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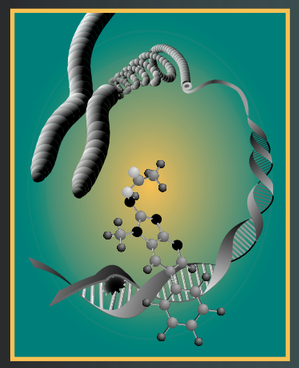

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Science & Technology REVIEW

September 1995

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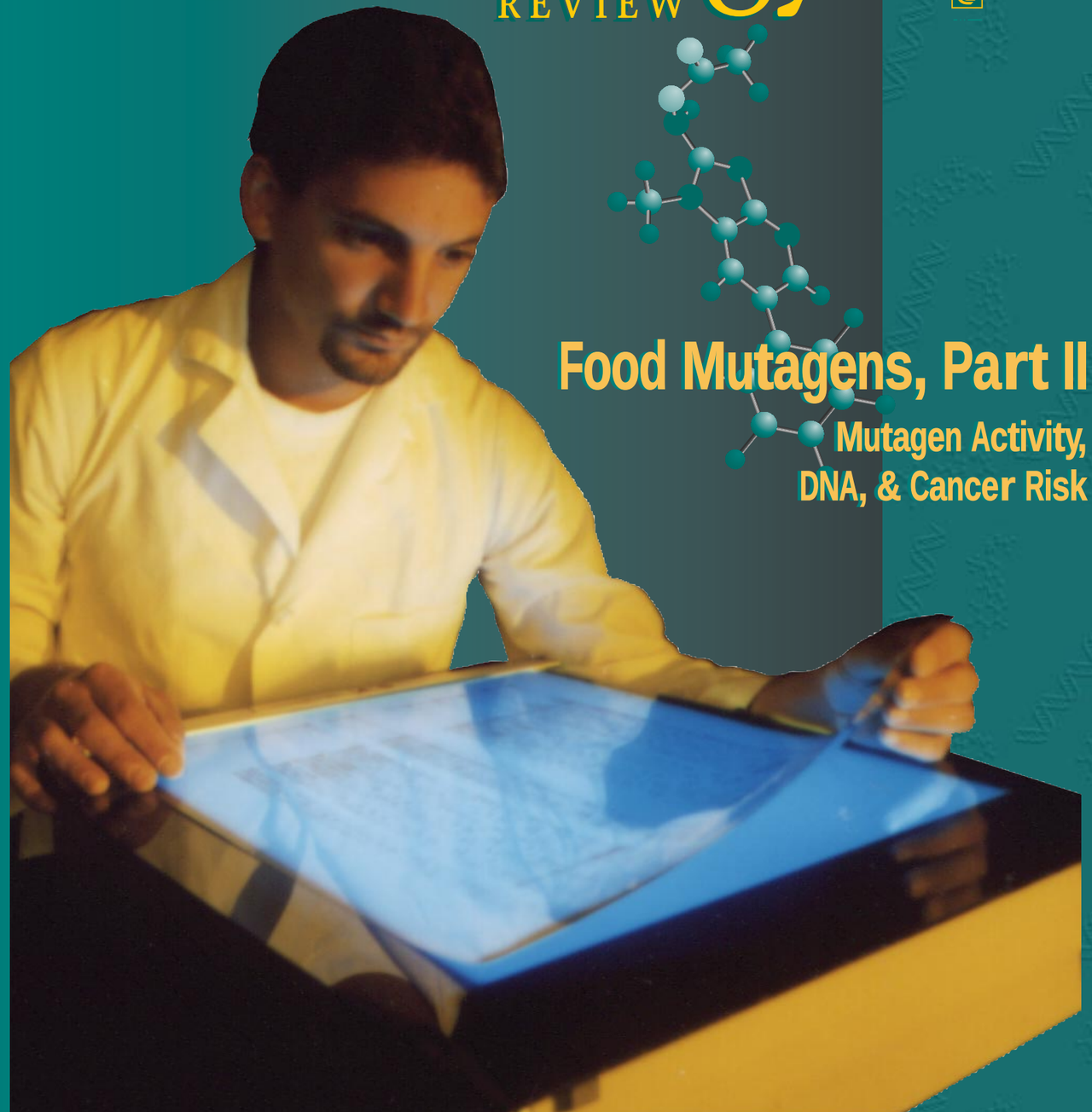
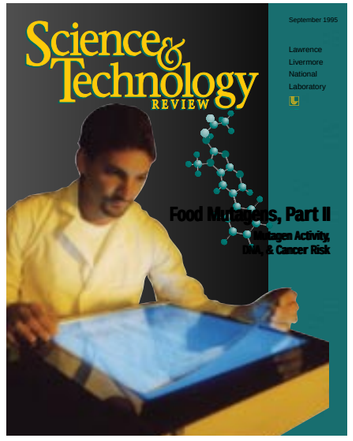


Food Mutagens, Part II

Mutagen Activity, DNA, & Cancer Risk

About the Cover

The DNA strand pictured on this month's cover is one of the primary concerns of the second and final installment of our report on 17 years of food mutagen research at the Laboratory. Beginning on p. 6, we discuss what happens when food mutagens, particularly those in fried meat, are metabolized and how these toxic compounds affect DNA to produce mutations that can lead to cancer.



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About *S&TR*

The Lawrence Livermore National Laboratory, operated by the University of California for the United States Department of Energy, was established in 1952 to do research on nuclear weapons and magnetic fusion energy. *Science and Technology Review* (formerly *Energy and Technology Review*) is published monthly to communicate, to a broad audience, the Laboratory's scientific and technological accomplishments, particularly in the Laboratory's core mission areas—global security, energy and the environment, and bioscience and biotechnology. The publication's goal is to help readers understand the accomplishments and appreciate their value to the individual citizen, the nation, and the world.

Please address any correspondence concerning *S&TR* (including name and address changes) to Mail Stop L-664, Lawrence Livermore National Laboratory, P.O. Box 808, Livermore, California 94551, or telephone (510) 422-8961. *S&TR* is also available on the Internet at *http://www.llnl.gov/str/str.html*, and our electronic mail address is *hunter6@llnl.gov*.

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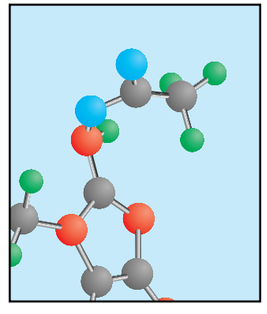

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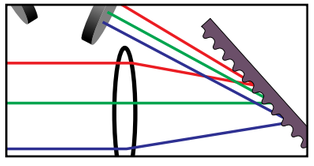

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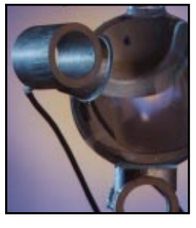

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Lab receives five R&D 100 awards

R&D 100 awards will be presented to Laboratory researchers this month in a ceremony at the Museum of Science and Industry in Chicago. Handed out annually by Chicago-based *R&D Magazine*, the awards go to what are judged to be the year's best 100 new technical products offered for purchase or licensing. Since the Lab began participating in R&D 100 competition, it has garnered 55 awards—five of them this year.

The Laboratory received a shared 1995 award for two of its aerogel processes: **Injection Molding Process for Rapid Production of Net-Shaped Aerogels** and **Capacitive Deionization with Carbon Aerogel Electrodes.**

The other Lab winners were: **Sealed-Tube Electron Beam Gun for Material Processing; Miniature Ion Cyclotron Resonance Mass Spectrometer; All-Solid-State Laser with Diode Irradiance Conditioning;** and **High Average Power Solid-State Laser with High Pulse Energy and Low Beam Divergence**.

Summaries of the Laboratory's 1995 R&D 100 winners appear below. The technologies will be discussed in more detail in the November 1995 issue of *Science and Technology Review.*

Injection Molding Process for Rapid Production of Net-Shaped Aerogels: Similar to the molding process used to manufacture certain types of plastics, injection molding permits the production of aerogels 30 times faster than any other existing process. Such mass-produced aerogels can have precise sizes and shapes for a variety of applications. The production time has been shortened through invention of a way to eliminate stress in the drying gel. Through the unique drying process, liquids can be purged without causing cracking to a confined gel's delicate structure.

The process reduces liquid waste by 40% and consumes about ten times less energy. Laboratory researchers estimate that aerogel production cost per unit can be eight times less than it would be using conventional processes.
Contact: Lawrence Hrubesh (510) 423-1691 (hrubesh1@llnl.gov).

Capacitive Deionization with Carbon Aerogel Electrodes: This patented process enables the efficient and economical removal of salt and impurities from water. It generates no secondary waste because, unlike ion exchange, it requires no acids, bases, or salt solutions for regeneration of the system. Capacitive deionization (CDI) also is more energy efficient than competing technologies. Applications include waste treatment, water purification and softening, and desalination.

The electrochemical process at the heart of CDI is rather straightforward. What makes the approach promising is the use of carbon aerogel, developed at the Lab, as the agent for absorbing ions. In the CDI process, water containing salt, heavy metals, or even radioactive isotopes can be pumped through a series of electrochemical cells. An electric potential is then applied across the carbon aerogel electrodes, which attract the negatively and positively charged ions. The trapped ions can be released into a relatively small stream of rinse water.
Contact: Joseph C. Farmer (510) 423-6574 (farmer1@llnl.gov).

Sealed-Tube Electron Beam Gun for Material Processing: The Laboratory and American International Technologies, Inc., have teamed up to develop a low cost, sealed-tube electron beam gun capable of shooting electron beams several inches into the air for industrial materials processing applications as well as DOE waste treatment and weapon dismantlement uses. The sealed-tube gun is expected to lower the cost of electron beam processing of materials to less than a tenth of present-day costs.

The tube is a low cost replacement for large electron beam processing systems that require a dedicated vacuum pumping system and extensive x-ray shielding. Tubes produced to date have operated at power levels of up to 140 watts. The project's key technical challenge has been development of a reliable thin membrane window capable of transmitting electron current densities of several milliamperes per square centimeter into the air with 90% efficiency at 50 kilovolts.
Contact: Booth Myers (510) 422-7537 (myers5@llnl.gov).

Miniature Ion Cyclotron Resonance Mass Spectrometer: This is a totally portable, battery-operated, hand-held mass spectrometer. Based on the principle of ion cyclotron motion, the system combines the ion source and mass analyzer/detector in a mechanically simple device with an integral vacuum system. The power budget is less than one quarter of a watt when not active and less than 20 watts when fully operational.

The prototype system has a total length of 29 centimeters and a total weight of 5.6 kilograms. It was created by using a simple, permanent magnet designed for optimal field homogeneity, coupled with ion trap electrodes that are integral with the vacuum housing. Developers say that in spite of its simplicity, the system achieves sufficient mass resolution to detect contaminants or trace compounds in air samples. It has the broad mass range capability and ultrahigh sensitivity of laboratory-based ion trap mass spectrometers.
Contact: Daniel D. Dietrich (510) 422-7868 (dietrich1@llnl.gov).

All-Solid-State Laser with Diode Irradiance Conditioning: This diode-pumped, lens-duct-concentrated, solid-state laser system constitutes a new class of lasers. The invention offers a simple, inexpensive, and commercially attractive technique for scaling the pulse energy and/or average output power of a laser system—up to 100 times higher pump intensity and laser output energy.

The three key laser components that enable the design of the all-solid-state system are: (a) a laser diode pump array assembly, (b) a light delivery optic, dubbed the "lens duct," and (c) a laser gain medium. The laser system is useful for a variety of applications, in fields as diverse as medicine and laser welding.
Contact: Ray Beach (510) 423-8986 (beach2@llnl.gov).

High-Average-Power, Solid-State Laser with High Pulse Energy and Low Beam Divergence: This flashlamp-pumped, neodymium-glass, zig-zag slab laser system produces 25- to 30- joule laser pulses at a repetition frequency of 6 hertz, resulting in an average power of more than 150 watts. This system, with greater than ten times the commercially available average power at these pulse energies, can operate with pulse widths ranging from 10 to 0.01 nanoseconds and has near-diffraction-limited divergence. In addition, the laser output has the very narrow spectral bandwidth required for high-resolution imaging.

The laser system is being used in two new, important applications: the efficient generation of x rays for advanced integrated circuit production and long-range visible coherent laser radar. The laser's potential for environmentally sound paint removal in the aircraft and ship industries as well as for the removal of lead-based paint in public and private buildings is being evaluated.
Contact: C. Brent Dane (510) 424-5905 (dane1@llnl.gov).

Laser surgery removes tumor from rare fox

Researchers from the Laboratory and the Beckman Laser Institute and Medical Clinic (BLIMC) at the University of California at Irvine are continuing their work on a diode-laser-assisted, experimental surgery technique used in June in a two-and-a half hour operation to remove a golfball-sized cancerous tumor from the ear of a rare Santa Catalina Island fox.

Under a Cooperative Research and Development Agreement between the Lab and Beckman, a 10-watt diode laser is being developed for use in photodynamic therapy. The Lab has the responsibility for designing and building the prototype diode laser and the fiber-optic delivery system. Beckman's responsibilities under the agreement include evaluating the medical laser's performance in clinical trials.

In photodynamic therapy, lasers are tuned to specific wavelengths and shined at photosensitizers that have been introduced into cancerous areas. The photosensitizers, excited by the laser, interact with oxygen in the tumors, converting the oxygen into an active form that kills the cancerous cells.

The surgery on "Fauna," the 13-year-old fox, was part of a veterinary outreach program at BLIMC that transfers medical technology being developed for humans to the treatment of animals. Fauna recovered completely and has been returned to the island. Researchers hope to see the procedure approved for human use within three to five years.
Contact: Ray Beach (510) 423-8986 (beach2@llnl.gov).

