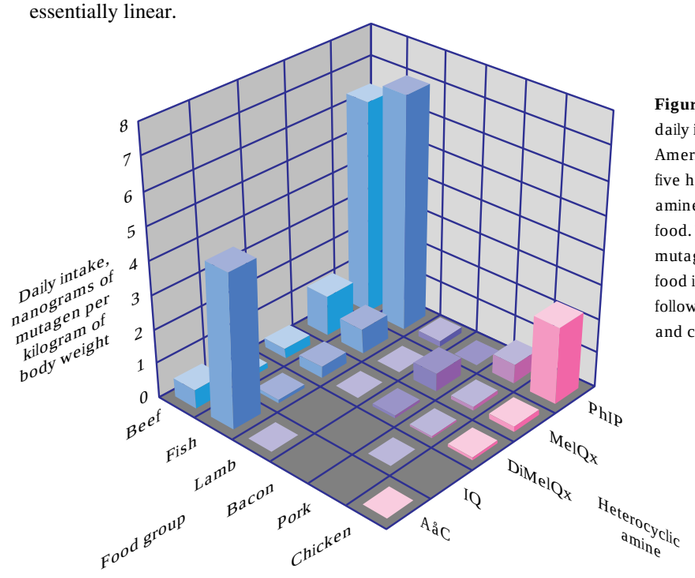
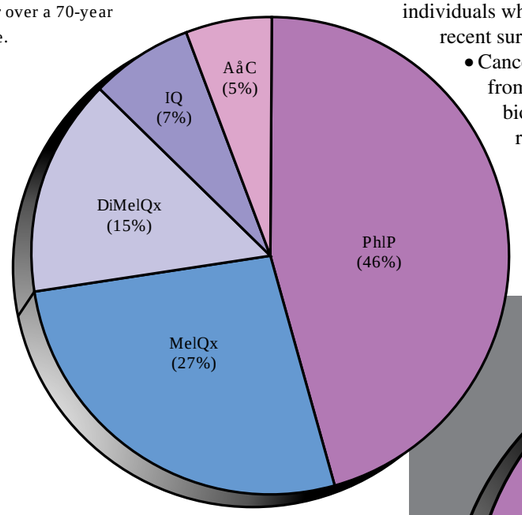


Figure 9. Average daily intake in the American diet of five heterocyclic amines by type of food. The primary mutagen-bearing food is fish, followed by beef and chicken.

How Big Is the Risk?

We recently made an improved estimate of the cancer risk to humans posed by the presence of heterocyclic amines in the U.S. diet.[4] This work was done in collaboration with Ken Bogen and Dave Layton in LLNL's Health and Environmental Assessment Division. More refined estimates are now possible because research over the past few years has given us better values for the levels of mutagens in foods, the genetic toxicity of mutagens, and their carcinogenic potency.

Figure 10. Total calculated cancer risk to humans from the intake of the five principal heterocyclic amines in the U.S. diet is represented as a circle. The entire circle represents nearly 28,000 cases of cancer for the U.S. population, or a one in ten thousand chance of developing cancer over a 70-year lifetime.



We based our new estimates on:

- Actual measurements of the heterocyclic amines in a variety of foods common in the U.S. diet.
- The average intake of these common foods in the U.S. diet. This estimate was based on a random dietary survey conducted under the auspices of the U.S. Department of Agriculture. We computed the average daily intake of meat and fish consumed by 3563 individuals who completed the most recent survey.
- Cancer potencies derived from the results of animal bioassays, or established relations between the mutagenic activity of heterocyclic amines and their carcinogenic potency (for example, in the case of 4,8-DiMeIQx).

For many years, we have been adding information on food mutagens to a database that now contains some 261 records classified by food item, cooking method, and type of mutagen. Of the 13 different food mutagens in our database, we found that only five are commonly consumed at significant levels in the U.S. diet. Therefore, in estimating average dietary intake for the U.S. population, we considered only five heterocyclic amines. They are, in descending order of exposure: PhIP, AαC, MeIQx, DiMeIQx, and IQ.

Figure 9 is a convenient way to show the average daily intake of these five heterocyclic amines by type of food. The graph shows that the primary mutagen-bearing food, based on the literature values, is fish, followed by beef and chicken. However, we must remember that cooking methods and cancer potencies of the heterocyclic amines are extremely important variables in estimating risk.

