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ENVIRONMENTAL HEALTH PERSPECTIVES

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A Not-So-Sweet
Sweetener?

Bans, Bans, Good
for the Heart!



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STUDENT EDITION

In a purely technical sense, each species of higher organism is richer in information than a Caravaggio painting, Bach fugue, or any other great work of art.
Edward O. Wilson (1985)

POLICY

New Environment Law for Afghanistan

In April 2005, Afghan president Hamid Karzai established the National Environmental Protection Agency (NEPA), the country's first such entity. The event was strictly ceremonial, since Afghanistan still had no legal tool for environmental management. Eight months later, however, on 18 December 2005, the Afghan cabinet approved legislation that for the first time gives Afghanistan the legal power it needs to begin bettering its environment.

Known as the Environment Act, the law clarifies administrative roles at the national level and coordination with provincial authorities. It spells out frameworks for managing natural resource conservation and biodiversity, drinking water, pollution control, and environmental education. Equally as important, say its supporters, the law provides tools for enforcement.

"It seems to be a pretty sensible act," says David Hanrahan, lead environmental specialist for South Asia at the World Bank. For example, the law's environmental impact assessment process was guided by a review of

10 countries' experience and vetted by environmental law experts at the World Bank, the World Conservation Union, and the UN, as well as by Afghan groups.

NEPA proposed the legislation based on recommendations issued in 2003 by a team of experts from the UN Environment Programme (UNEP). The UNEP findings were alarming: after two decades of conflict and drought, Afghanistan had lost nearly all of its wetlands and much of its forests, and its citizens were increasingly at risk for infections and epidemics caused by poor waste management and unequal access to fresh water. [For more on the UNEP findings, see "Environmental Triage in Afghanistan," *EHP* 111:A470–A473 (2003).]

For two years, Asif Ali Zaidi, UNEP's program manager in Afghanistan, has worked to help the government respond. UNEP facilitated consultations on the draft legislation with various agencies, citizen groups, and international officials, and funded translations of the draft law into Dari and Pashto, the country's official languages. Besides NEPA (which emerged from the former Ministry of Irrigation, Water Resources, and Environment), other agencies with key roles under the new law include the Ministry of Justice and the Ministry of Agriculture, Animal Husbandry, and Food.

In Afghanistan, more than 80% of the population relies directly on natural resources such as rangelands and water bodies for their livelihood and daily needs, and only 12% of the land is arable. Thus, widespread environmental degradation poses a threat to livelihoods and places the poorest Afghans at particular risk, wrote Zaidi and Belinda Bowling, UNEP's environmental law expert in Afghanistan, in the Fall 2005 issue of *Sustainable Development Law & Policy*.

Zaidi and Bowling linked environmental issues directly with Afghans' top concern, security, observing that environmental degradation in Afghanistan—often the consequence of socioeconomic inequities—is a factor contributing to prevalent insecurity. In one example, desperate subsistence farmers displaced by desertification and crop failure are more likely to cultivate poppies as part of the drug trade, which is intrinsically linked to insecurity in Afghanistan.

Protecting natural resources in a country lacking basic infrastructure has posed a serious challenge. The National Development Framework, a 2002 map for Afghanistan's economic development, did not mention the environment, although Zaidi says it was understood as "an important cross-cutting issue." In 2004, another approach emerged in the Afghanistan National Development Strategy, an Afghan-specific version of the UN Millennium Development Goals couched as a five-year plan. These overlapping schemes have caused confusion among planners, compounded by a lack of baseline data on forest cover, energy use, and other indicators.

According to Bowling, the new law will help spur institutional reform and the development of regimens for pollution control and environmental impact assessments, among other things. "Like most fledgling institutions," she says, "NEPA now requires time to establish itself properly within the new government structure." UNEP plans to support much of the reform through its Post-Conflict Branch.

Hanrahan is cautiously optimistic about the recent developments. Most Afghans, he says, care about their environment. He notes the people have a history of cooperative practices, for example in ancient irrigation systems, adding, "This is where civilization comes from." —David A. Taylor



Thirsting for reform. The extinct Hamoun wetlands (top right in 1976, while still thriving; bottom right in 2001, as a desiccated salt flat) and Kole Hashmat Khan wetlands (above) are just two illustrations of how years of drought and conflict have stripped Afghanistan of its natural resources. A new agency and guiding law offer hope that life can be breathed back into Afghanistan's environment.

Left: UNEP, right: H. Partow/UNEP

ASTHMA

Bowled Over by Dust

A common substance in house dust is a major risk factor for asthma, according to work reported in the 1 December 2005 issue of the *American Journal of Respiratory and Critical Care Medicine*. The study charted room-by-room distribution of endotoxin, a lipid-like material that comes from the surface of bacteria, in the first nationwide snapshot of exposure in American homes. Then researchers looked at the relationship between household endotoxin exposure and the presence of allergic symptoms (such as hay fever) and asthma.

The field sampling showed that endotoxin exposure “isn’t something that’s only limited to inner-city homes or to homes that are dirty or to homes in certain parts of the country,” says coauthor Darryl Zeldin, a senior investigator in the NIEHS Laboratory of Respiratory Biology. “Almost all the homes in the nation have detectable levels of endotoxin in multiple locations.”

The study used data from the U.S. National Survey of Lead and Allergens in Housing, which ran from July 1998 through August 1999. In this survey, field personnel collected dust samples (and later analyzed their composition), recorded demographic and health data, and conducted visual inspections of a nationwide representative sample of 831 dwellings. Zeldin and colleagues were then able to analyze the health impacts of individual dust components from sources including not only bacteria, but also dogs, cats, rodents, cockroaches, dust mites, and fungi.

Although endotoxin was found in all of the homes studied, concentrations varied considerably from home to home and from room to room within a given home. Concentrations were on average highest on kitchen floors, but it was the bedroom exposure that was most highly associated with residents having doctor-diagnosed asthma, experiencing asthma symptoms such as wheezing, or using asthma medications.



Nothing to sneeze at. Endotoxin, commonly found in household dust, is a ubiquitous risk factor for asthma.