Environmental Directorate gets new leader

Jay C. Davis is the Laboratory's new Associate Director (AD) for Environmental Programs, a directorate of some 400 scientists, engineers, and support staff conducting environmental research, development, and demonstration. The directorate is currently operating under an annual budget of approximately $50 million.

Davis was named acting head of the newly established directorate last year while a search was launched for a permanent associate director. Citing Davis's excellent job in leading the new directorate in an acting capacity, Laboratory Director Bruce Tarter announced Davis's selection as AD in July.

Tarter noted that during the past year, the directorate made significant advances in the development and demonstration of new remediation technologies, in regional precipitation and climate change modeling, and in continuing development of diagnostic and risk assessment technologies.

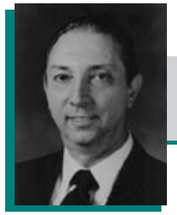
Davis has worked in fields ranging from nuclear physics to magnetic fusion to arms control. He was the founding director of the Lab's Center for Accelerator Mass Spectrometry, establishing the center as a leader in the application of accelerator analytical techniques to problems in biomedicine, geophysics, arms control, and the environment. Previously, Davis played significant operational and scientific leadership roles in the nuclear inspections of Iraq by the United Nations following the Gulf War.

A Fellow of the American Physical Society, Davis has been with the Laboratory since 1971. He holds a B.A. and M.A. in Physics from the University of Texas and a Ph.D. in Physics from the University of Wisconsin.
Contact: Gordon Yano (510) 423-3117 (yano1@llnl.gov).

Patents

Each month in this space we report on the patents issued to and/or the awards received by Laboratory employees. Our goal is to showcase the distinguished scientific and technical achievements of our employees as well as to indicate the scale and scope of the work done at the Laboratory.

Patent issued to	Patent title, number, and date of issue	Summary of disclosure
William McLean, II, Philip E. Miller, and James A. Horton	**Compact Reaction Cell for Homogenizing and Down-Blending Highly Enriched Uranium Metal** U.S. Patent 5,411,722; Issued May 2, 1995	Specialized reaction cell for converting uranium metal to uranium oxide and down-blending highly enriched uranium metal by the simultaneous conversion of highly enriched uranium metal and natural or depleted uranium metal to uranium oxide.
Arthur W. Coombs, III	**Ultra High Vacuum Heating and Rotating Specimen Stage** U.S. Patent 5,412,180; Issued May 2, 1995	Simultaneous heating and rotating specimen stage ideally suited for operation in ultrahigh vacuum (UHV) but also useful at atmosphere and in pressurized systems. The stage is made of materials compatible with UHV and able to withstand high temperatures and electrical loading without overheating.
Randall L. Simpson and Cesar O. Pruneda	**Method for Fabricating Non-Detonable Explosive Simulants** U.S. Patent 5,413,812; Issued May 9, 1995	Method of making a simulator that is chemically equivalent to an explosive but is not detonable or explodable. The materials contain small amounts of the actual energetic material as a minor component overall in a nonreactive matrix or as a thin coating. The simulator has particular use in the training of explosives-detecting dogs and in calibrating sensitive analytical instruments.
Troy W. Barbee, Jr., Gary W. Johnson, and Dennis W. O'Brien	**High Performance Capacitors Using Nano-Structure Multilayer Materials Fabrication** U.S. Patent 5,414,588; Issued May 9, 1995	A high-performance, very high energy-density capacitor fabricated by using nanostructure multilayers of dielectric and conductor materials deposited atom-by-atom to form multiplate capacitors with very large area-to-surface ratios.
Anthony M. McCarthy	**Transistors Using Crystalline Silicon Devices on Glass** U.S. Patent 5,414,276; Issued May 9, 1995	A method for producing single-crystal silicon transistors in a silicon-on-glass substrate wherein device components are formed in a silicon substrate and transferred to glass. This method overcomes the damage that may be caused to the device during high-voltage bonding and employs a metal layer that may be incorporated as part of the transistor.
Raymond D. Scarpetti, Jr., Clarence D. Parkinson, Vernon A. Switzer, Young J. Lee, and William C. Sawyer	**Self Aligning Electron Beam Gun Having Enhanced Thermal and Mechanical Stability** U.S. Patent 5,416,381; Issued May 16, 1995	A compact, high-power electron gun that incorporates a mechanically coupled, self-aligning structure for the anode and cathode using a common support structure and modular design.
Craig R. Wuest, Thomas M. Tillotson, and Coleman V. Johnson, III	**Aerogel-Supported Filament** U.S. Patent 5,416,376; Issued May 16, 1995	The containment of thin wire filaments in a supportive aerogel matrix to reduce tension and stresses on the filaments, particularly for use in radiation detection instruments and incandescent lamps. The design circumvents the problem of wire breakage and device failure.
Daniel W. Shimer and Arnold C. Lange	**High Voltage DC-DC-Converter with Dynamic Voltage Regulation and Decoupling During Load-Generated Arcs** U.S. Patent 5,418,707; Issued May 23, 1995	A high-power solid-state power supply for producing a controllable, constant-high-voltage output under varying and arcing loads suitable for powering an ion source, such as an electron beam gun in a vacuum furnace or one used in an atomic vapor laser isotope separation process.
Clinton M. Logan	**High Performance X-Ray Anti-Scatter Grid** U.S. Patent 5,418,833; Issued May 23, 1995	An x-ray antiscatter grid, particularly for screening mammography, and method of fabrication that reduces or eliminates the problem of image degradation due to the scattering of x rays within the object being imaged. The function of the grid is to pass image photons but to block the scattered photons.
James A. Horton and Howard W. Hayden, Jr.	**Process for Producing Enriched Uranium Having ^{235}U Content of at Least 4 Wt.% Via Combination of a Gaseous Diffusion Process and an Atomic Vapor Laser Isotope Separation Process to Eliminate Uranium Hexafluoride Tails Storage** U.S. Patent 5,419,820; Issued May 30, 1995	A uranium enrichment process that will consume less energy and produce metallic uranium tails having a lower uranium-235 content than normally produced in a gaseous diffusion separation process.
Steven T. Mayer, James L. Kaschmitter, and Richard W. Pekala	**Method of Low Pressure and/or Evaporative Drying of Aerogel** U.S. Patent 5,420,168; Issued May 30, 1995	A process whereby resorcinol/formaldehyde (RF) aerogel having a density of about 0.4–1.2 g/cm^3 can be manufactured using a simple air drying procedure. The method is simpler, quicker, and less expensive than the more conventional supercritical or subcritical carbon dioxide extraction procedures.
Philip E. Harben, Peter W. Rodgers, and Daniel W. Ewert	**Seismic Switch for Strong Motion Measurement** U.S. Patent 5,420,380; Issued May 30, 1995	An earthquake alert system using a distributed network of strong motion measurement stations to warn an area prior to a large earthquake by simultaneously switching out the signal from an existing microseismic station seismometer and switching in the signal from a strong ground motion measuring instrument.



Tony Carrano
Associate Director,
Biology and Biotechnology Research Program

A Revolution in Biological Knowledge

CHANGING international, political, and economic priorities have caused Laboratory planners to rethink many mission objectives. The Laboratory's biological research has also undergone major shifts in response to changing national needs. However, many projects are fundamentally shaped by the emergence of new technologies and by discoveries that those technologies make possible. Much of our planning today is driven by a vision shared by many investigators in the field of biological research, namely that the 21st century will be the century of biology.

We already have evidence for this prediction. Until several decades ago, the intricate architecture of the human genetic blueprint, the DNA molecule, remained unknown even to experts. Indeed, the now-familiar double helix of the DNA molecule was discovered by Watson and Crick only about 40 years ago. Today, ordinary citizens watching television or reading newspapers daily encounter an explosion of information in genetics as they learn about new genetically engineered crops and hear detailed forensic evidence offered in courtrooms. In response to the rapid pace of technological innovation, biological researchers at LLNL are placing increased emphasis on biotechnology—new tools of the trade—and on biological processes that take place at very small scales, often at the molecular or atomic level.

We are experiencing a revolution in molecular biological information. The human genome project, for example, is an ambitious international program to map the entire human genetic blueprint. Understanding the genetic code—and extending our knowledge to other species, from microbes to maize to mice—will have enormous payoffs for improving health, agriculture, the environment, and the economy in the next century. What we learn can potentially affect every aspect of our lives. In the area of human health alone, more than 4000 diseases, including cancer and heart disease, are now linked to a breakdown in the genetic process. With each advance in knowledge comes choices, including medical and ethical questions about how the new information should be applied.

Even though our mission is not aimed at addressing some of the more difficult philosophical or ethical issues, we see ourselves as having an important role in educating the public about the scientific basis of our work and about how our research can benefit individuals. It is appropriate that this month's issue of *Science and Technology Review* features a topic that relates to everyone who has ever eaten a hamburger, a piece of fried chicken, or a slice of bread. The article on food mutagens describes how certain compounds formed in the cooking—especially overcooking—of foods that are typical of the American diet can potentially lead to cancer. As this article shows, we are learning that cancer and other human diseases may be caused by molecules that can bind to DNA, and we are looking at ways to minimize health risks.

Much of the Laboratory's biological research is directly aimed at improving human health and minimizing the costs associated with health care, from the diagnosis of ill health, through the prevention of disease, to the delivery of medical treatment. LLNL is a truly multidisciplinary environment, and we have always fostered and profited from team research. Nonetheless, we have been surprised at the groundswell of interest in how some technologies that have been traditionally associated with defense work can be applied to health care. To better coordinate efforts across the Laboratory, to initiate new research, and to serve as a single point of contact for outside organizations, we have formed the Center for Healthcare Technologies. Our new Center is already making important contributions in addressing the national need for more cost-effective health care technologies.

The products we expect to deliver in the next few years will include both tangible materials and basic knowledge that can be directly returned to the public. In a real sense, taxpayers who ultimately support the Laboratory will benefit from our research through better health and health care.

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

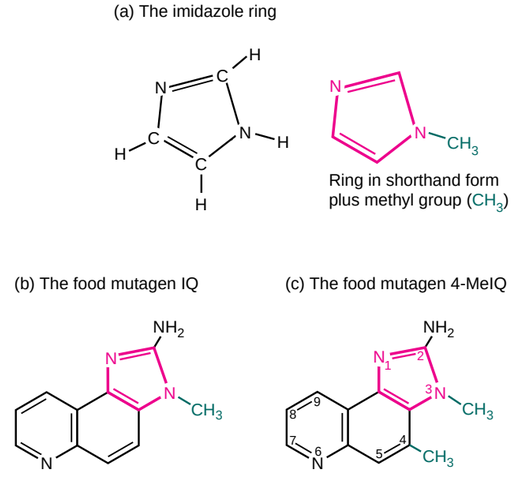


Figure 1. (a) The imidazole ring is a common feature in all of the heterocyclic amines. (b) IQ is a highly potent food mutagen that has been widely studied. (c) When a methyl group (CH_3) is added to the number-4 position of the molecule to make 4-MeIQ, the mutagenic activity is increased.

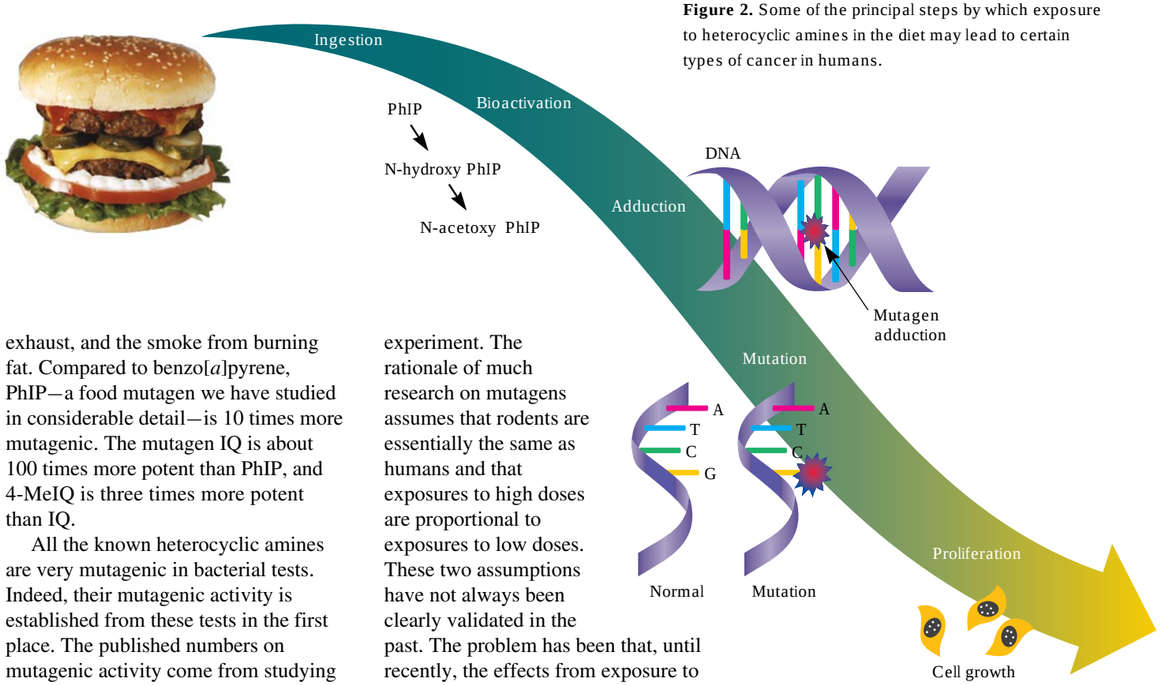


Figure 2. Some of the principal steps by which exposure to heterocyclic amines in the diet may lead to certain types of cancer in humans.