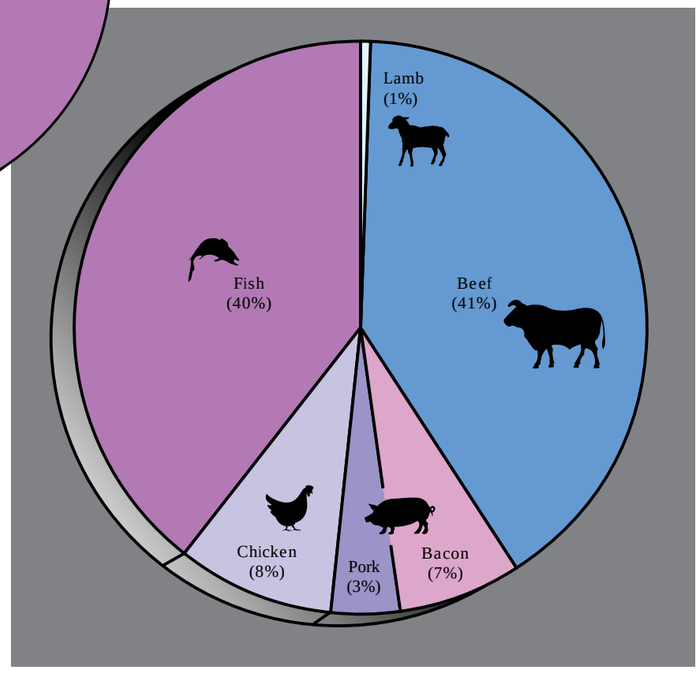


Figure 11. Principal food types contributing to the total calculated cancer risk from ingesting the five heterocyclic amines shown in Figure 10. The highest cancer risk results from eating beef products and fish.

In contrast to daily *intake*, we find that the carcinogenic *potencies* of the five heterocyclic amines have almost the reverse order of their prevalence in the diet: IQ, DiMeIQx, MeIQx, PhIP, AαC. Thus, whereas average Americans consume less IQ by weight than, say, PhIP, IQ is much more potent. Cancer potency depends on several factors, including the effective biological dose within target tissues (such as the liver, colon, or pancreas) and the likelihood of tumors actually being induced in those tissues.

We estimated the risk of cancer by multiplying the intake of the five major heterocyclic amines by their cancer potencies. Figure 10 shows the incremental risk of cancer from each of the five principal heterocyclic amines. PhIP accounts for nearly half (46%) of the total risk, followed by MeIQx (27%) and DiMeIQx (15%). The mutagens IQ (7%) and A*a*C (5%) contribute the least risk of the five substances.

Another way to view the results is by looking at the risks arising from each of the food groups we studied. Figure 11 shows that the highest cancer risks, by far, result from eating beef (steaks and ground beef) and fish.

We estimate that the overall cancer risk to the U.S population is one in ten thousand. Put another way, our latest prediction is that about 28,000 people living in the U.S. today will develop cancer during their lifetime (over 70 years) from dietary exposure to the five heterocyclic amines included in our calculations. For comparison, the American Cancer Society estimated that 149,000 cases of colorectal cancers occurred in the U.S. in the single year 1994. From a public health standpoint then, the magnitude of the cancer risk we currently predict from eating heterocyclic amines is not alarming, but it is certainly not negligible.

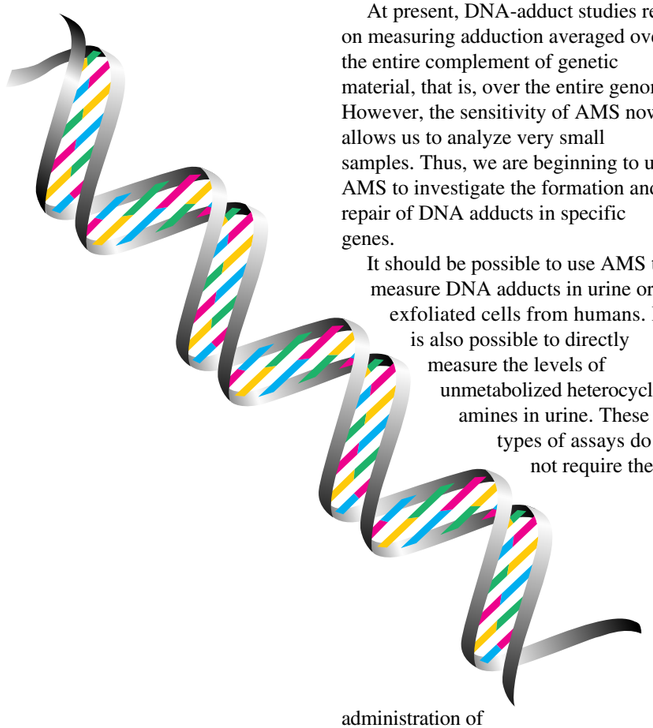
Table 1. Estimates of some lifetime cancer risks and cancer-related regulatory guidelines in the U.S.

Risk to U.S. females of developing breast cancer	**1 in 9**
Risk in the U.S. of developing colorectal cancer	**1 in 15**
Estimated average cancer risk in the U.S. from eating heterocyclic amines in cooked foods	**1 in 10,000**
Risk of cancer after consuming average amounts of fish containing the pesticide dieldrin*	**1 in 10,000**
Risk of cancer after consuming average amounts of fish containing the pesticide DDT*	**1 in 100,000**
Risk level deemed significant by the EPA in cancer etiology	**1 in 1,000,000**

*These pesticides have been banned for about 20 years. However, declining amounts of residues are still sometimes found in foods like fish at levels that exceed the EPA's negligible risk standards.

