On the Trail of Mutations at MIT

"Basically, the way this center operates is that it starts with human disease and works backwards to a clear and probable cause," says Director William Thilly describing MIT’s Center for Environmental Health Sciences (CEHS). "If the problem stems from a genetic mutation, then we try to figure out what caused that mutation—exogenous chemicals, endogenous chemicals, or a replication error."

Although much public policy is now based on the premise that the mutations occurring in people are caused by environmental chemicals, nobody has yet proven that hypothesis, Thilly maintains. "The hypothesis is widely believed but has barely been tested, mainly because there’s little or no technology available to test it."

The center, a consortium of faculty members with backgrounds in food chemistry, combustion engineering, water resources, analytical chemistry, and toxicology, has set out to determine whether environmental chemicals cause mutations and, if so, to link specific chemicals to specific mutations and to correlate those mutations, in turn, with particular diseases. CEHS researchers have had to develop an array of new tools to trace chemicals and their byproducts from the source of contamination through the environment and into the human body. "You’ve got to find a means of getting inside people and seeing what’s going on, firsthand, because extrapolations from animal studies or cell culture experiments are scientifically dubious," Thilly stresses. "New methods of analytical chemistry can tell us whether or not certain chemicals are making their way into the body."

The long-term goal, according to Thilly, is to protect people from mutations, "because we know that’s how a lot of diseases start. But we’ve got to figure out what’s causing these mutations before we can be in a position to protect anybody."

A Confluence of Ideas

The MIT CEHS came together over a number of years, as interrelated research strands became entwined. Among the earliest multidisciplinary efforts was a research project in food toxicology supported by an NIEHS program grant and led by Gerald Wogan, current director of the Division of Toxicology at MIT. Starting in the early 1960s, Wogan and his colleagues discovered that eating food laden with aflatoxin B1, a fungal agent that grows on damp peanuts, can put people at risk for liver cancer. The finding eventually led to standards which protect consumers from the fungus, as well as a way of monitoring peanuts, corn, rice, wheat, and other grains for the presence of this mold contaminant. Because the aflatoxin molecule is fluorescent, the toxin can be detected by scanning peanuts with a fluorometer. The work also led to the establishment of a graduate toxicology program at MIT.

In the mid-1970s, Thilly and Ronald Hites—an Indiana University professor who chairs the CEHS Advisory Committee—team up to identify the chemicals coming out of fossil fuel combustion systems that were capable of causing genetic changes in human cells. At the time, most people believed that the compound benzo[a]pyrene was largely responsible for the increased incidence of lung cancer in urban areas. However, Thilly and Hites learned that other compounds, cyclopenta[cd]pyrene and fluoranthene, were even more important mutagens because of their higher concentrations in combustion products. Work with combustion engineers revealed that adjusting home oil burners to eliminate visible smoke had no effect on the total amount of mutagens released.

This research program in combustion, which brought mechanical engineers, analytical chemists, and toxicologists together in a joint mission, led to the establishment in 1977 of an NIEHS Environmental Health Sciences Center at MIT which unofficially came to be known as the "Soot Center." Wogan was named director and Thilly became responsible for the center’s engineering/toxicology collaborations.

In the mid-1980s, with programs underway in food toxicology and air pollution, the center’s leaders decided to launch a new program in water quality. CEHS projects would then cover the three main avenues—food, air, and water—by which exogenous chemicals enter the human body. In 1985, Thilly took over the director’s post and began work with MIT geochemist Harold Hemond to develop CEHS’s contribution to the NIEHS Superfund Basic Research Program. The Aberjona Watershed Study, directed by Hemond, was initiated in 1987 to find out which chemicals are entering the waters of the Aberjona Basin, where these chemicals are moving to, and the threat they pose to humans. The watershed lies within 10 miles of MIT and is home to about 70,000 people. The project is a large undertaking, involving the cooperative effort of geologists, hydrologists, chemists, civil engineers, air pollution specialists, and toxicologists.

"These big, interdisciplinary ventures don’t come together overnight," says Thilly, who has been building the center’s programs since the mid-1970s. "It takes several years of working together before scientists and engineers with such diverse backgrounds can develop a common language that enables them to understand each other." The CEHS is still trying to expand its reach, with ongoing attempts to bring materials scientists, aeronautical engineers, and other specialists into the fold. "We’re especially looking to attract more young people," Thilly says. "The ‘heavy hitters’ in this field are starting to get gray."