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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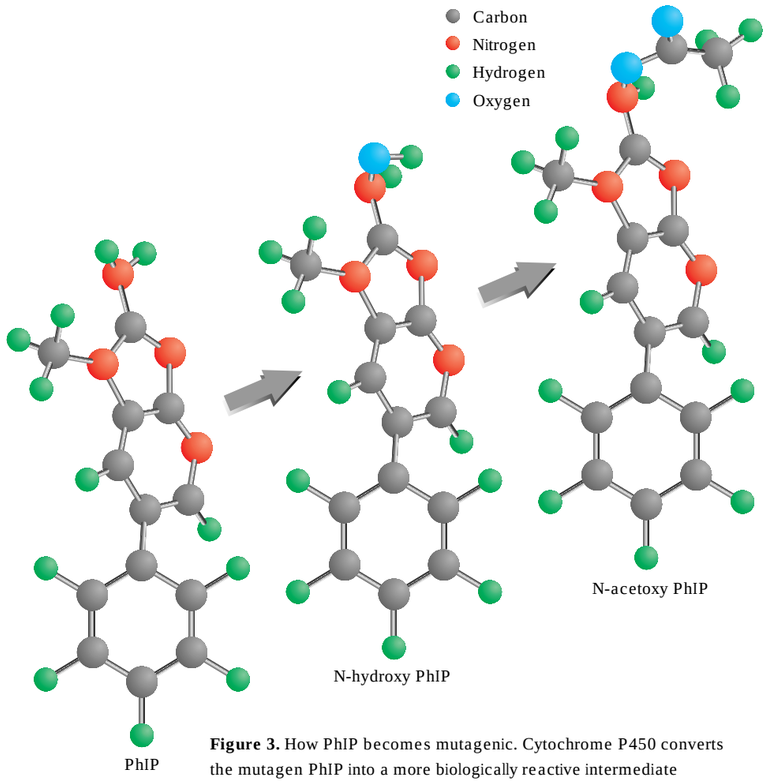


Figure 3. How PhIP becomes mutagenic. Cytochrome P450 converts the mutagen PhIP into a more biologically reactive intermediate molecule containing the hydroxy (OH^-) group. N-hydroxy PhIP is transformed into an acetate, called N-acetoxy PhIP, which is highly reactive with DNA.

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

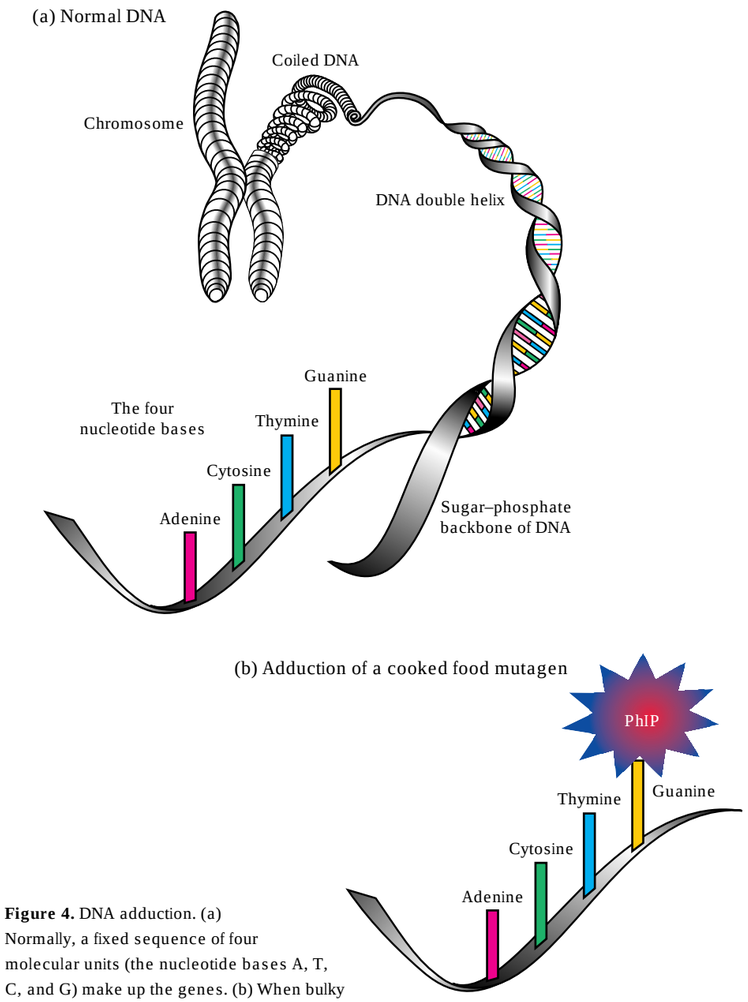


Figure 4. DNA adduction. (a) Normally, a fixed sequence of four molecular units (the nucleotide bases A, T, C, and G) make up the genes. (b) When bulky molecules (adducts) chemically bond to DNA, they distort the helix and can block the ability of DNA to replicate or can cause errors in replication.

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

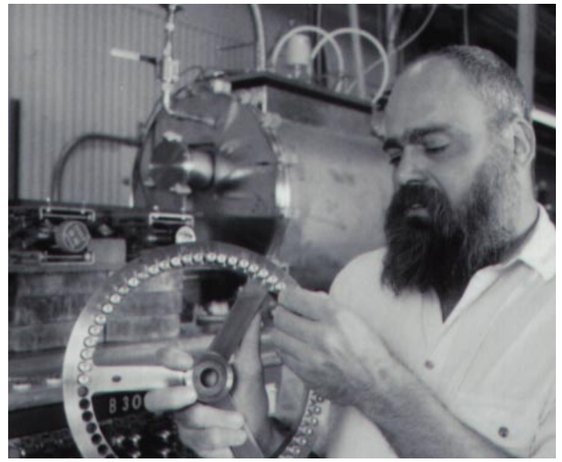


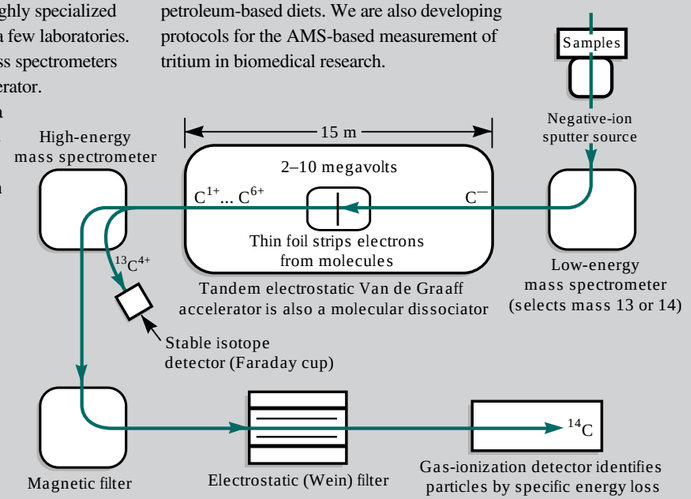
Figure 5. LLNL researcher John Vogel loads samples for analysis by accelerator mass spectrometry (AMS). Originally designed for measuring radioisotopes in nuclear physics experiments, AMS has proven to be precise and versatile in tracking very low doses of radioisotope-tagged food mutagens in laboratory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



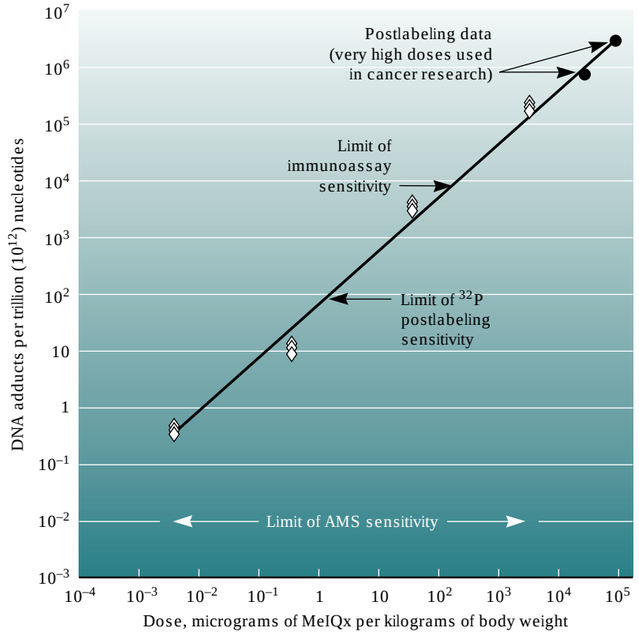


Figure 6. Using accelerator mass spectrometry (AMS), we analyzed the levels of DNA adducts in the livers of rodents that had been exposed to varying doses of the food mutagen MeIQx for seven days. Doses ranged from the amount in about one bite of a hamburger to the very large amounts used in cancer studies. 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

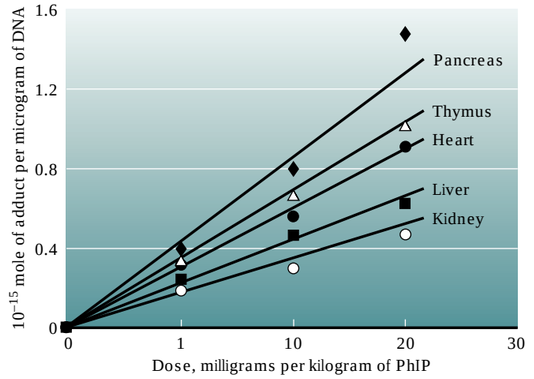


Figure 7. The response to varying doses of the mutagen PhIP depends on the tissue type and species. For mice, the pancreas had the greatest level of adducts with PhIP, followed by the thymus, heart, liver, and kidney. Rats show a different profile.

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

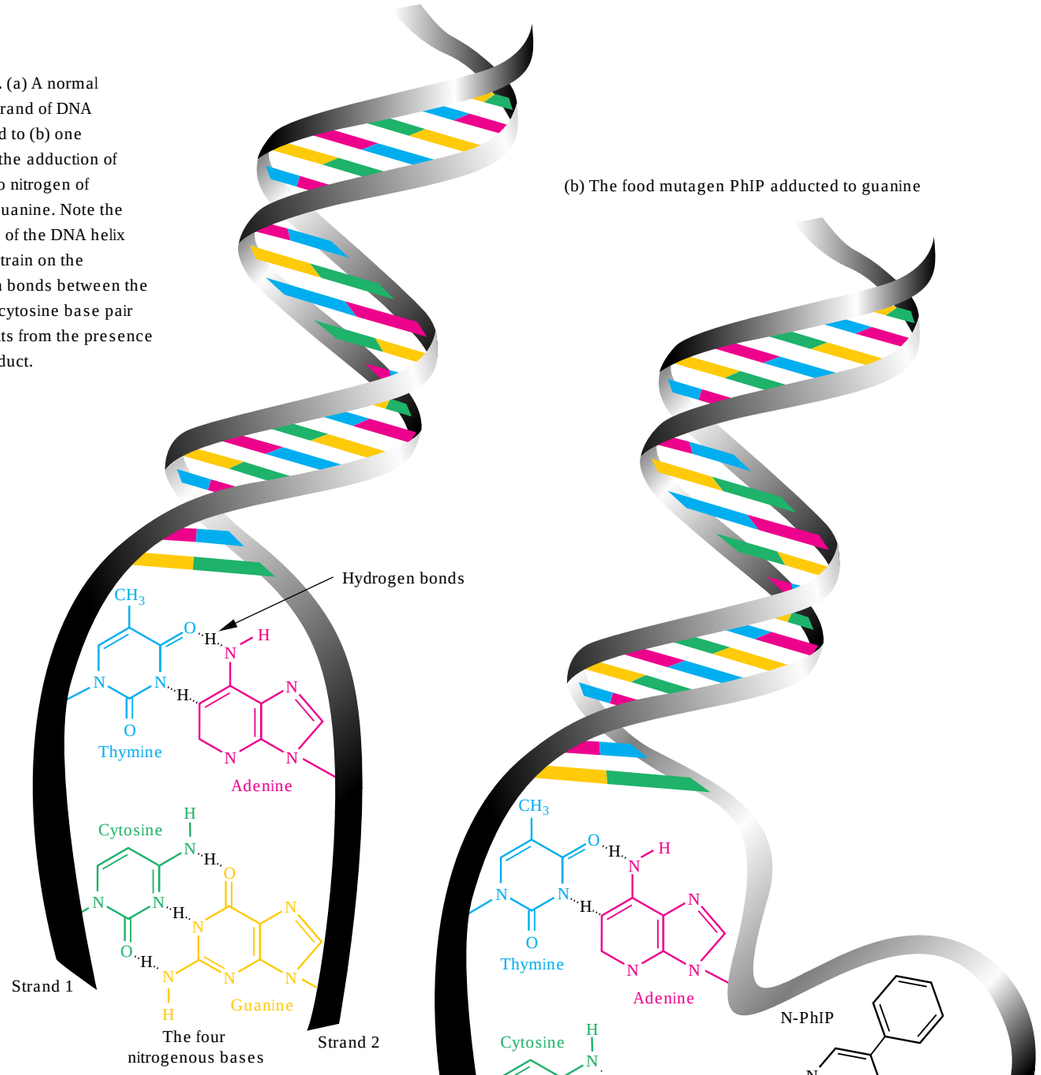


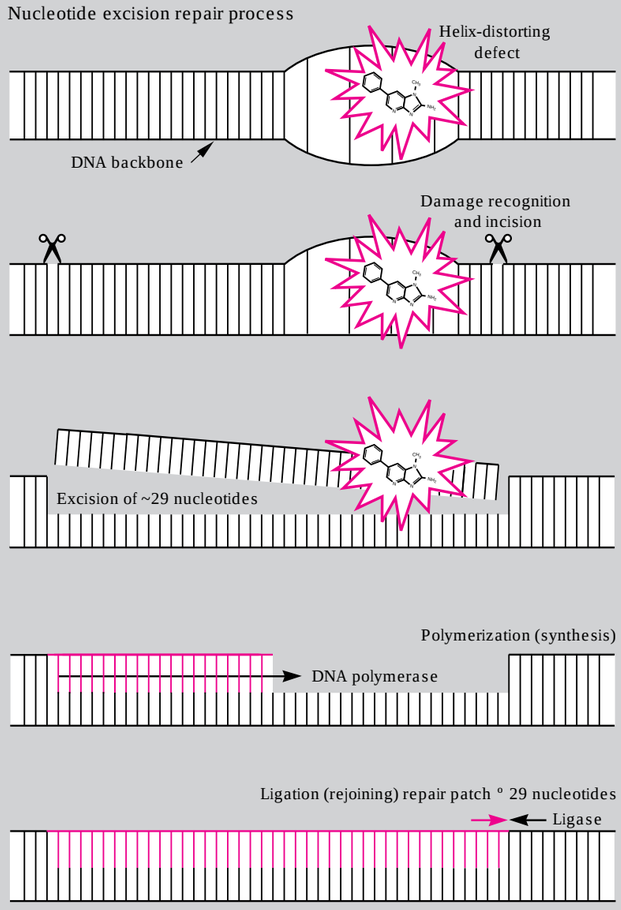
Figure 8. (a) A normal double strand of DNA compared to (b) one showing the adduction of the amino nitrogen of PhIP to guanine. Note the distortion of the DNA helix and the strain on the hydrogen bonds between the guanine-cytosine base pair that results from the presence of the adduct.