Zeldin says, “Endotoxin exists on [dust] particles that can go airborne with activity—vacuuming, sleeping on their bed or with their face in the bedding, kids playing on their beds or on the floor.” The asthma–endotoxin link persisted at all exposure levels, with symptoms more likely with higher exposure.

The relationship of asthma to endotoxin was strong for adults in the study, but not for children. However, the authors say the original survey was not designed to collect information on when or how long people had been exposed, nor did it have sufficient statistical power to examine these relationships in young children.

So far, other work in children has mostly led to more questions. The answers may lie in inflammation of the airways rather than activation of allergy’s histamine responses. “What is well understood is that endotoxin exposure worsens airway inflammation and symptoms in people with asthma,” says Andy Liu, an asthma researcher at the National Jewish Medical and Research Center in Denver. “This investigation . . . supports endotoxin’s relationship with asthma as a toxic one, and is consistent with what has been reported with infant wheezing. What is perplexing is that, in older children, the association of allergy-associated asthma with endotoxin becomes a negative one: more exposure, less disease.”

That kind of observation forms the root of the hygiene hypothesis—that exposure to infections in early life helps build an immune system that is less prone to allergic diseases in later life. But does an exposure–allergy relationship tie in with asthma?

“The question with the hygiene hypothesis is, can we extend it from ‘infection’ to endotoxin—which comes from bacteria—and can we extend it from allergy to asthma?” says lead author Peter Thorne, director of the Environmental Health Sciences Research Center at the University of Iowa. The current study suggests not, he says: “What we found is that endotoxin is causing symptoms of asthma in both those who are allergic and those who are not. So endotoxin exposure is not protecting people from asthma.”

—Victoria McGovern

Supersized Sun Power

At the end of 2004, the United States had 397 megawatts of solar energy capacity. Now two Southern California utility companies plan to harness the state’s abundant sunshine for two solar-powered plants that will produce more electricity than all of those solar energy projects combined. The new plants will use 40-foot dishes to focus the sun’s energy onto Stirling engines, sealed systems filled with hydrogen that, when heated with the solar energy, drive four pistons. A 500-megawatt power plant of 20,000 dishes will be located in the Mojave Desert, while a 300-megawatt plant of 12,000 dishes will call the Imperial Valley home. Construction will begin on the sites in 2008.



Green Buses for Beijing Olympics

Beijing has signed a contract to replace 7,277 older city buses with new ones that meet higher environmental standards as part of its plan to host a “green” Olympic Games in 2008. The November 2005 decision is also in line with the city’s effort to combat heavy air pollution, which, due to temperature inversions, hangs in a cloud over the metropolis for three months of the year. Currently 17,507 buses operate in Beijing, and 4.36 billion person-rides were reported in 2004, making bus travel the city’s most popular mode of transport. The new buses will meet stringent Euro III standards for carbon monoxide, smoke, particulate matter, nitrogen oxide, and hydrocarbon emissions.

West Africa Adopts Fishing Plan

Fish currently provides nearly a quarter of the protein in the African diet, yet sub-Saharan Africa is the only region in which the per-capita availability of fish is declining. To help ensure the security of this food source, government officials meeting at the August 2005 Secretariat of the Economic Community of West African States endorsed the Abuja Declaration on Sustainable Fisheries and Aquaculture in Africa. The declaration includes a five-point action plan to support capture fisheries, develop aquaculture, improve fish market chains, increase the benefits from the fish trade, and support government decision makers with information. Speaking at the meeting, Nigerian president Olusegun Obasanjo pointed out that for Africa’s fish consumption to remain at its already-low present level, fish production must increase by more than 250% by the year 2015.



ECONOMICS

The Clear Advantage of Clean Air

Regulations addressing environmental problems such as air pollution might be dismissed as a luxury for rich countries that can afford it, but members of the UN Environment Programme (UNEP) insist that such measures actually represent the most effective means of lifting countries out of poverty. At a major gathering of environment ministers in the United Arab Emirates in February 2006, UNEP executive director Klaus Töpfer cited the increasing cost and demand for fossil fuels as key reasons why many rapidly developing countries were taking a closer look at environmental degradation, which can have profound consequences. “That is now the bottleneck to future economic development,” he said.

The extent of the economic implications of this bottleneck is being outlined by researchers at the Massachusetts Institute of Technology (MIT), who have expanded a well-established economic model to put some numbers to the economic disadvantages created by unregulated air pollution. Those numbers are quite big, as it turns out. In a July 2004 report titled *Economic Benefits of Air Pollution Regulation in the USA: An Integrated Approach*, they calculated that the United States enjoyed an additional \$5.4 trillion in market consumption between 1975 and 2000 that would not have been available without the implementation of air pollution controls.

For coauthor John Reilly, associate director of research with MIT’s Joint Program on the Science and Policy of Global Change and the Laboratory for Energy and the Environment, that amount dwarfs the less than \$1 trillion that the EPA estimates the controls cost. More importantly, this financial benefit contrasts sharply with the

widespread health effects of leaving remaining air pollution unregulated. “It’s causing your workers to be ill, and your children not to be able to advance,” he argues. “It’s going to slow the economy.”

Using the Emissions Prediction and Policy Analysis (EPPA) model, which has already been used to estimate the costs of reducing carbon dioxide emissions, Reilly and his colleagues incorporated measures of human health in order to consider the benefits. Rather than employing only basic multipliers for illness, lost lives, or medical expenditures, the algorithms were broadened with published health data on the occurrence of specific diseases that could be linked to air pollution, such as asthma.

By including pollutant exposures, ability to work, and health effects on different age groups over time, the model compared actual economic performance with what would have happened in the absence of regulations. The tally wound up representing 3–4% of the entire U.S. market consumption over a quarter of a century. By comparison, the entire agricultural sector accounts for just 1%.