Structure

The CEHS has an annual research budget of about $8.6 million, 95% from the National Institutes of Health (about 83% of which comes from NIEHS). The Department of Energy contributes the remaining 5%. The work is divided into four areas—air quality, water quality, analytical chemistry, and genetics and toxicology—which are in almost constant collaboration.

The airborne toxins program serves as a good example of this multidisciplinary approach. Combustion engineers study engines and emissions. Atmospheric chemists sample emissions at the source and then see what happens in the air as chemicals react with water, oxygen, and sunlight. Analytic chemists conduct further studies in the lab to identify compounds that enter the human body through food, water, or air. Toxicologists then try to determine what kind
of damage may result from the exposure. “Everything is tightly integrated,” Thilly notes.

The CEHS has three core laboratories. The Analytical Chemistry Laboratory, directed by Arthur Laflour, uses sophisticated instrumentation to analyze the complex chemical mixtures contained in air, water, and soil samples. A group located in the MIT Nuclear Reactor Laboratory, headed by Ilhan Olmez, performs neutron activation analysis to identify metals and trace elements found in environmental samples. The Genetic Toxicology Laboratory, led by John Hanekamp, coordinates collection of surgical discard specimens through a variety of clinical collaborators. Human cell mutation assays are performed by Gentest, a private company founded by graduates of the MIT program, which specializes in genetically engineering human cell lines to express drug metabolizing enzymes found in human tissues.

Air Quality
Adel Sarofim, an MIT professor of chemical engineering, heads the Air Quality Program. The research covers three basic fronts: mechanisms by which organic and inorganic pollutants reach the atmosphere; processes which transform these chemicals in the atmosphere; and the means by which the resulting chemical mixtures are deposited on the lungs. Much of Sarofim’s effort has been devoted to a study of combustion-generated aerosols, including polycyclic aromatic hydrocarbons [PAHs]. Among other questions, he and his colleagues are trying to find out which size particles pose the greatest health hazard.

John Durant, a civil and environmental engineer at Tufts University who collaborates with the CEHS, got involved in air quality research as a result of his prior work on the Aberjona Basin. In 1992, Durant discovered that the majority of mutagens in the watershed come from air pollution, not from direct discharges into the water. This finding contradicted the assumption that all of the chemical contaminants in the region were mainly of industrial origin, dumped into nearby streams over the course of many decades.

One class of airborne chemicals, PAHs, stand out in exhausts from various combustion systems, according to Durant, as “the most ubiquitous, abundant, and potent [in human cell mutation assays].” Durant has worked with Professor Glen Cass of CalTech to set up an air monitoring program at the Aberjona watershed and at other sites in Massachusetts, Rochester, New York, and the Los Angeles, California area. Using bioassay directed chemical analysis, the researchers have found a wide variety of highly mutagenic PAH derivatives are apparently created in atmospheric transport.

Water Quality
The Water Quality Program, led by Harold Hemond, draws on hydrology, geology, and microbiology to study the fate of chemicals introduced into ground and surface waters, especially from hazardous waste sites. The Aberjona Basin offers a case study for determining which chemicals, if any, in the region have adverse effects on human health, as well as the environment. A broad range of research programs are underway to explore these questions.

Although Hemond and colleagues have shown that metals such as chromates and arsenites continue to move into the surface waters of the aquifer from a local Superfund site, a major study using neutron activation analysis of hair samples from children living on the watershed showed that the water supply did not significantly contribute to the presence of metals in children.

Another project is investigating the fate of arsenic, chromium, and mercury in the lakes of the watershed. “The concentrations of these metals in lakes and rivers fluctuate during the course of the year, and we’re trying to understand the processes that bring these materials up from the sediments,” Hemond explains. To date, researchers have identified three different strains of bacteria which affect the mobility and solubility of arsenic in the Aberjona Basin. One organism converts arsenic to a more toxic and soluble form called arsenite. Another organism converts arsenic to a relatively immobile form that binds to soils on the bottom and could potentially be utilized for bioremediation purposes.

Another study is trying to ascertain pathways by which contaminants in ground water can infiltrate municipal wells. The MIT team has developed a new tool called a “piezocene penetrometer” to probe soils and wetlands in search of conduits to the wells. In two municipal wells in the Aberjona Basin, the team has found layers of sand that could have provided access between the river and the wells, and is performing hydraulic tests on these layers to
determine if they were the actual pathways of water flow when the wells were in use.