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.

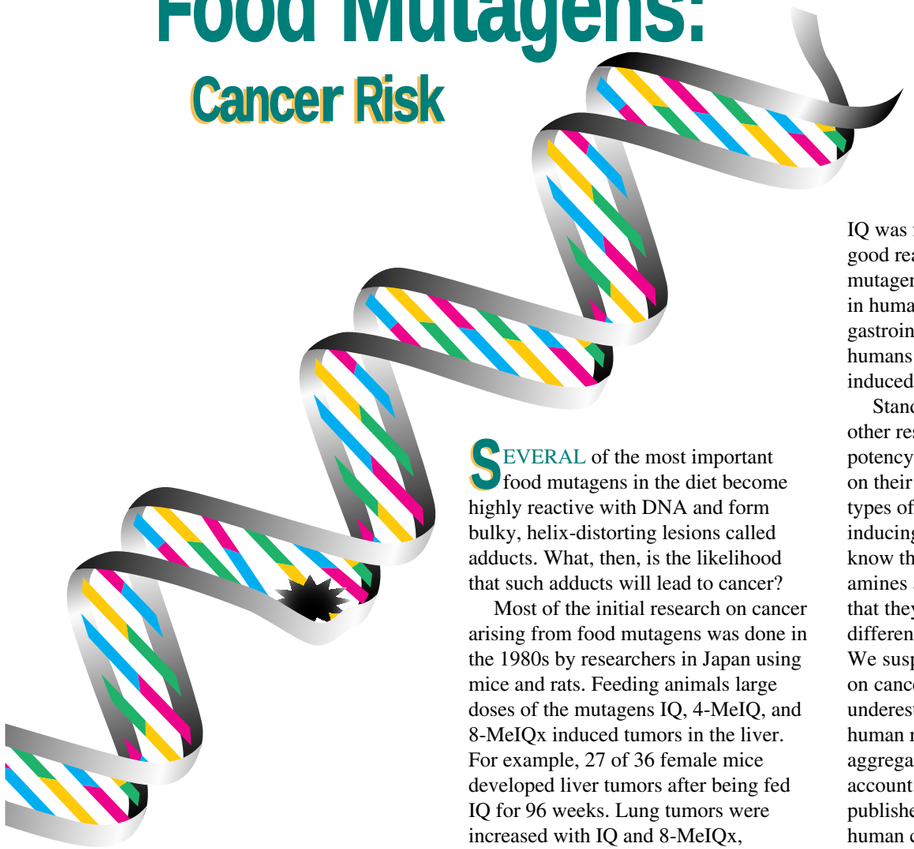
After being subjected to certain types of "insult," the usual double-helix structure of DNA can be changed. In the case of many environmental pollutants and food mutagens, bulky molecules (adducts) become attached to DNA and distort the phosphate backbone of this macromolecule. However, DNA has the ability 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.

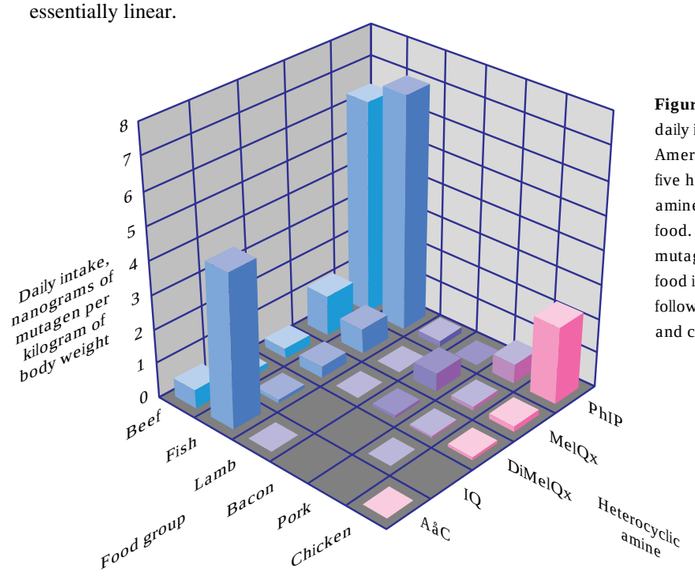
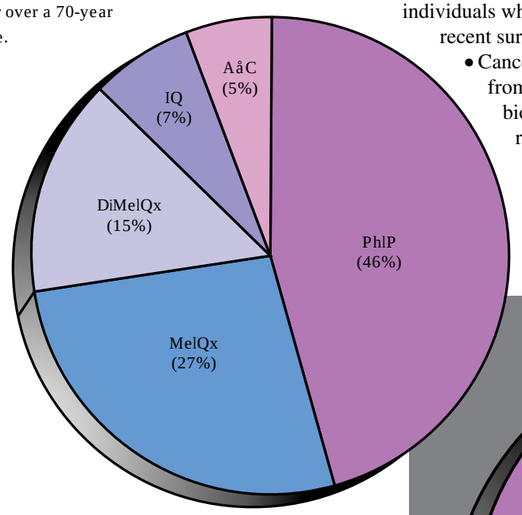


Figure 9. Average daily intake in the American diet of five heterocyclic amines by type of food. The primary mutagen-bearing food is fish, followed by beef and chicken.

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.

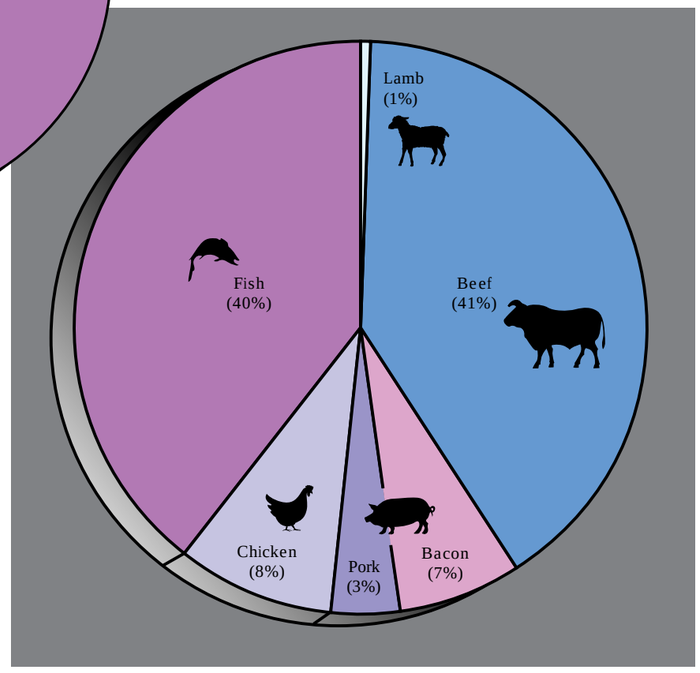


Figure 11. Principal food types contributing to the total calculated cancer risk from ingesting the five heterocyclic amines shown in Figure 10. The highest cancer risk results from eating beef products and fish.

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.

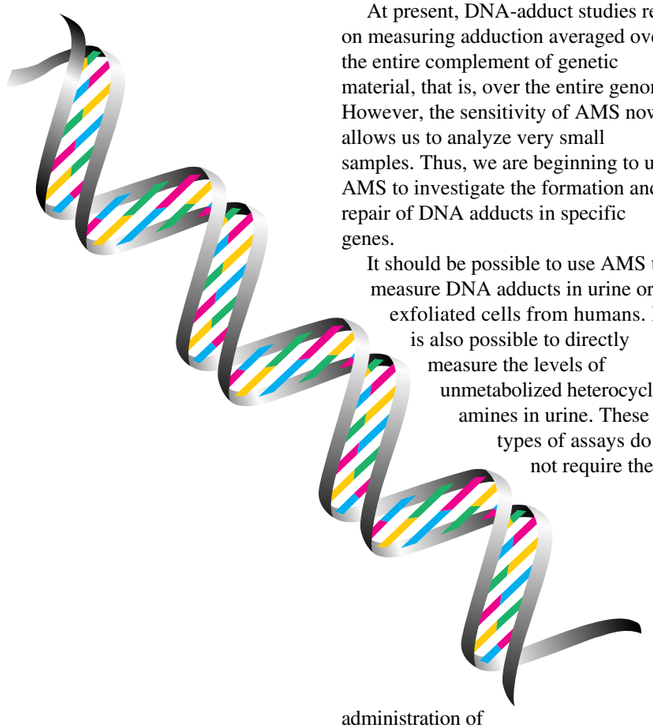
Table 1. Estimates of some lifetime cancer risks and cancer-related regulatory guidelines in the U.S.

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.

Notes and References

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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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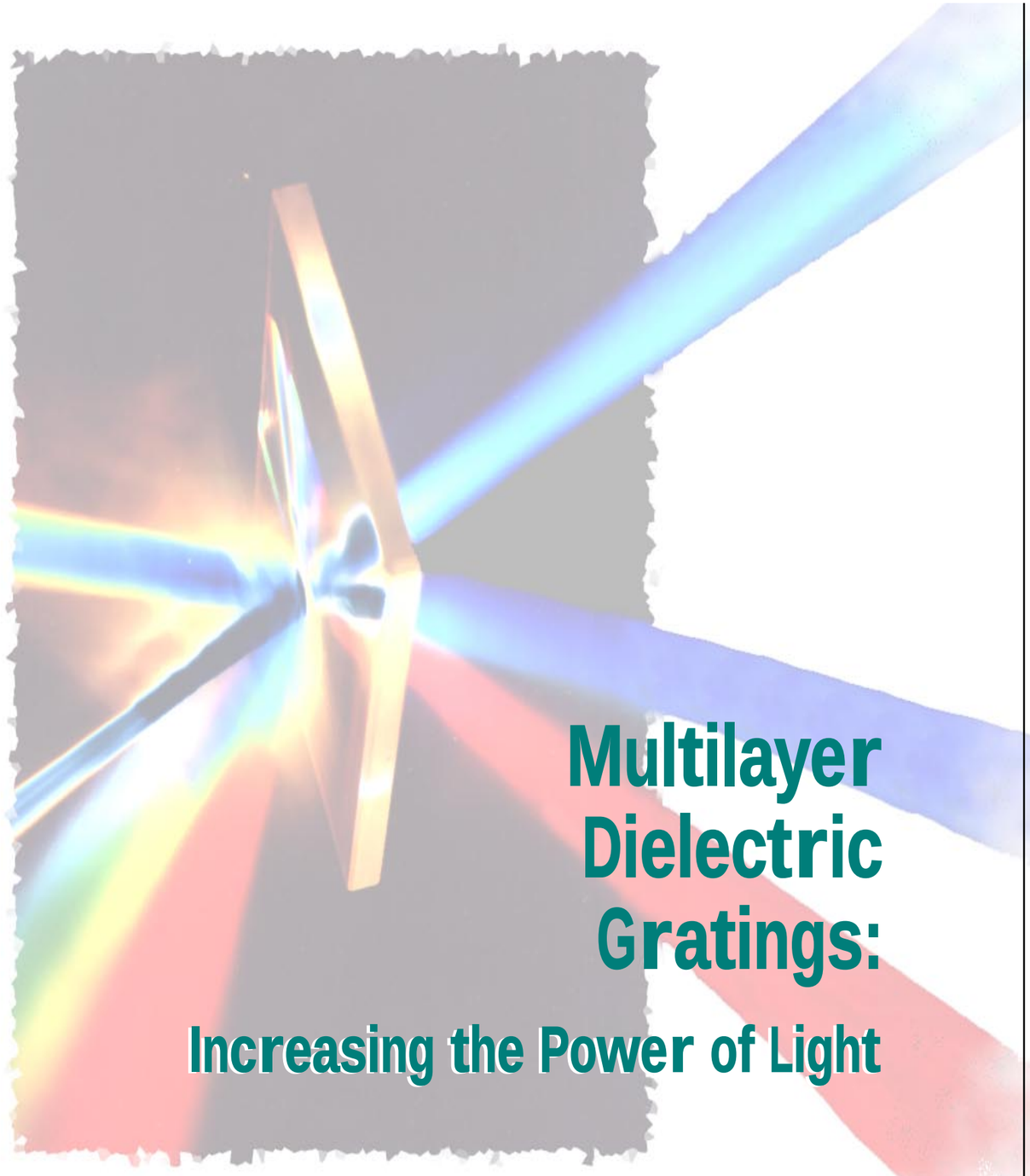
About the Scientist

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.



Multilayer Dielectric Gratings:

Increasing the Power of Light

Diffraction gratings are an essential component of high-power, short-pulse lasers. Novel grating designs using dielectric materials (insulators) rather than on traditional metallic (conducting) surfaces can significantly increase the output of high-power pulsed lasers. Their unique characteristics give these gratings numerous potential applications.