That result should grab the attention of observers in developed as well as developing countries, says Kristie Ebi, an epidemiologist with the scientific consultancy Exponent Health Group. “Health is often not at the table,” she says, referring to the negotiation of policies that treat the physical well-being of people as a separate priority incurring expenses that are not considered part of the general economy. “Many calculations of the costs and benefits of regulations include limited consideration of health because health is not traded in a market and so is hard to value.”

For Ebi, the expanded EPPA model provides all the more reason to make health an integral part of such negotiations and look closely at the potential advantages conferred by environmental regulations. “There’s very little work out there that you can take to policy makers and say ‘if you don’t regulate, this is the cost,’” she concludes. “It’s really nice to see that taken up in a rigorous way.” —**Tim Lougheed**

SMOKING

Bans, Bans, Good for the Heart!

Could moving to Pueblo, Colorado, be a new way to reduce the risk of suffering a heart attack? A study reported at the American Heart Association’s Scientific Sessions 2005 Conference in Dallas last November suggests that the city’s ban on smoking in all public buildings helped reduce the local heart attack rate by 27% over a year and a half. The finding appears to bolster recent decisions to ban smoking in workplaces and other public buildings across the United States, Spain, Ireland, and parts of the United Kingdom.

Tobacco smoke—including secondhand smoke—activates blood coagulation pathways that could lead to coronary thrombosis, or heart attack. Smoke is particularly dangerous for people whose arteries are already hardened by age, cholesterol deposits, or smoking itself. Indeed, some 30% of heart attacks are thought directly related to smoke-induced

thrombosis. In the United States, the annual direct medical cost of coronary heart disease caused by secondhand smoke alone is some \$2.45 billion, according to the U.S. Society of Actuaries.

In an attempt to reduce the incidence of heart attacks and other smoking-related illnesses, Pueblo introduced a ban 1 July 2003 on smoking in all indoor public areas within the city limits. Eighteen months later the Pueblo Public Health Department instigated an observational study to determine whether the ban had had any effect on the incidence of coronary events.

“We recorded the number of heart attacks that occurred in the city for the eighteen months before and after the introduction of the ban,” explains Mori Krantz, director of the Prevention Department of the Colorado Prevention Center in Denver, “and found a post-introduction fall of around twenty-seven percent. This was significantly greater than the small reduction we saw among the population living outside the city limits, and much greater than the virtually unchanged rate among residents of [adjacent] El Paso County, which does not have such an ordinance.”

Similar findings were reported in an earlier study published 24 April 2004 in *BMJ* after a smoking ban was imposed in Helena, Montana. “Our study builds on this work by involving a sample three times as large, and suggests that these ordinances may be having a positive effect on cardiac health,” says Krantz.

“This observational study is limited in that it does not distinguish between smokers and nonsmokers, and did not check the medical records of those involved,” remarks Jose Ramón Banegas, a professor of preventive medicine and public health at the Universidad Autónoma in Madrid, Spain. He adds, however, that “even if the effect were half that reported, it would mean this type of ban could save many lives while reducing health spending.”

Banegas points out that the results are also relevant given the new Spanish ban, enacted 1 January 2006, which still allows restaurateurs to declare their locales either smoking or nonsmoking. “Unfortunately,” he says, “ninety-eight percent are scared of losing income by going smoke-free.”

—**Adrian Burton**

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Wellcome Trust Sanger Institute

The Cambridge, United Kingdom-based Wellcome Trust Sanger Institute (WTSI) was founded in 1993 to provide a center for research on the genomes of humans and other organisms. The institute has been a leading contributor to the Human Genome Project and now focuses much of its work on studying human DNA sequence variations and how these correlate with genetic diseases. The institute has developed a website located at <http://www.sanger.ac.uk/> to serve as a central repository for information on its varied areas of study.

The densely packed website offers a wealth of scientific resources related to its research on human genetics, model organisms, pathogens, bioinformatics, sequencing, and proteomics. Visitors also have several points of access to databases including Ensembl, COSMIC, Pfam, GeneDB, Wormbase, Vega, MEROPS, and DECIPHER.



Clicking on any of the topic headers in the center of the homepage leads to an assemblage of resources,

project information, and laboratory homepage links related to the WTSI's six main areas of study. Clicking on Human Genetics, for example, takes visitors to a lengthy menu of team links, each of which leads to information on what that team is working on, along with relevant references and illustrations. The Model Organisms link yields resources categorized by organism: mouse, zebrafish, *Caenorhabditis elegans*, *Schizosaccharomyces pombe*, and *Xenopus tropicalis*. The Pathogens link leads to organism-specific sequencing information for more than 100 bacteria, fungi, protozoa, helminths, vectors, and plasmids, as well as a variety of computational tools. The Bioinformatics header leads to links for downloading a number of programs, including packages for production sequencing, physical mapping, informatics analysis, and special data descriptions and specifications used at the WTSI. The Sequencing link pulls together in one area those resources specific to sequencing of various organisms. And the Proteomics link offers information on three groups at the institute that are working in this field of study.

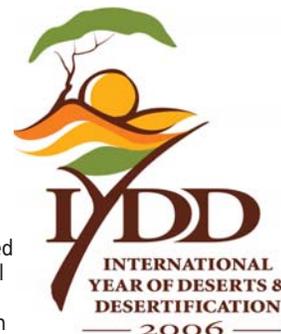
Visitors can also navigate through a bar down the left-hand side of the homepage, which offers quick links to biological resources, the database resources mentioned above, and news releases from the institute. Biological resources listed here include the Mutagenic Insertion and Chromosome Engineering Resource and the Sanger Institute Gene Trap Resource (both of which aid in developing genetically modified animal models), the WTSI Microarray Facility (which produces arrays for a range of organisms largely for use by Sanger researchers), and Clone Ordering (which allows external scientists to order mouse and human clone cells free of charge).