Yet another project is examining volatile organic compounds [VOCs], chemical solvents which, according to Hemond, “are widespread in this watershed and virtually every industrialized watershed.” MIT investigators are using a newly devised “dual trace technique” for measuring the amount of solvents coming into a given stretch of stream. “This measurement was not possible in the past,” Hemond says. “This technique provides an estimate of where ground water contaminants are, how fast they are leaving through discharge to streams and rivers, and how long it will take for ground and surface waters to clean themselves up. We can determine whether it’s a one-year problem, a multi-year problem, or a century-long problem.”

Analytical Chemistry
The Analytical Chemistry Laboratory is a central resource, acting as a point of connection between several diverse projects within the CEHS. Considerable effort has been devoted toward characterizing the chemicals emanating from Superfund cleanup sites in the Aberjona Basin. From its beginning, the lab has focused on the wide variety of PAH compounds and their derivatives which are discharged to the environment by combustion systems.

“Isolating the mutagens in air samples is an involved process,” explains Lefleur. First, particulates are placed in an organic solvent. “These particulates are coated with everything—protein, pollen, and dust—but only a small percentage of the stuff consists of active compounds,” he says. The sample is fractionated, or split into many parts, and then tested for mutagenicity. Fractions which show no signs of mutagenic activity are discarded; active samples are split and tested again. This process, known as bioassay directed chemical analysis, was pioneered by Hites and Thilly.

After compounds are shown to be mutagenic by tests on human cells, two complementary analytic methods are used to identify them—GS-MS [gas chromatography-mass spectrometry] and GC-FTIR [gas chromatography-fourier transform infrared spectrometry], the latter reserved for bigger molecules. About 1000 different compounds have been tested, some of which have led to the discovery of chemicals that significantly contribute to the mutagenicity of air mixtures. According to Lefleur, most of the mutagens identified so far are PAHs or other aerosol derivatives.

“We’ve identified the important chemicals that are produced by fossil fuel burning,” he says. “Now we’re considering the next steps: what happens to this stuff in the air, how does it get inside people, and what happens there?”

Genetics and Toxicology
Toxicology research draws on basic studies of genetic changes in bacteria, yeasts, human cell cultures, and rodents while moving toward the ultimate goal of directly observing genetic changes in humans. Organic chemist John Wishnok has shown that nitric oxide (NO), a compound involved in many normal physiological functions, may become a dangerous human toxin under some circumstances. Recent data suggest that NO can damage DNA when the body produces it in quantities associated with inflammation. “In test tube reactions and experiments with live human cells, we’ve identified at least two mechanisms by which NO damages DNA,” Wishnok says. “There is some evidence of this occurring in lab animals. Now we’re trying to develop methods at the spectroscopy lab to show that this is actually happening in people.”

Senior research scientist Paul Skipper is part of a group investigating the link between PAHs and lung cancer. In particular, they are looking at adducts—new chemical structures formed between a carcinogen and biomolecules—created when a PAH molecule lodges in the lungs. Adducts in DNA, according to the conventional wisdom, lead to mutations which may result in cancer. Adducts formed by benzo[a]pyrene can be detected in these samples by laser-induced fluorescence, a technique developed at MIT.

Meanwhile, Thilly and his colleagues have devised a new technique called mutational spectroscopy that can identify DNA alterations inside human organs. The method is now sensitive enough to detect one point mutation (a change in a single DNA base) in one million cells. A ten-fold increase in sensitivity is still needed to study genes that are mutated to produce human cancers, Thilly says, but he is encouraged by the fact that the approach is already a thousand times better than it was in 1993. The center’s program in Genetics and Toxicology links MIT toxicologists with molecular genetics faculty to study the pathways of spontaneous mutation. In 1996, Steven R. Tannenbaum will succeed Wogan as head of the Toxicology Division at MIT.

Bringing It All Together
The various programs of the CEHS complement each other considerably. For example, studies of pond sediments reveal that some of the most important contaminants originate as air pollutants. Efforts to control air emissions, conversely, can have a critical bearing on water quality. The crossover between these different fields may be the most important legacy of the CEHS—setting up a mechanism whereby the collective talents of experts in a variety of disciplines can be brought to bear on complex problems which had previously been intractable. “Before the CEHS was formed, the people who monitored smokestack emissions, the people who analyzed water samples, the people who studied exposures to toxic chemicals, and the people who investigated the theory of cancer had, for the most part, all worked separately,” Thilly notes. “That’s what this center is all about—brought these disciplines together.”

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