OVER the years, pulsed lasers, such as the Shiva and Nova lasers at Lawrence Livermore National Laboratory, have become ever more powerful. We have built laser systems that generate more than a trillion watts (a terawatt, or 10^{12} W) and will soon complete a quadrillion-watt (10^{15}-W) laser. The duration of these pulses is less than a trillionth of a second (a picosecond, or 10^{-12} s). At the Laboratory, we have completed construction of a short-pulse 100-terawatt (TW) laser, an important step toward a petawatt laser, which is scheduled for completion by the end of 1995.*

The development of short-pulse, high-power lasers is important in the continued progress of our inertial confinement fusion program. The fast ignitor concept, described on p. 36, would use high-power laser pulses to advance our fusion power efforts. We are also studying the propagation of strong shocks, such as those generated by high-power laser pulses, to provide information on the fundamental properties of matter (for example, the equation of state of a material). These investigations are particularly important for the Laboratory's science-based stockpile stewardship efforts. The technology of short-pulse lasers also has applications to materials processing, medicine, and dentistry.

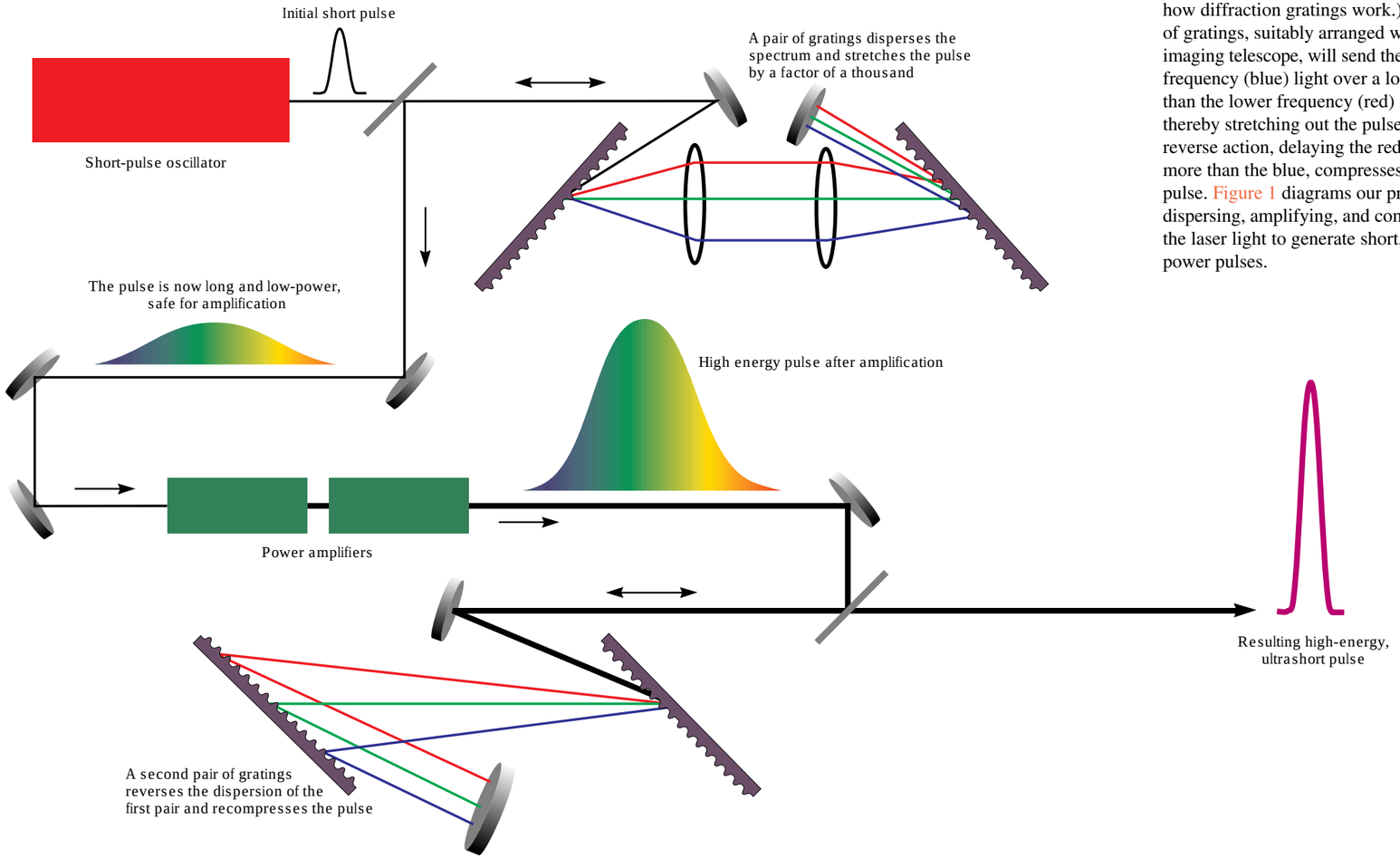
Recent developments in solid-state laser materials and the use of chirped pulse amplification[1] (CPA) have dramatically increased the intensity available with pulsed lasers. To recognize the importance of this new technology, one must appreciate some of the constraints that have previously limited the output of pulsed lasers. A laser pulse gains electromagnetic energy by extracting energy stored (as atomic excitation) in an optical amplifier. Solid-state amplifier materials make it possible, in principle, to extract several joules of energy from laser systems of modest size. In certain laser materials, the energy is available over a broad bandwidth of frequencies, an important prerequisite for short pulses. However, as the intensity of the light grows, it induces changes in the optical response of the amplifier medium. The consequent alterations of the refractive index of the amplifier medium cause the laser beam to self-focus inside the laser system. This self-focusing can result in catastrophic damage to the laser. To avoid self-focusing, it is necessary to limit the intensity that is present in amplifiers of reasonable length to less than a few gigawatts per square centimeter (1 gigawatt = a billion watts, or 10^9 W).

* For more information on the 100-TW laser, see the Research Highlight beginning on p. 34 of this issue.

Because the detrimental effects of self-focusing are proportional to instantaneous intensity rather than to accumulated pulse energy (fluence), it is possible to overcome this obstacle by amplifying a long-duration pulse and then compressing the pulse to the desired duration. Briefly stated, we first generate a broad-bandwidth seed pulse, typically 100 femtoseconds in duration (1 fs = 10^{-15} s). We then stretch the duration of this pulse by a factor of ten thousand or more, pass it through amplifiers where it grows in energy by as much as a trillion, and compress it back into a short pulse of extremely high intensity.

The first step is to produce the broad-bandwidth pulse and to impose on it a controlled frequency sweep or "chirp," in which the different frequencies occur at different times. The bandwidth of the pulse is critical to the chirped pulse amplification technique because pulse stretching or compression relies on manipulating the various frequencies or "colors" contained within the pulse. Any device that delays certain frequencies relative to others could stretch a short pulse over a longer time or, alternatively, compress a long broad-bandwidth pulse into a short one. We use diffraction gratings for this purpose, sending light rays of different frequencies in different directions. (The box on p. 28 describes how diffraction gratings work.) A pair of gratings, suitably arranged with an imaging telescope, will send the higher frequency (blue) light over a longer path than the lower frequency (red) light, thereby stretching out the pulse. The reverse action, delaying the red light more than the blue, compresses the pulse. Figure 1 diagrams our process of dispersing, amplifying, and compressing the laser light to generate short, high-power pulses.

Figure 1. Schematic of beam stretching, amplifying, and compressing system used to give different beams longer or shorter paths.



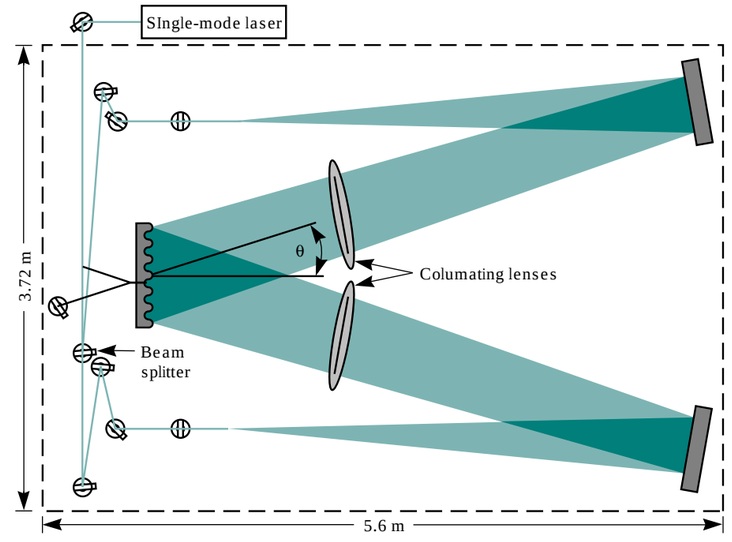


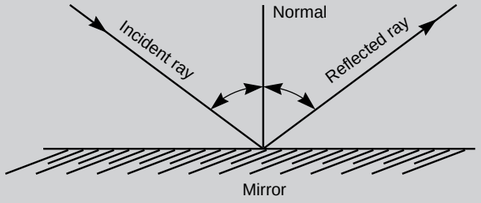
Figure 2. Schematic layout of the equal-path, fringe-stabilized interferometer used for grating exposure. Light from a single-mode laser, is divided into two paths by a beam splitter and passes, via small turning mirrors, spatial filters, larger turning mirrors, and lenses, onto the surface of a grating blank. The angle between the two beams fixes the groove spacing.

Reflecting Light Off Metal

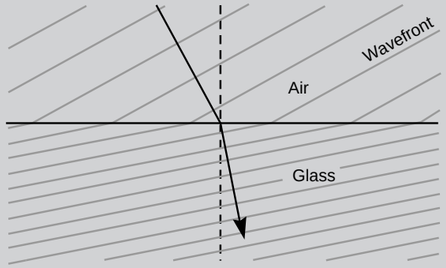
Traditional diffraction gratings, widely used in spectrometers to analyze broadband low-intensity light sources, have a corrugated metallic surface of precisely parallel periodic grooves. The metallic surface reflects the light, and the periodic groove structure diffracts the light, sending different wavelengths, or colors, back at different angles. For many decades, such gratings were produced by engraving with an extremely precise tool called a mechanical ruling engine. Today grating manufacturers often use holographic techniques, relying on the stability of continuous-wave laser wavefronts to provide the precision. Figure 2 shows a schematic diagram of our holographic exposure facility. A photosensitive surface is exposed to a standing wave pattern created by interfering two highly coherent laser beams. The latent image of the periodic interference pattern is then developed (essentially as photographic film is developed) to create a corrugated surface. Coating this grooved surface with a thin metallic film can create a highly efficient reflection grating. Alternatively, the pattern can be transferred into a more robust underlying dielectric substrate by chemical etching or ion etching

How a Diffraction Grating Works

Light is an electromagnetic wave that travels through free space in a straight line, or ray. When light waves encounter a surface, they change direction. If the surface is a mirror, nearly all the light is reflected; each incoming ray reflects at an angle equal to its angle of incidence.



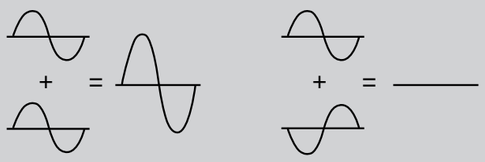
When the surface is smooth and transparent, such as a windowpane, most of the light will pass through. However, because wavefronts travel more slowly in glass than in air, the rays of obliquely incident light will become bent as they enter the glass.



The refractive index of the material—the ratio of the speed of light in a vacuum to the speed in the material—determines how much bending (refraction) occurs.

Light also bends when it scatters from the edge of a small object; this is diffraction. When the light has a narrow range of colors, the scattered waves will interfere.

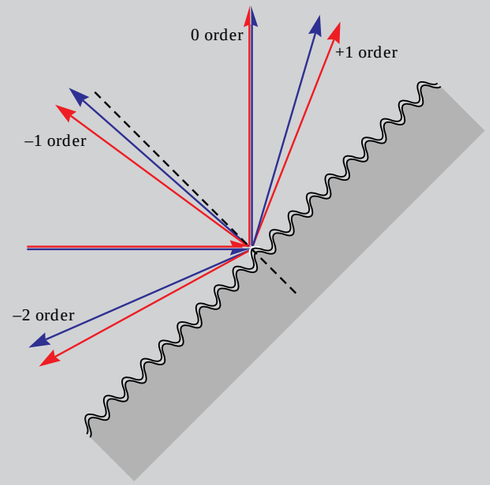
In some directions, wave crests will overlap and reinforce one another; this constructive interference will enhance the intensity of the light. In other directions, crests will match troughs, canceling one another as destructive interference and producing a dark region.



When the surface has periodic ridges (a grating), then an incoming single-color ray produces scattered rays (of the same color) at only specific discrete directions. Each of these directions is a diffraction order for the specified color and incident angle.

The color of the light is not changed by diffraction. However, blue light diffracts by smaller angles than red light, so that a ray of white light (blending many colors) will emerge from a grating with each constituent color diffracted into a different direction. The resulting display is the familiar rainbow of colors. The periodic grooves on the surface of a compact disk provide an everyday example of this phenomenon.

The color and direction of a light ray incident on a grating, together with the spacing of the periodic grooves, determines how many diffracted orders are present. If the grooves are far apart, then one color may emerge into many diffraction orders. If the grooves are closely spaced, as they are for our gratings, then only two orders occur: one emerges from the grating as it would from a mirror (the zero order), and the other order is retroreflected back along the incident direction (order –1).



When a collimated beam (a ray) of single-color light strikes a grating, the periodic grooves scatter the light into all angles. At certain angles, the outgoing waves add constructively to create an outgoing beam (a diffraction order). The outgoing wave has the same color as the incoming wave. If the incoming beam contains two or more colors, the outgoing angles differ for the different colors, and a multicolor beam is separated into single-color outgoing beams.

procedures. Transfer etching tends to produce rectangular or trapezoidal grating profiles from the original rounded patterns of developed photoresist (see Figure 3).