Our estimates are highly conservative, representing average exposures and risks for the U.S. population based on available data in the literature. Depending on individual dietary habits, some people could have 10 to 50 times higher doses of food mutagens in their diet. Such people would be at much higher risk of developing cancer (up to one chance in 200). Other considerations that could lead to even higher risk include the possibility that some individuals have an increased susceptibility to cancer and that humans might be even more sensitive to food mutagens than rodents.

Table 1 provides an additional perspective on our new estimates of cancer risk associated with food mutagens. In cancer etiology, a risk greater than one in a million is deemed significant by the Environmental Protection Agency (EPA). A cancer risk of one in ten thousand would be high enough to trigger regulatory action if the substance in question were an environmental contaminant, such as a pesticide.



Future Research

To date, few studies have even attempted to measure all of the known heterocyclic amines present in cooked foods. No systematic studies on the mutagen content of cooked foods have been reported. Additional research in these areas would give us improved assessments of exposure and risk.

At present, DNA-adduct studies rely on measuring adduction averaged over the entire complement of genetic material, that is, over the entire genome. However, the sensitivity of AMS now allows us to analyze very small samples. Thus, we are beginning to use AMS to investigate the formation and repair of DNA adducts in specific genes.

It should be possible to use AMS to measure DNA adducts in urine or exfoliated cells from humans. It is also possible to directly measure the levels of unmetabolized heterocyclic amines in urine. These types of assays do not require the administration of radioisotopes to humans and could be used to estimate the variability of human susceptibility to carcinogens.

Our work on risk assessment carries with it some important implications that warrant follow-up. In particular, it may be possible to identify strategies to manage potential cancer risks associated with food mutagens. One key objective would be to develop guidance on cooking methods that could reduce the concentrations of PhIP and MeIQx, which contribute the most to the predicted cancer risks.

Summary

Based on the research reported in the first installment of this two-part series on food mutagens,[4] diets rich in well-done meat cooked (especially fried) at temperatures over 200°C will have significant levels of the heterocyclic amines. Meats cooked to rare or medium-rare (below 150°C, or hotter for short periods) have markedly less mutagen content than well-done meats. Pretreatment of ground beef by microwave cooking, then discarding the clear fluid before frying, lowers the mutagen content of even well-done meat.

A comparison of fried meats shows that beef and chicken are the most mutagenic. When account is taken of the relative amounts of different meats consumed by Americans, and of the potency of mutagens in them, ground beef is probably the most important source of food mutagens in the U.S. diet.

As reported in this installment, we are beginning to understand the process by which food mutagens become adducted to DNA, an important step that can lead to cancer. The binding of mutagens depends on the formation of intermediate, biologically reactive molecules. The intermediate forms appear to link preferentially to the DNA base guanine in many cases. The extent of DNA adduction, and the subsequent occurrence of tumors, varies considerably in different types of tissues and in different animal species.

The overall, average, upper-bound lifetime cancer risk in the U.S. from eating heterocyclic amines in cooked foods is estimated to be about one in ten thousand. The consumption of muscle meats contributes most to the total risk. Specific subgroups of the population eating large amounts of muscle food cooked well-done may be at much higher risk.

Key Words: accelerator mass spectrometry (AMS); adduct dosimetry; amino-imidazoazaarene (AIA); bioactivation; cancer risk assessment; carcinogen; carcinogenicity; DNA adducts; food mutagen; mutagens—2-amino-9H pyrido[2,3-b]indole (AαC); 2-amino-3,4,8-trimethylimidazo[4,5- f]quinoxaline (DiMeIQx); 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), 2-amino-1-methyl-6-phenyl-imidazo[4,5-b]pyridine (PhIP); ^{32}P-postlabeling.

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The following scientists contributed to this article: Kenneth Bogen (risk assessment), Mark Knize (chemical analysis), David Layton (risk assessment), James Tucker (cytogenetic analysis), Kenneth Turteltaub (DNA adducts), Lawrence Thompson (DNA repair), John Vogel (accelerator mass spectrometry), and Rebekah Wu (mutation analysis).

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About the Scientist

JAMES FELTON joined the Biomedical Sciences Division of Lawrence Livermore National Laboratory as a Senior Biomedical Scientist in 1976. He is currently the Group Leader of the Molecular Toxicology Group of the Biology and Biotechnology Program at the Laboratory. He received his A.B. in Zoology from the University of California, Berkeley, in 1967 and his Ph.D. in Molecular Biology from the State University of New York at Buffalo in 1973. From 1973 until 1976, he was a Fellow at the National Institutes of Health in Maryland.

In more than 147 professional publications, James Felton has explored the role of diet in carcinogenesis and mutagenesis. He has been a part of the Laboratory's research on food mutagens since it began 17 years ago and has led it for the past 8 years.