Delving deeper into the site through the Site Map, visitors will find a page on Functional Genomics projects including the Human Epigenome Project (HEP), a public-private consortium of the WTSI, Epigenomics AG, and France's Centre National de Génotypage. The goal of the HEP is to locate, identify, and catalog methylation variable positions within the human genome. The partners have conducted a pilot study of the methylation patterns within the major histocompatibility complex, a region of chromosome 6 that is associated with more diseases than any other region of the human genome. An overview of this pilot study is available within the HEP section, along with a look at the data analysis process employed by this study, information on epigenotyping, and links to the HEP partners. —Erin E. Dooley

Top to bottom: UN; WTSI; Corbis

International Year of Deserts

Arid land covers one-third of the Earth's surface. Each day, more arable land is lost to advancing deserts and more people fall victim to drought. In recognition of these problems, the UN has declared 2006 the International Year of Deserts and Desertification. Planned activities, including a week-long film festival, will explore ways to protect the biological diversity of areas affected by desertification as well as the knowledge and traditions of the 2 billion people living in those areas. The UN Convention to Combat Desertification reports that desertification and drought shave \$42 billion off agricultural production each year. The resulting food insecurity, famine, and poverty foster social, economic, and political tensions that perpetuate the cycle of degradation.



Mercury Warnings Go Multilingual

In November 2005, the San Francisco Board of Supervisors unanimously passed an ordinance requiring that markets and restaurants post warnings in English, Chinese, and Spanish that certain fish may contain harmful levels of mercury. The measure is authorized under California's Proposition 65, which requires that consumers be warned of toxicants in the products they buy; it is the first to mandate multilingual warnings. Mercury is a potent neurotoxicant, and fetuses and young children are particularly sensitive to its effects. Studies have demonstrated that low-level exposures are linked with small changes in learning and intelligence.

Detoxifying Dust Bunnies

Nearly one-fourth of the housing units in the United States have significant levels of lead present in dust, soil, or paint. Now researchers at the Saint Louis University School of Public Health report in the 15 January 2006 issue of *Environmental Science & Technology* that all-purpose floor and counter detergents remove lead-bearing dust from wood, wallpaper, and vinyl flooring as effectively as detergents developed specifically for removing lead. These findings contradict earlier recommendations that only lead-specific cleaners or high-phosphate detergents are effective for this purpose. According to lead author Roger Lewis, these findings will be incorporated into new HUD guidelines to be released in 2006.



Moving On in Vietnam

War and unrest mark the history of Vietnam. For hundreds of years, Vietnam has fought a number of foreign invaders including the French, Japanese, and Chinese. The U.S.–Vietnam War was just one of several chapters in this history, ending in 1973 with the Paris Peace Accords. The American War, as it is referred to in Vietnam, has been estimated to have resulted in the deaths of 1.5 million Vietnamese and 58,000 Americans. The countryside is still dotted by unexploded mines, and questions remain about environmental health concerns such as the lasting effects of dioxin-containing herbicides used during the war.

But as a bumper sticker spotted on a Hanoi street pointed out, “Vietnam is a country, not a war,” and today many of Vietnam’s most pressing concerns are related to post-war growth. About half of Vietnam’s current population of 80 million people is under 30 years old. This young generation, many of whom have a limited recollection of wartime, drives a sense of optimism about the future of the country. Foreign investment in this country for 2005 is estimated at US\$5.4 billion, only a little less than investment in India, and Vietnam is expected to enter the World Trade Organization in mid-2006.



State of flux. Billboards towering over slums along the banks of the Saigon River demonstrate how Vietnam’s infrastructure struggles to keep up with the nation’s rapid industrialization.

This growth makes Vietnam a duality of a country, on the one hand facing the environmental problems typical of the rural countryside (such as those related to poor sanitation), and on the other facing the occupational and environmental health consequences of rapid industrialization.

This was the setting of the joint Second International Scientific Conference on Occupational and Environmental Health and Sixth National Scientific Conference on Occupational Health, held in Hanoi on 16–18 November 2005. The conference gathered 176 participants with 194 abstract presentations. Most of the participants were from Vietnam; 44 were from abroad, mostly from other Asian countries. The conference was organized by the Vietnam Association of Occupational Health and the University of Washington, with the sponsorship of numerous Vietnamese, international, and U.S. agencies, including the NIEHS, the National Institute for Occupational Safety and Health (NIOSH), and the John E. Fogarty International Center of the NIH.

Early in the conference NIEHS director David Schwartz and Anne Sassaman, director of the NIEHS Division of Extramural Research and Training, presented the vision of the NIEHS for expanding global health as part of its five-year strategic plan and bringing the institute’s expertise to bear on the problems facing this growing country. “NIEHS-supported research has shown that air pollution, which is a big problem in Vietnam,

can impact cardiovascular health and increase susceptibility to respiratory infections,” said Sassaman. “These and other environmental health concerns are a growing problem in the developing world.”

Powering Up

The United States first established diplomatic relations with Vietnam in 1995, and a bilateral trade agreement signed by the two countries in 2000 opened the door to increasing economic and industrial development in Vietnam. Economic agreements have continued to strengthen with President Bush’s plan to participate in the Asia-Pacific Economic Cooperation Summit to be hosted later this year in Hanoi. In addition to a new wave of U.S. investors, Taiwanese and Japanese business ventures are eager to tap Vietnam’s wealth of cheap, relatively well-educated workers, who are willing to work six days per week, as opposed to the Chinese, who adhere to a five-day work week. Workers in traditional trade villages earn US\$60 per month, sometimes less, as the national minimum wage is US\$38 per month. (By comparison, the average rate in China is US\$90 per month.) Because of these low wages and the relatively new implementation of occupational health standards, Vietnam may be poised to compete with China in the manufacture of goods for export, further expanding environmental and occupational health concerns.

At this point, many multinational companies are adopting a “China plus one” strategy where they continue to support their main manufacturing centers in China and develop subsidiary ventures in



Families at risk. In traditional trade villages, manufacturing enterprises (like this brickyard in Hue Province) and attendant exposures are often located near or in villagers’ homes.

Top to bottom: Chris Stowers/Panos Pictures; Mark Henley/Panos Pictures

another country, increasingly Vietnam. This trend is exemplified in the industrial park located near Hanoi's international airport. Opened in 2000, the facility already houses 46 factories with more than 16,000 Vietnamese employees. If the Vietnamese government continues to liberalize the economy and improve its infrastructure, the country may significantly increase its share of the global market.