Metallic gratings have many useful attributes. A properly designed and carefully manufactured metallic coated grating can have a diffraction efficiency that exceeds 95% over a broad range of wavelengths (that is, more than 95% of the light is returned from the surface as diffraction into a single order). The behavior of a grating is primarily governed by the spacing and the shape of the grooves as well as by the optical properties of the metal. Gold, silver, and aluminum are typically used as coatings.

Because metallic diffraction gratings owe their highly reflective surface to the high conductivity of the metal, and the conductivity is relatively insensitive to wavelength, metallic gratings are inherently broadband devices. However, they have a low threshold for optical damage; heating of conduction electrons renders the surface susceptible to damage at fluences of around 0.8 joule per square centimeter (J/cm^2) for nanosecond laser pulses in the infrared and at much lower fluences for shorter wavelengths or shorter pulses. Metallic diffraction gratings have therefore been a limiting factor in the production of short, high-energy pulses.

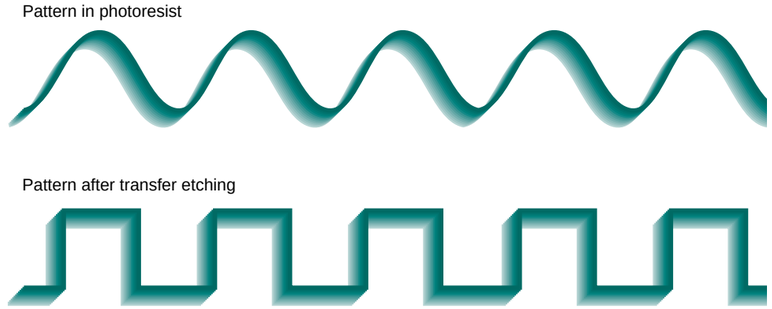


Figure 3. Schematic comparison of grating groove profiles before and after development of photoresist.

Making Insulators Reflect

Because transparent dielectric materials are insulators, they lack the conduction electrons that make metals reflecting. As a result, transparent dielectrics have intrinsically higher thresholds for laser-induced damage than do metals. Moreover, multiple layers of different dielectrics have long been known to produce highly reflecting structures. In 1991, reasoning from this well-established fact, researchers at LLNL undertook to develop a grating design by the addition of a grating to a multilayer dielectric stack.

Whereas metallic gratings achieve high reflectivity as a consequence of conduction electrons, a multilayer dielectric stack of alternating high and low refractive index layers achieves reflectivity by interference: light is reflected from the succession of surfaces whose separation is designed so that waves traveling forward and backward will destructively interfere. Each interface between a high-index and low-index pair has approximately 4% reflection, but the aggregate of many layers can approach complete reflection of light (over a range of wavelengths).

The manufacture of high-efficiency multilayer dielectric gratings draws on several subsidiary technologies in optics and chemistry and requires careful control of a number of steps: the creation of a stack of dielectric films, each of a specified thickness; the uniform coating of a photosensitive layer; the creation of a very precise interference pattern in this layer; and the transfer of the latent image into a permanent corrugation pattern in a dielectric layer. The transfer etching may be performed by a host of standard lithographic techniques, including conventional wet etching, ion sputter etching, and reactive ion etching. The choice of technique is governed by the choice of dielectric material and the desired groove spacing and depth.

Demonstrating the New Concept

In 1992, LLNL teamed with Hughes Electrooptic Systems of El Segundo, California, to demonstrate these multilayer dielectric gratings. Computer modeling provided target designs. These computations included examining how a variety of groove shapes and multilayer properties affect efficiency;

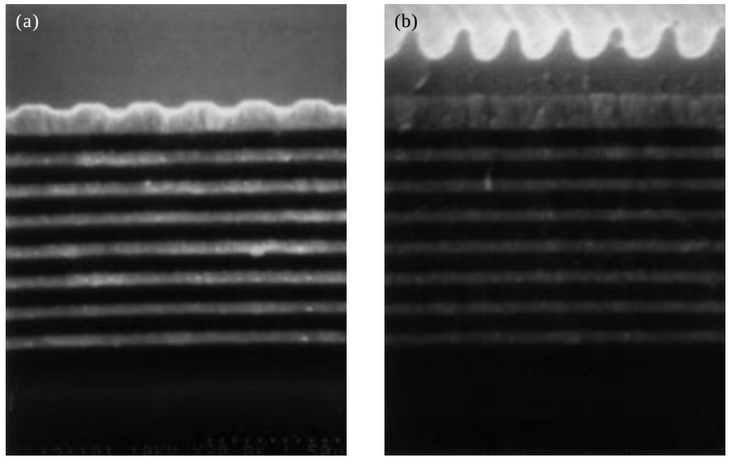


Figure 4. Scanning electron micrographs of two multilayer dielectric gratings: (a) one produced by ion etching, and the other (b) by the secondary layer technique.

for selected groove depths, diffraction efficiency exceeding 98% was predicted.

In 1993, for our first demonstration of this new type of grating, we sought extremely high efficiency in reflection for light with a wavelength of 1053 nanometers (1 nm = 10^{-9} m), the wavelength to be used in the 100-TW and petawatt lasers. We created a dielectric stack of eight pairs of high- and low-refractive-index materials deposited on borosilicate glass. Each layer pair was designed to provide high reflectivity. The grating structure was transferred into the multilayer by a multistep ion etching technique. Our computations indicated that grooves etched to the appropriate depth in the topmost, high-index layer should have an efficiency of 98%. The actual grating achieved a measured efficiency exceeding 97%.

We have continued the development of these gratings with a range of design objectives, various dielectric films, and gratings of larger size.

How the Gratings Are Made

A dielectric grating can be fabricated directly into the topmost layer of a dielectric stack, as shown in Figure 4a. This fabrication technique requires ion etching to transfer the grating pattern into the dielectric multilayer. It requires that the other layers in the stack be designed for use with the grating layer under consideration, since that layer is part of the stack.

An alternative method, called the secondary layer technique, is to fabricate a grating in a dielectric layer to be placed on top of an independently designed multilayer structure, as shown in Figure 4b. This method allows the multilayer stack to be designed either with or without the grating in place. The grating structure is constructed in a separate dielectric layer, of the necessary thickness, which is deposited on top of the multilayer. The grating pattern can be achieved by depositing the dielectric through a holographically produced mask, in photosensitive material directly, or by other lithographic techniques (e.g., lift off). As in the previous case, the grating shown in Figure 4b was designed to provide high diffraction efficiency at 1053 nm. It was fabricated in prepared photoresist on a conventional hafnium oxide/silicon oxide multilayer high reflector; it produced a diffraction efficiency of more than 96%.

Novel Grating Properties

Our primary motive for developing multilayer dielectric gratings was to enhance resistance to laser damage, an objective that has been realized. However, multilayer dielectric gratings have several novel properties that offer unique opportunities for new applications:

- The efficiency of a multilayer dielectric grating can be adjusted for any given wavelength and polarization by altering the phase retardation properties of the multilayer stack, the depth and shape of the grating grooves, and the beam's angle of incidence. We adjust these properties during manufacture to control the distribution of energy among the reflected, transmitted, and diffracted beams. Diffraction efficiency for specific incident radiation can be adjusted between 0.01% and 98%.
- The wavelength discrimination inherent in a multilayer stack makes it possible to build gratings that transmit or reflect light with high efficiency within a narrow optical wavelength band. A grating can be designed to have nearly any desired efficiency and bandwidth. This extreme optical selectivity, which is not possible with conventional metallic or bulk dielectric transmission gratings, allows a narrow spectral region to be selected to the exclusion of all others (see Figure 5).
- The damage threshold of our dielectric gratings for 3000 picoseconds (ps) laser pulses has been measured to exceed 5 J/cm^2, nearly ten times that of the best metallic gratings. For short pulses (0.1 ps), our standard multilayer dielectric gratings exhibit a damage threshold of approximately 0.6 J/cm^2, three times higher than the short-pulse damage threshold of commercially available metallic gratings. Further refinement of our multilayer design is expected to increase the short-pulse damage threshold to more than 1 J/cm^2. A grating that can withstand these high powers is essential to realize the compression of multikilojoule pulses that will be required for fast ignition of an inertial confinement fusion capsule.

Numerous Applications

Either independently or in combination, the unique capabilities of multilayer dielectric gratings allow new optical and laser products to be created. Manufactured in large size (a meter in diameter), these gratings are an enabling technology for the development of lasers with a petawatt of peak power and for the application of such high-energy lasers to inertial confinement fusion. Lasers that provide a petawatt of power in a picosecond may make it possible to achieve fusion using significantly less laser energy than currently envisioned. Useful fusion power could then be achieved perhaps years earlier than would otherwise be possible. (See p. 36 for a discussion of how the application of the 100-TW laser to the fast ignitor concept could accelerate our development of laser fusion.)

Figure 5. A multilayer dielectric diffraction grating designed to reflect yellow light, diffract broadband visible radiation (bottom left), eliminate all green and yellow light in the transmitted diffracted beam (at right), and transmit blue–green light. The grating pictured is 15 ¥ 20 ¥ 2.5 cm.

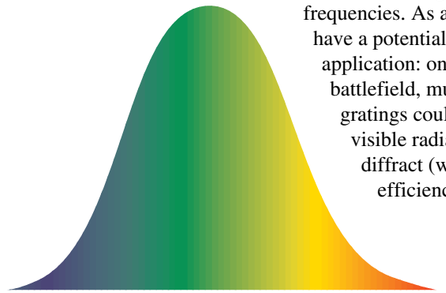
Multilayer dielectric diffraction gratings have many commercial applications. The high diffraction efficiency will find immediate use in commercial laser systems employing gratings for pulse compression, and the increased damage threshold will permit the size of the pulse compressor to fall below that of current metallic gratings.

The gratings' combination of high efficiency and high damage threshold for long pulses will make it possible to develop high-power, tunable, narrow-linewidth lasers using broadband solid-state materials with high-density energy storage, such as alexandrite, titanium-doped sapphire, and neodymium-doped glass. Compact lasers can now be made that have high pulse energies and

narrow linewidth outputs that are tunable over the gain bandwidth of the laser material. This new type of grating will also extend high-efficiency diffracting structures into the ultraviolet region (to wavelengths below 220 nm) where the reflectivity of metallic coatings drops precipitously.

Because multilayer dielectric gratings can be designed with an arbitrary bandwidth to reflect some frequency components, transmit others, and diffract still others in either reflection, transmission, or both, it is possible to select a narrow spectral region with the grating while discriminating against all others. Such sensitivity will find immediate use in high-contrast spectrometers, where discrimination often must be one part per million and is currently achieved only by the use of multiple conventional gratings.

Because of their spectral selectivity, these multilayer dielectric gratings are discrimination filters. Specifically, we designed the gratings to reflect undesirable narrow-line optical radiation (e.g., laser radiation) while transmitting most other frequencies. As a result, they have a potential military application: on the battlefield, multilayer gratings could transmit visible radiation and diffract (with high efficiency)



unwanted radiation from laser weapons or laser guidance systems. Finally, because the distribution of energy among the spectrally reflected, transmitted, and diffracted beams is controllable by adjusting the design of the multilayer and grating structure, we can use multilayer dielectric gratings as selective beam splitters in optical switches and distribution systems.

Summary

Our development of high-efficiency multilayer dielectric gratings is a technical innovation that opens the door to a host of new products and makes metallic gratings obsolete in many current applications. Its significance lies in the versatility of the device. By proper design, we can obtain a grating of almost any efficiency and bandwidth. For laser applications, the nearly tenfold increase in the optical damage threshold for long pulses over metallic gratings enables their use in high-power laser systems. These unique features, either independently or in combination, make possible the development of a new class of optical products.

We have demonstrated that such multilayer dielectric gratings can be produced, that they can reflect selected wavelength bands with high efficiency, and that they can be made in large sizes while maintaining high quality wavefronts. Manufactured in small size, these gratings can be used to create lasers with narrow linewidth and high pulse energy for such uses as directional beam splitters and efficient narrow- or broad-band filters.

Key Words: chirped pulse amplification; dielectrics; diffraction gratings–metallic, multilayer dielectric; petawatt laser; short-pulse laser.

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About the Scientist

MICHAEL PERRY joined Lawrence Livermore National Laboratory as a physicist in October 1987. He is a graduate of the University of California at Berkeley with a B.S. in both nuclear engineering and chemical engineering (Summa Cum Laude, 1983), an M.S. in nuclear engineering (1984), and a Ph.D. in nuclear engineering/physics (1987). He is currently the project leader for the Petawatt Laser Project at the Laboratory and Group Leader of the Short-Pulse Laser and Diffractive Optics groups. He is the author or coauthor of more than 70 professional publications.