"These investments should include protections for workers in the cities as well as in the rural areas," said Bui Thanh Tam, who cochaired the session on environmental health and industrial hygiene with conference co-organizer Matthew Keifer, director of the International Scholars in Environmental and Occupational Health program at the University of Washington. With this rapidly expanding industrialization of Vietnam, environmental and occupational health protections and improved scientific capacity in these areas will become increasingly important.

Health for Workers

The conference therefore focused heavily on occupational health. The establishment of safety codes and means for enforcing occupational and environmental health standards that protect workers and communities was a concern that resonated throughout the conference. A study presented by Ta Quang Buu of the Hai Phong Preventive Medicine Center showed that only 3% of enterprises in the region implemented guidelines for injury prevention. The average number of workdays missed due to occupational injuries was 14.5 per person between the years 2000 and 2004, with 70% of these injuries attributable to violations of occupational safety rules.

These data will eventually form the basis of a workplace health promotion program for workers and employers.

However, it is likely that even these figures are underreported. Another presentation described a collaboration between investigators of the Vietnamese National Institute of Occupational and Environmental Health, the Liberty Mutual Research Institute for Safety in the United States, and the University of Massachusetts Lowell that aims to develop a comprehensive injury reporting system in Vietnam. This project will provide information about the scale of the burden of work injuries in the country.

Other presenters described working conditions and the health of workers in the chemical industry. Authors Nguyen The Cong and colleagues at the National Institute of Labour Protection studied data obtained from 24,482 annual medical checkups of workers in this industry. They showed that the most prevalent diseases in this sector were acute ophthalmologic diseases, followed by respiratory and allergic conditions (from 26.8% to 75.1%). There were also findings of silicosis, lead poisoning, and hearing loss among these workers, and the health status of as many as 15.9% of workers was categorized as "unacceptable." The presenters added that, as Vietnam becomes home to more technical types of ventures that require increased use of industrial chemicals and heavy machinery, these kinds of occupational injuries can be expected to increase

unless worker protection guidelines are implemented and enforced.

Much of the manufacturing activity is currently concentrated in traditional trade villages, where families work together to produce one specific commodity, such as pottery, silk garments, furniture, or paper. "Low cost is both a strength and a weakness of this manufacturing enterprise," said Nguyen Duc Hung of the Institute of Labour Science and Social Affairs in his presentation. In an investigation of six traditional trade villages, Hung found that most of the manufacturing enterprises were located within or in close proximity to the villagers' living quarters. Most of these settings lacked proper ventilation, chemical disposals, or other protections. Levels of respiratory toxics such as carbon monoxide, sulfur dioxide, and nitrogen dioxide were found to be high in these homes. Hung suggested that labor inspections and regulations that are applied to industrial sites should be expanded to traditional trade villages, where occupational exposures can affect whole families, including children.

A Need for Sanitation

Despite advances in industrialization, rural and some suburban areas of Vietnam still



Meeting demand. Improved occupational health standards and safety codes are essential for Vietnam, a country poised to become one of the world's great manufacturing centers.



face a lack of basic sanitation, which affects the availability of safe potable water and causes other environmental health problems. Two studies presented by Le Thi Song Huong and colleagues from the Hai Phong Preventive Medicine Center investigated levels of water contamination with fecal coliforms. Analysis of water samples showed that those with low chlorine residues had high levels of coliforms.

Water sources are likely contaminated by effluent from poorly designed household latrines. These latrines are often located near the house kitchen and have poor drainage. The investigators helped villagers in the An Duong District to build latrines with better drainage and ventilation, located farther from the kitchen, which reduced the contamination of wells and vegetable gardens with fecal microbes. They were able to increase the number of households throughout the area that had these latrines from 19% to 29%. "Rural sanitation is a big concern and a big challenge, and we need implementation of creative new ways to solve these problems," said Huong.

Another study of fecal contamination of drinking water was conducted in the Cu Jut District in Dac Lac Province by Vuong Tuan Anh and colleagues from the National Institute of Hygiene and Epidemiology of Hanoi. The authors showed that a common source of household drinking water contamination occurred when family members dipped their unwashed hands into water vats stored in the home. The authors speculated that the simple use of long-handled ladles to scoop water combined with hand-washing campaigns could significantly reduce contamination.

On the Rise

Vietnam, while undeniably on the rise, has far to go to ensure the environmental and occupational health of its citizens. "After visiting Vietnam," said Schwartz, "it's abundantly clear that . . . we need to build research capacity in environmental health to effectively address the global health disparities caused by environmental exposures."

The emergence of international ventures that is permeating Vietnam may yet tip the balance toward a healthier country. Said Le Van Trung, president of the Vietnam Association of Occupational Health and co-organizer of the conference, "This conference is a useful scientific forum and an opportunity for scientists in the local country, in the region, and over the world to exchange information and to discuss occupational and environmental health issues that need to be resolved in the first years of the new millennium."

—Luz Claudio

BEYOND THE BENCH

Tracking Clues with Environmental Health Sleuths

Who doesn't love a good mystery? Add in detailed, interactive information on the latest environmental health research, and you've got the basic concept behind *Unsolved Mysteries of Human Health: How Scientists Study Toxic Chemicals*, a project developed by the Community Outreach and Education Core (COEC) of the Environmental Health Sciences Center (EHSC) and Marine and Freshwater Biomedical Sciences Center (MFBS) at Oregon State University.

The *Unsolved Mysteries* website (<http://www.unsignedmysteries.oregonstate.edu/>) was launched in 2005 as a supplement to provide information on the analytical instruments and techniques featured in the Hydroville Curriculum Project, a problem-based environmental health science curriculum for high school students [see "Beyond the Bench: Welcome to Hydroville!" *EHP* 112:A166 (2004)]. Since then, the *Unsolved Mysteries* website has grown into an independent, informative teaching tool in its own right. "Unsolved Mysteries offers a great opportunity to highlight research at the EHSC and MFBS at Oregon State while delivering a balance between scientific accuracy and a nontechnical explanation of very complex concepts," says COEC program coordinator Sandra Uesugi.