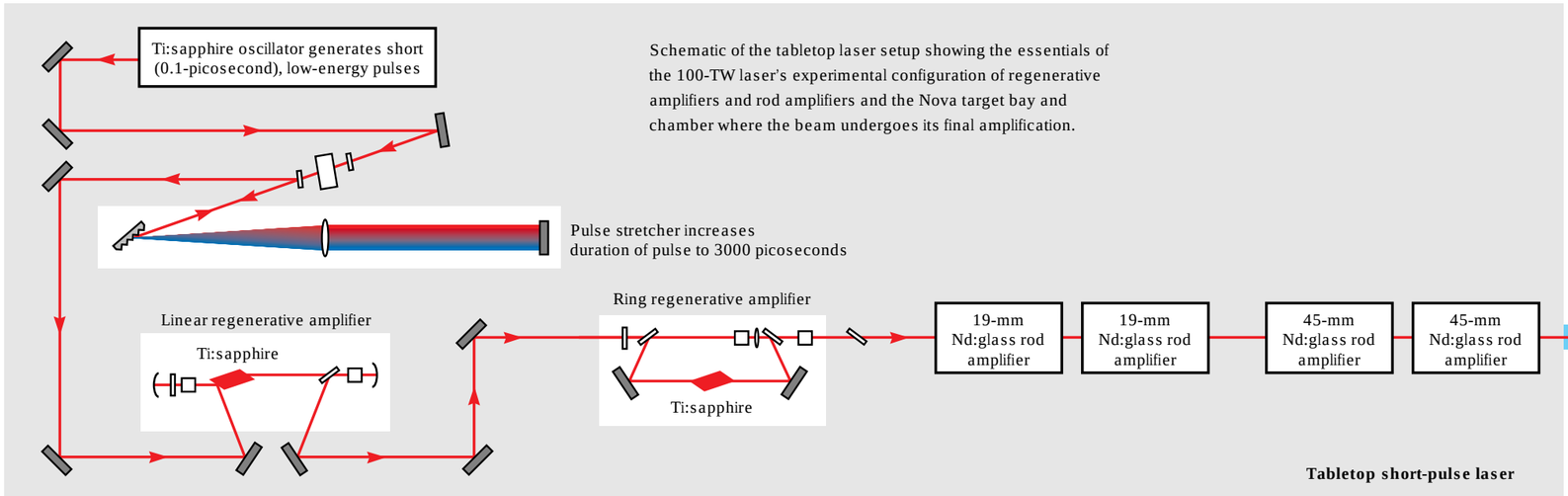
Taking Short-Pulse Laser Energy to New Peaks

IT is an extraordinary accomplishment in itself—a tabletop laser system that delivers ultrashort pulses with a peak power of 100 trillion watts, each lasting 400 quadrillionths of a second. But the contribution of this new 100-terawatt (TW) laser, whose power is equal to our Nova laser's for a fraction of the time, is that it is a stepping stone to the Laboratory's quadrillion-watt (petawatt) laser system, which is scheduled for completion late this year. The 100-TW system will help us perform preliminary tests of certain concepts and components that we hope to investigate later on the petawatt laser. One such concept is that of the fast ignitor, a promising method for achieving fusion ignition at lower laser energy than was previously thought possible. Confirmation of this concept might hasten the day when controlled inertial confinement fusion will be used for commercial power production.

How the System Works

The 100-TW system has two main sections (see the figure below). The pulsed beam is formed in the first section, a 1.5- × 6.6-m tabletop laser setup in a separate room adjacent to the Nova laser. The beam is piped to the second section, a target bay that is also fed by two of Nova's ten beams. (The 100-TW system uses a few Nova components in the target bay—most notably the two-beam target chamber itself—but it can operate either independently of Nova or in conjunction with it.)

Solid-state amplifier materials, such as titanium-doped sapphire (Ti:sapphire) and neodymium-doped glass (Nd:glass), make it possible to extract several joules of energy from modest-scale laser systems. Ti:sapphire also makes the energy gain available over the large wavelength range that is needed to amplify pulses lasting less than a picosecond (1 ps = 10^{-12} s, or a trillionth of a second). However, all amplifier materials have nonlinear components in their indexes of refraction; that is, they bend the light path by degrees that vary with the intensity of the beam. Because of this nonlinearity, intense beams tend to self-focus destructively. We must therefore limit the beam's intensity (the energy in the pulse per unit of area *and of time*) within amplifiers. One way to decrease the intensity is to make the area very large. Nova decreases intensity by expanding the beam diameter to 74 cm through its amplifier chain. However, the intensity of subpicoseconds pulses would remain too high even if the beam were spread to a Nova-size diameter.



Schematic of the tabletop laser setup showing the essentials of the 100-TW laser's experimental configuration of regenerative amplifiers and rod amplifiers and the Nova target bay and chamber where the beam undergoes its final amplification.

Therefore, we must increase the pulse duration in order to decrease the energy per unit of time.

To alter the pulse duration, we use the technique of chirped pulse amplification. We stretch the duration of the pulse before amplification and then compress it back down to approximately its original duration after amplification. In a chirped pulse, the frequency of the laser pulse changes throughout the pulse. By delaying the frequencies late in the pulse, we can stretch a short pulse into a long one. By delaying the frequencies early in the pulse, we can compress a long, broad-bandwidth pulse into a short one. Diffraction gratings separate different frequencies, send them in different directions, and vary their delays. In a pulse stretcher, sending the higher frequency (blue) light over a longer path than the lower-frequency (red) light stretches out the pulse. (Diffraction gratings are described in the article beginning on p. 24 of this issue.)

Following the Pulse

In the case of our 100-TW laser, a commercial Ti:sapphire oscillator produces 0.1-ps pulses with energies at the nanojoule level—short pulses at low energy. As indicated in the figure below, we stretch their duration to 3000 ps—a factor of 30,000. Then, we amplify the stretched pulses with a linear regenerative amplifier using Ti:sapphire. The pulse enters the linear amplifier at 10 to 100 picojoules (pJ), makes about 130 passes through it, and comes out at approximately 7 millijoules (mJ). This is an amplification of a billion—a huge portion of the total energy gain needed to produce 100 TW of power.

At 7 mJ, the intensity is getting high enough that self-focusing can occur in the Ti:sapphire crystal. Having pushed this limit in the first amplifier, we expand the beam in diameter to lower its intensity without decreasing its energy and send it into a second Ti:sapphire amplifier, a regenerative ring amplifier. The 7-mJ beam makes approximately 15 round-trips and is amplified to about 60 mJ.

At this point, we have amplified the beam as far as is practical in Ti:sapphire and have maintained its bandwidth (frequency range). We must then use two pairs of Nd:glass amplifiers for higher energy extraction. The first pair are 19 mm in diameter, the second are 45 mm. The beam is expanded in diameter at each stage to maintain a reasonably low intensity. To maximize the efficiency of these amplifiers, we clip the edges off the beam, giving it a "top hat" or rectangular crossectional profile. Clipping the beam halves its energy to about 25 mJ. The first pair of rod amplifiers increases the energy to 2 J and the second pair to 15 J. This beam is then piped from the tabletop setup to the two-beam target bay in Nova.

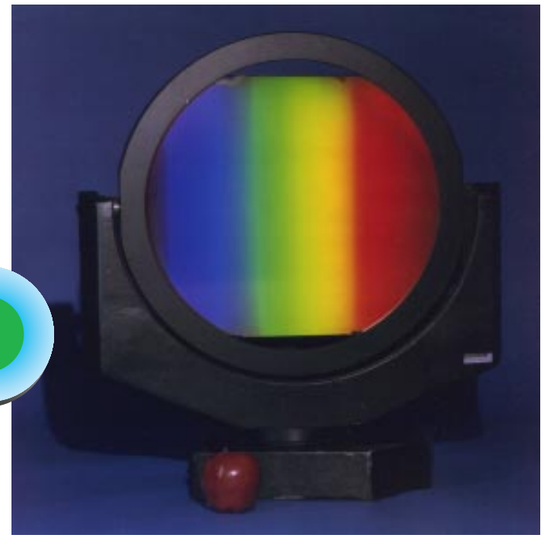
In the target bay, the beam undergoes its final amplification. Passage through a standard 9.4-cm-diameter Nova disk amplifier brings the beam energy to about 60 J—a fivefold gain. The beam passes through a spatial filter, is expanded to about 14 cm in diameter, and enters the pulse compressor. The two 40.6-cm-diameter gold diffraction gratings (see photo on p. 36) within the compressor undo the stretching that we did at the beginning (these Laboratory-designed and -made gratings are superior to commercial gratings both in wavefront quality and damage threshold). Delaying the red light more than the blue compresses the pulse from 3000 ps to about 0.4 ps. We cannot achieve full compression to the original 0.1 ps because of the narrowing of the pulse spectrum in the glass rod and disk amplifiers.

The beam loses some energy on the gratings and mirrors in the compressor and comes out at about 40 J. The beam enters the target chamber, where it is focused by an off-axis parabolic mirror from 14 cm in diameter to a 10-micrometer (μm) spot, yielding an intensity of 100 quadrillion watts of power per square centimeter (10^{20} W/cm^2) when it strikes its target, higher than any short-pulse system has ever achieved.

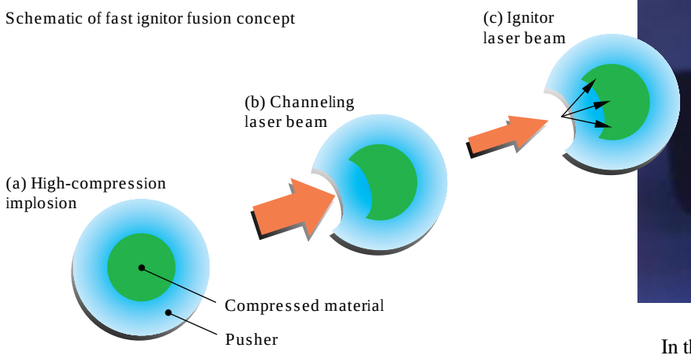
The Uses of Short, Intense Pulses

The ultrashort pulses and extremely high intensities of the 100-TW laser will enable us to advance our understanding of laser–matter interactions. A pulse of high-density light impinging on a solid surface drives an ultrahigh-pressure shock wave into the material. Studies of the propagation of such strong shocks can provide information on the fundamental properties

One of the two gold gratings in the compressor chamber of the Nova target bay. These gratings return the stretched pulse to about 0.4 picoseconds in duration before it enters the Nova target chamber.



Schematic of fast ignitor fusion concept



of matter—for example, the equation of state of a material, investigation of which is particularly important to the Laboratory's science-based stockpile stewardship work. These studies promise to further our understanding of how pressure is developed in a given material when a given amount of energy is added to it—that is, its equation of state, which is the thermodynamic relationship between the energy content of a given mass of material and its pressure, temperature, and volume.

The electric field of our 100-TW, 0.4-ps laser is many orders of magnitude larger than the fields that bind electrons within the atoms of the target material. A near-instantaneous liberation of a large number of outer-shell electrons from those atoms can create a highly charged plasma or "gas" of electrons or ions. The plasma is "cold" because there is no time during the picosecond pulse for the ions or electrons to interact and thermalize. Cold plasmas are ideally suited to test the concepts on which certain x-ray laser schemes are based.

Fast Ignitor

The Laboratory's 100-TW facility provides us with our first tools to begin to address the fast ignitor fusion concept, which requires extremely high powers on the order of a petawatt (10^{15} W). Focusing a high-power laser pulse gives rise to an extremely high density of energy, or light pressure. When a pulse of high-density light impinges on a solid surface, an ultrahigh-pressure shock wave can be driven into the material. These intense pulses also generate large amounts of energetic (about a megaelectron-volt) electrons. These high-intensity phenomena form the basis of the fast ignitor fusion concept.

In the fast ignitor scheme (see schematic at left), laser energy compresses a spherical volume of fusion fuel to high density—exactly as in the conventional approach to inertial confinement fusion. However, in the conventional approach, the fuel must be compressed to the point that it ignites. By contrast, the fast ignitor concept will add two laser beams to the fusion process. The first, a channel beam made up of 100-ps pulses, bores through the plasma created by the conventional laser driver and pushes the fuel in its path toward a higher density near the core of the fuel. The second, an ignitor beam, interacts with this density gradient and generates hot, high-energy electrons that penetrate the core and instantaneously raise its temperature, hastening ignition. The relation between conventional fusion ignition and the fast ignitor concept is roughly analogous to the relation between the diesel engine and the gasoline engine: the diesel uses compression ratios of about 100:1 to ignite the fuel in the absence of a spark; the typical gasoline engine uses a spark to ignite its fuel at a compression ratio of about 10:1. The fast ignitor technique, if proven successful, will not only offer the advantage of circumventing the rather difficult task of ignition by compression alone but also promises fusion gain at significantly less input laser energy than in conventional inertial confinement fusion.

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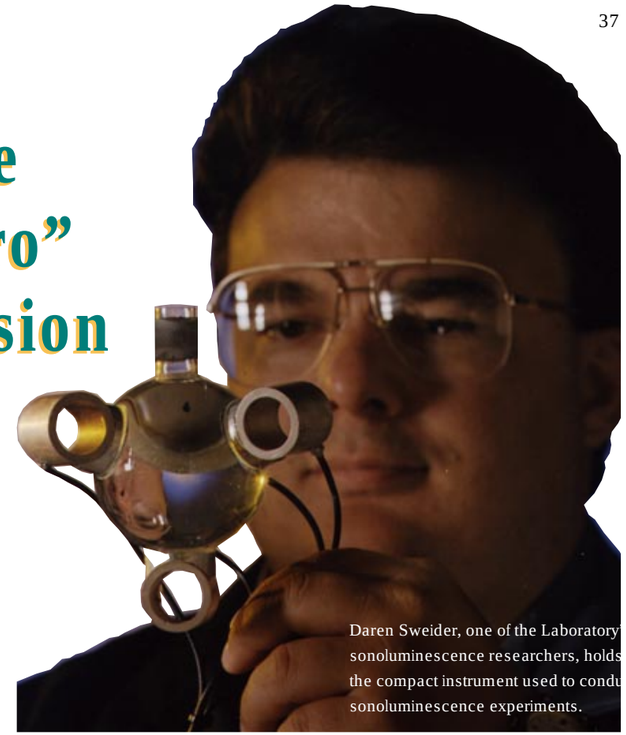
Sonoluminescence and Tabletop "Micro" Thermonuclear Fusion



Daren Sweider, one of the Laboratory's sonoluminescence researchers, holds the compact instrument used to conduct sonoluminescence experiments.

SONOLUMINESCENCE is the curious phenomenon of converting acoustic energy to optical energy—literally, turning sound into light. Although first observed more than 60 years ago, only recently have we begun to understand the phenomenon. We know that the light observed does not result from the acoustic field directly; rather, it results from a process called cavitation, in which gas-filled bubbles in a liquid form, grow, and collapse in response to the pressure waves generated by the sound pulses; as the bubbles collapse, they compress or implode and heat the gas to the point that it emits light.