The core used input from EHSC investigators to develop two modules to date. Each provides information on techniques used in basic chemistry, environmental health research, and toxicology via an "unsolved mystery" based on real research scenarios.

In "The Answers Are Blowing in the Wind," the reader meets Jenny, who hikes to the top of a mountain but finds the view blocked by haze. While atop the hazy mountain, Jenny encounters Robert, an Oregon State graduate student taking air samples. Jenny's experience with unexpected haze at the mountaintop sets the stage for a discussion of semivolatile chemicals, their atmospheric transport across long distances to high elevations, and the use of gas chromatography-mass spectrometry to analyze trace amounts of chemicals in the air. Then students analyze the mass spectrum data from Robert's air samples to solve the puzzle of whether the haze blew in over the water to the west or came from forest fires to the east.

In "Going With the Flow," the reader meets Joe, who plans to catch some fish for dinner. At the fishing spot, he notices a sign warning that the fish in that area may contain elevated levels of dioxin and should be eaten only once a month. Joe wants to learn more about dioxin and its health effects, so the game warden refers him to the Oregon State EHSC. The module tells how the scientists there use flow cytometry to study immune damage. Students then compare mouse histogram data to unravel the puzzle of dioxin's effects on the immune system.

Each module includes a "Meet Real Scientists!" section that introduces the

Applied tools for learning. The *Unsolved Mysteries* modules show students how scientists in the real world use tools like gas chromatography-mass spectrometry (right) to better understand the relationship of toxicants to our health.



investigators behind the research. The website also features virtual tours of each researcher's lab so visitors can see examples of the tools discussed. In addition, there is a detailed glossary of technical terms and a link to additional resources.

Although the content is written for high school students, the information is also appropriate for the general public, and the site has proved to be popular not only with students but also others surfing the web. "Our inquiries have come from many diverse backgrounds including an agriculture professor in Oman teaching an instruments course, a medical school professor giving a lecture on endocrinology, a hematologist at a hospital in Cambridge, United Kingdom, a home-schooled student, and a graduate student considering a future in toxicology research," says Uesugi.

As a testament to its value, Unsolved Mysteries received the Digital Dozen Award from the Eisenhower National Clearinghouse in March 2005, an honor given to exemplary websites for educators that feature current, accurate math and/or science content, that support school improvement efforts, and that have useful multimedia features or helpful navigation. The Oregon State COEC continues to build on the website's accomplishments by keeping the information fresh and engaging. An upcoming Unsolved Mysteries module will highlight the use of microarray techniques, focusing on zebrafish embryos as a model for understanding chemical toxicity and the impact of developmental toxicants on human health. Be ready to solve the next Unsolved Mystery in spring 2006! **—Tanya Tillett**

Top to bottom: Stockbyte; BPA

Headliners

NIEHS-Supported Research

Pesticides



Neurobehavioral Deficits in Children from Agricultural Communities

Rohlman DS, Arcury TA, Quandt SA, Lasarev M, Rothlein J, Travers R, et al. 2005. Neurobehavioral performance in preschool children from agricultural and non-agricultural communities in Oregon and North Carolina. *Neurotoxicology* 26:589–598.

Most research on the neurobehavioral effects of organophosphate (OP) pesticides has focused on adult occupational exposures. However, the developing organ systems of children can be especially sensitive to these chemicals. Now NIEHS-supported scientists Linda McCauley of the University of Pennsylvania, Thomas A. Arcury of Wake Forest University, and Joan Rothlein of Oregon Health & Science University, with their colleagues, report modest differences in neurobehavioral performance between young children from agricultural communities and those from nonagricultural communities.

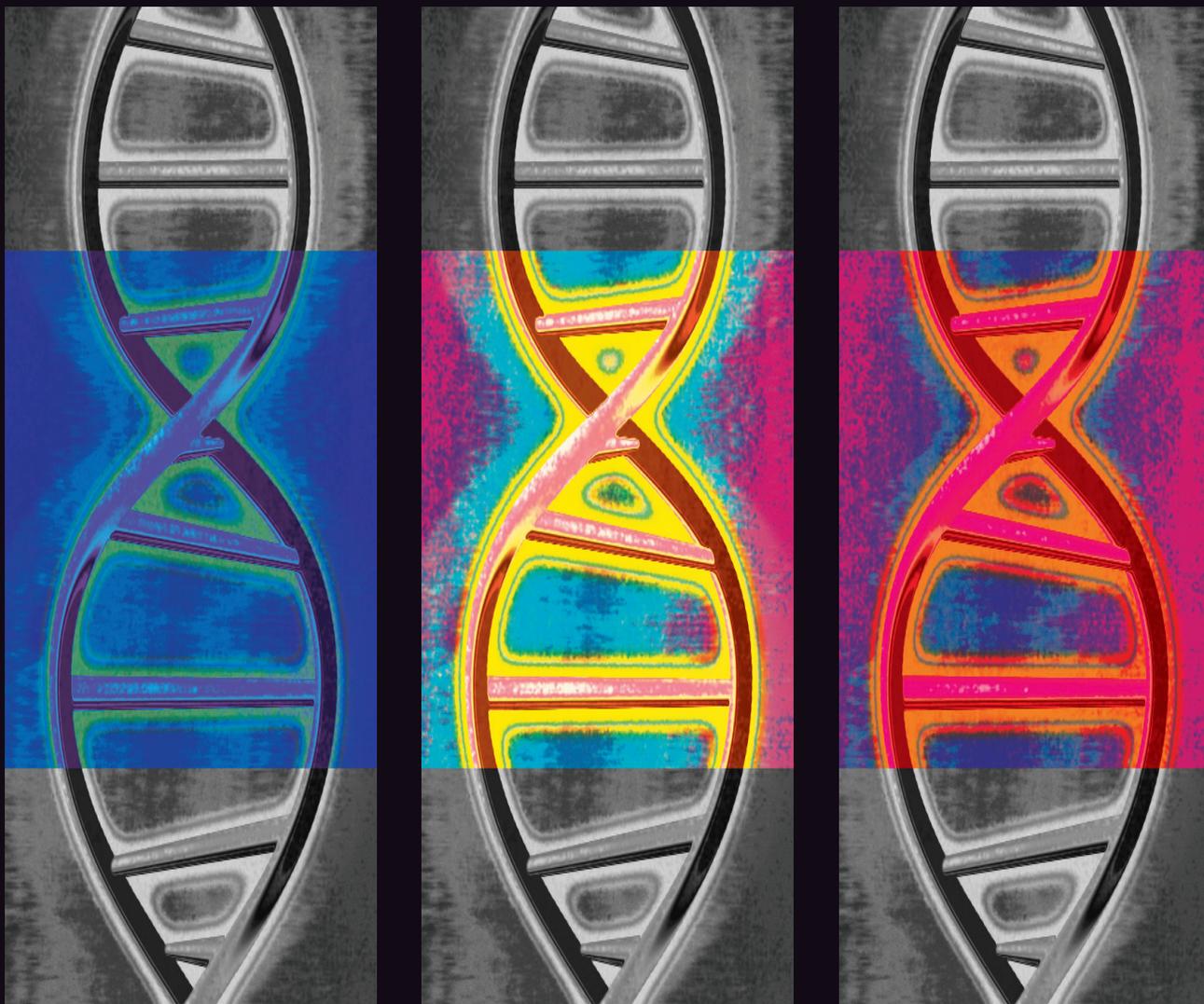
Research has shown that children may be chronically exposed to low doses of pesticides that do not cause symptoms evident in routine examinations. These exposures can result from hand-to-mouth behavior and more time spent on the floors of their homes and in contact with soils. They can also occur through food, drinking water, and indoor and outdoor use of pesticides. In general, children of agricultural workers are at special risk of pesticide exposure because their homes are usually close to fields where application occurs, and they can encounter take-home exposure on parents' clothing.

The researchers recruited children of Latino immigrants. All of the children recruited were aged 48 to 71 months. At least one parent of each child from the agricultural communities worked in agriculture at the time of the study. Neither parent of children from nonagricultural communities had worked in agriculture in the past year.

The researchers used a battery of behavioral tests to measure the children's cognitive and neurobehavioral function. Eleven of the measures showed no significant deficit between the two groups. However, the agricultural children did perform significantly worse on two tests: finger tapping (which measures response speed) and a test for visual memory. The results are consistent with effects seen in previous research on adults with documented low-level exposure to OP pesticides.

This study points out the need for additional larger studies aimed at determining whether low-level OP pesticide exposures produce deficits in standardized test performance among children of agricultural workers. It also illustrates the importance of proper pesticide application and good hygiene in pesticide applicators to prevent exposures in their children. **—Jerry Phelps**



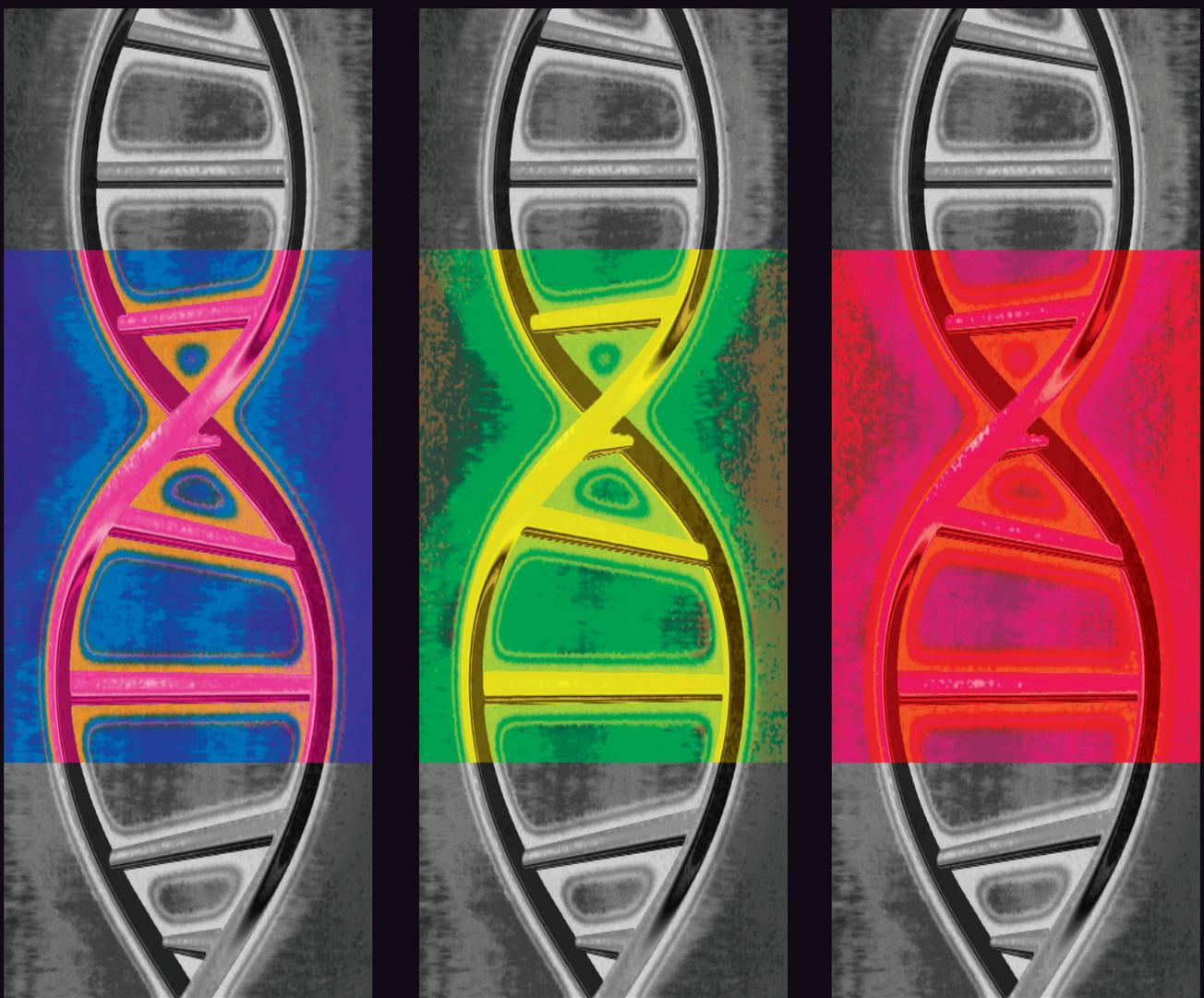


EPIGENETICS

For nearly a century after the term “epigenetics” first surfaced on the printed page, researchers, physicians, and others poked around in the dark crevices of the gene, trying to untangle the clues that suggested gene function could be altered by more than just changes in sequence. Today, a wide variety of illnesses, behaviors, and other health

indicators already have some level of evidence linking them with epigenetic mechanisms, including cancers of almost all types, cognitive dysfunction, and respiratory, cardiovascular, reproductive, autoimmune, and neurobehavioral illnesses. Known or suspected drivers behind epigenetic processes include many agents, including heavy metals,