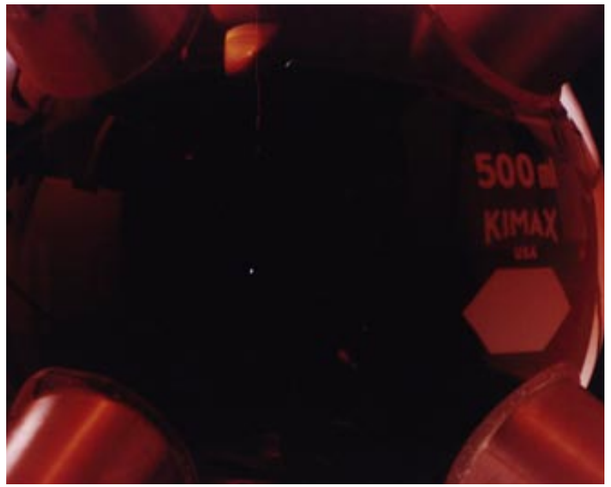
Scientists at Lawrence Livermore National Laboratory have been investigating the physics of implosions experimentally and calculationally for more than 40 years, first for nuclear weapons and then for inertial confinement fusion. These fusion studies previously involved nuclear detonations or massive laser systems. Imagine the savings and advances possible if tabletop sonoluminescence systems could generate the pressures and temperatures necessary to study nuclear fusion. Today, Livermore researchers are pursuing such possibilities using complex numerical models to investigate the effects of spherical convergence on the dynamics of bubbles. These are the same numerical models developed initially to simulate the implosion phase of nuclear weapons. The results of sonoluminescence simulations reveal the underlying physics of the phenomenon. What is especially intriguing about spherical convergence as related to sonoluminescence is the realization that it happens so intensely, yet nondestructively, in a tabletop experiment (see the photograph on p. 38).

Many Bubbles vs One Bubble

The first observation of sonoluminescence was recorded in 1934 by H. Frenzel and H. Schultes, of the University of Cologne, who found that photographic plates submerged in a water bath and irradiated with ultrasonic waves became exposed. The early research that followed concentrated on the random growth and collapse of large numbers of cavitation bubbles and involved fairly general analyses of a cavitation field containing many bubbles of various sizes. The analyses were helpful in uncovering gross aspects of sonoluminescence but did not reveal much about the physics of the individual cavitation events or the resulting bursts of light.

In 1990, D. Felipe Gaitan, now at the Jet Propulsion Laboratory in Pasadena, and Lawrence A. Crum, now at the University of Washington, discovered the conditions under which a single bubble could be trapped in water in a flask and made to stably expand and contract and emit light synchronously with the applied sound pulses. They found that an acoustic field equivalent to about 110 decibels is required to trap a bubble and cause it to expand and contract. This sound intensity is comparable to that of a smoke detector alarm held an inch or so from one's ear, but the frequency of the sound is beyond the range of human hearing. The Gaitan method, which involves carefully tuning both the amplitude and the frequency of the acoustic field, provided a simple and inexpensive experimental setup for studying sonoluminescence in detail.

A year later, researchers at the University of California at Los Angeles, headed by Seth Putterman, applied the Gaitan method to determine the duration of the sonoluminescence flashes and the flash-to-flash interval. Studying, in Putterman's words, this "hydrogen atom of sonoluminescence," they found that each light pulse lasts less than 50 picoseconds (1 ps = 10^{-12} s) and that the time between flashes was extremely stable and nearly synchronous with the frequency of the sound pulse. In addition, measurements of the spectrum of emitted light indicated



Single-bubble sonoluminescence. A single, stable bubble (visible near the center of the photograph) oscillates about an equilibium radius of a few micrometers, expanding and contracting and emitting light each acoustic cycle.

extremely high temperatures—at least several tens of thousands of degrees.

Previous explanations of sonoluminescence could not account for the extremely short pulses of light or the high temperatures. The calculations by Livermore scientists, however, provide the best explanation to date of the origin of both by showing that a spherically converging shock wave, generated within the collapsing bubble by the acoustic pulse, creates a relatively high energy density at the center of the bubble.

Simulations at LLNL

Beginning in 1992, a group of Livermore researchers performed numerical simulations to study the hydrodynamics that govern the growth and collapse of an air bubble in water. Unlike previous simulations done elsewhere, they treated both air and water as compressible fluids. These simulations showed that the acoustically driven compression is nearly isentropic until the final 10 nanoseconds (1 ns = 10^{-9} s). During this very brief time, strong spherically converging shock waves evolve in the bubble (see the graph on p. 39). As a result, the central region heats to very high temperatures—about 350,000 kelvin (K), or 30 electron volts (eV)—and emits light. Reflection of the shock from the center of the bubble produces a diverging shock wave. The subsequent flow behind the shock wave quenches the high temperatures and pressures—and thus the light pulses—in a few picoseconds.

These data are consistent with Putterman's measurements. In addition, the calculated temperatures for an air bubble in water are only one or two orders of magnitude lower than the ten-million-degree temperatures required to fuse deuterium.

Laboratory researchers then simulated the growth and collapse of a bubble containing pure deuterium (an isotope of hydrogen) and one containing a mixture of deuterium and water vapor containing deuterium. In this simulation, they found that pure deuterium in deuterated water alone cannot exhibit picosecond sonoluminescence because the speed of sound in deuterium is too rapid to sustain a shock in the collapsing bubble. However, because the bubble actually contains pure deuterium plus deuterated water vapor, the sound speed is lowered so that the mixture can support a sustainable shock. When the contents of the bubble are modeled as this mixture, calculated temperatures and pulse widths are consistent with preliminary experimental data collected at Livermore—that is, measured pulse widths of less than 15 ps and violet light emissions, corresponding to temperatures of a few electron volts (30,000–40,000 K).

Agreement between the deuterium-plus-vapor simulations and experimental data provides a starting point for examining practical methods to enhance an implosion. Many parameters can be varied, including the size and composition of the flask, the composition of the liquid, the size of the bubble, the ambient temperature and pressure, and the characteristics of the applied acoustical field. To minimize the perturbations to the "standard" sonoluminescing system, the Livermore researchers first studied the effect of "shaping" the acoustical pulses.

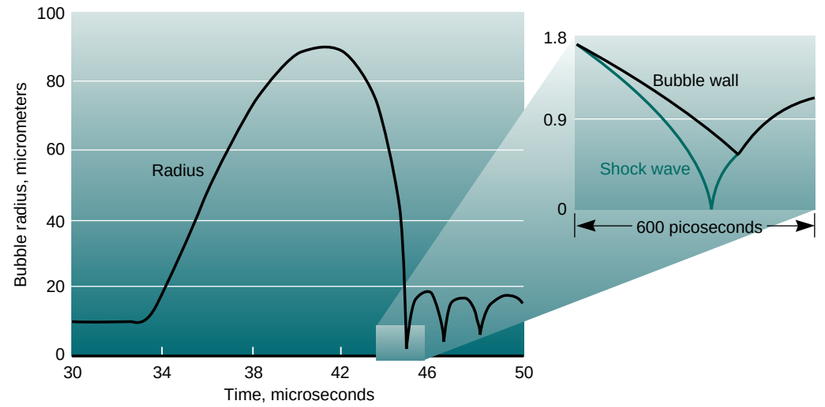
Spiking the Field

For another simulation using deuterium bubbles, they hypothesized that an acoustic "spike" of pressure, superimposed on the sinusoidal drive, would enhance the implosion by supplying extra energy without affecting the mechanisms that allow sonoluminescence to occur. Sonoluminescence is known to be extremely sensitive to the overall system acoustics that result from the periodic driving wave generated by the applied pulse. The preliminary experimental data show that deuterium bubbles are indeed brighter when the driving acoustical field has a spike.

Calculations reveal that a peak central temperature exceeding 500 eV (5.8 million K) may occur for a modest spike. The spike greatly accelerates the liquid–gas interface early in the implosion. Because acceleration occurs early in the implosion, there is ample time and distance for spherical convergence to increase the amplitude of the shock greatly and, therefore, the pressure and temperature in the center of the bubble.

It must be noted, however, that because these calculations currently do not include radiant energy transport and thermal conduction, the calculated pressure and temperature increases may be somewhat overestimated. Despite this simplification, fusion of the deuterium may occur under these intense pressure and temperature conditions.

Deuterium fusion produces neutrons. An estimate of the number of neutrons produced is 0.1 neutron per hour, neglecting the unrealistically high calculated temperatures at the very center of the bubble. Although this count rate is low, it should be measurable. Because neutron production is coincident with the flashes of light, the arrival of neutrons at the detector can be determined accurately. In addition, the energy spectrum of fusion neutrons is well defined. Thus, by temporal and energy gating (a mechanism similar to a camera shutter), most of the spurious background signals can be removed, making it possible to accurately detect fusion neutrons produced from sonoluminescence.



A graphic simulation of the bubble wall over time during a growth and collapse cycle. The enlarged area shows the critical time when the shock wave slams into the center of the bubble and light is emitted, indicating high implosion temperatures and pressures.

Fusion from Sound

At least theoretically, tabletop fusion appears possible. The numerical simulations done at the Laboratory provide the foundation for a serious attempt to attain thermonuclear fusion from a sonoluminescencing bubble. They indicdate that under specific, extreme experimental conditions, such as those simulated at Livermore, a very slow but scientifically interesting reaction rate is possible.

The Livermore numerical simulations also show many ways to improve the experimental results. For example, the neutron production rate can be increased by at least a factor of 50 if the deuterium is replaced by a mixture of deuterium and tritium. Modifications of the spike amplitude, timing, and shape may provide further enhancements. Raising the ambient pressure of the system to increase the bubble mass could be another enhancement.

Sonoluminescence experiments to date have produced light emissions lasting less than 50 picoseconds. Calculations indicate that single-bubble sonoluminescence may result in temperatures of near 1000 eV (more than 11 million K), pressures greater than 10 million atmospheres, and mechanical energy concentrations of up to 12 orders of magnitude. Further calculations and more sophisticated experiments are needed to demonstrate the feasibility of producing tabletop "micro" thermonuclear fusion.

The Livermore researchers caution that this approach offers no shortcuts to achieving fusion. Rather, it is a clever idea for doing some of the same physics that the Laboratory's Nova laser does but on a much smaller scale and at a relatively low cost. At the very least, applying the spiked driving pressure to any sonoluminescing system may provide the general scientific community with easy and inexpensive access to pressures, temperatures, and time scales that have been unattainable previously.

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Food Mutagens: Mutagenic Activity, DNA Mechanisms, and Cancer Risk

Heterocyclic amines are potent mutagens, and they are carcinogenic in laboratory animals. We have shown that typical Western diets rich in well-done meat cooked at high temperatures have significant levels of at least five different heterocyclic amines. We are beginning to understand the process by which food mutagens become adducted to DNA. This molecular binding to DNA is an important step that can lead to cancer. The binding of mutagens depends on the formation of intermediate, biologically reactive molecules. The intermediates appear to link preferentially to the DNA base guanine. The extent of DNA adduction and the subsequent occurrence of tumors vary considerably in different types of tissues, such as the liver and pancreas, and in different animal species.

A comparison of fried meats shows that beef and chicken are the most mutagenic types of food in a typical Western diet. When account is taken of the relative amounts of different meats consumed by Americans and of the potency of mutagens in them, ground beef may be the most important source of food mutagens in the U.S. diet. The overall, average, upper-bound estimate of cancer risk in the U.S. from eating heterocyclic amines in cooked foods is about one in ten thousand. The consumption of meat and chicken contributes most to the total risk. Some individuals eating large amounts of well-done muscle foods may be at much greater risk.

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Multilayer Dielectric Gratings: Increasing the Power of Light

High-power, short-pulse laser systems are vital to continuing inertial confinement fusion research. We have completed a system that can produce 100 terawatts (100 trillion watts) of pulsed power lasting about a trillionth of a second and will soon complete another system that will produce a petawatt (a quadrillion watts, or 10^{15} W) of power in a similarly short pulse.

High-power lasers present a special problem, however. As amplifiers increase the energy of the beam, the laser pulse intensity drives nonlinear changes in the refractive index of the amplifier material, causing the beam to self-focus and damage the amplifier. To avoid this effect, a pair of gratings is used to stretch the pulse and lower it speak intensity. The stretched or "chirped" pulse is then amplified many thousands of times and is finally passed through a second grating pair that compresses the pulse, shortening it almost to its original duration. When the laser pulse strikes its target, it is at its power-amplified, time-compressed peak intensity.

Traditionally, diffraction gratings have been made of metal—gold, silver, or aluminum. However, because electrical conductivity in the skin of the metal is the basis of their reflectivity, they are susceptible to optical damage. Multilayer dielectric gratings, by contrast, are made of transparent, insulating materials. They are reflective through interference from many layers and distribute the reflection through the bulk of the material. They are thus not easily subject to optical damage and can be used in carefully constructed pairs to first stretch and then compress the short, high-energy pulses of today's most powerful lasers.

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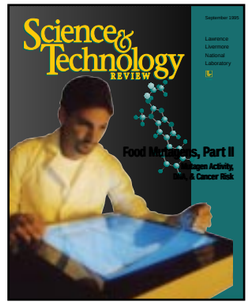

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	very interesting	interesting	minimal	none
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Multilayer Dielectric Gratings	❑	❑	❑	❑
Short-Pulse Laser	❑	❑	❑	❑
Sonoluminescence	❑	❑	❑	❑

How would you rate the technical level of the content?

	acceptable	too high	too low	didn't read
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Multilayer Dielectric Gratings	❑	❑	❑	❑
Short-Pulse Laser	❑	❑	❑	❑
Sonoluminescence	❑	❑	❑	❑

How interesting and valuable do you find our new sections?

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