Matt Ray/EHP



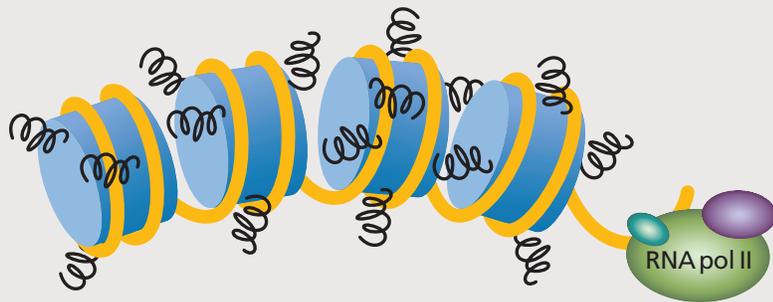
THE SCIENCE OF CHANGE

pesticides, diesel exhaust, tobacco smoke, polycyclic aromatic hydrocarbons, hormones, radioactivity, viruses, bacteria, and basic nutrients.

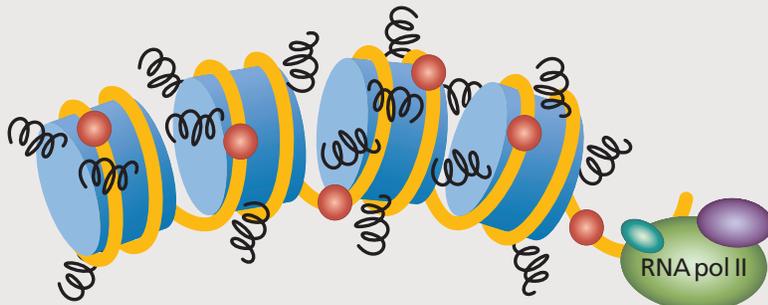
In the past five years, and especially in the past year or two, several groundbreaking studies have focused fresh attention on epigenetics. Interest has been enhanced as it has become clear

that understanding epigenetics and epigenomics—the genomewide distribution of epigenetic changes—will be essential in work related to many other topics requiring a thorough understanding of all aspects of genetics, such as stem cells, cloning, aging, synthetic biology, species conservation, evolution, and agriculture.

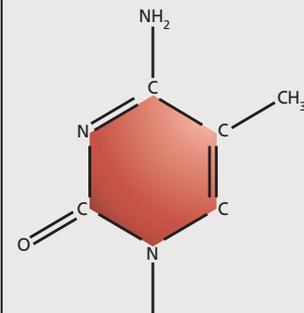
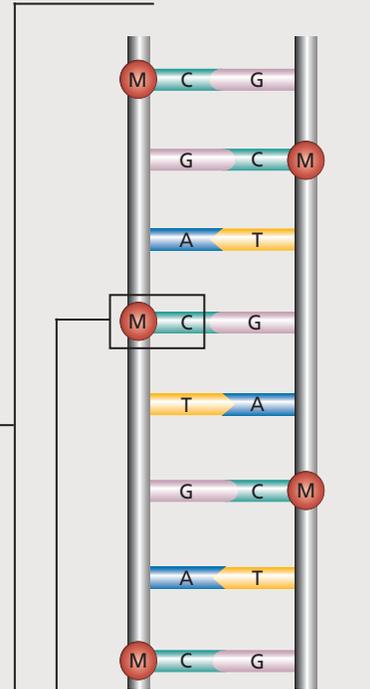
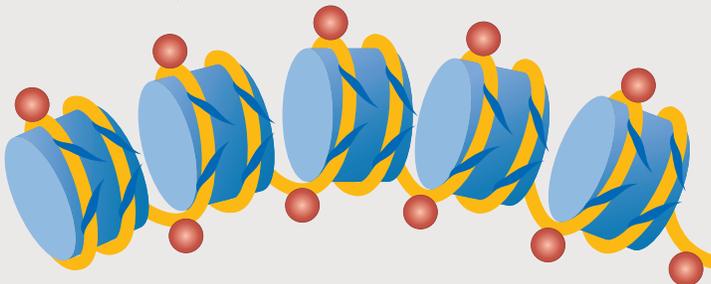
One Epigenetic Mechanism for Repressing Transcription



Methyltransferases attach methyl groups to DNA



Protein complexes, recruited to methylated DNA, remove acetyl groups and repress transcription



Methyl groups (CH₃) attach to cytosine bases

-  Deacetylated histone tail
-  Methylated CpG pair
-  Acetylated histone tail

Repression of transcription—the transfer of genetic information from DNA to RNA—is one route by which epigenetic mechanisms can adversely impact health.

Multiple Mechanisms

The word “epigenetic” literally means “in addition to changes in genetic sequence.” The term has evolved to include any process that alters gene activity without changing the DNA sequence, and leads to modifications that can be transmitted to daughter cells (although experiments show that some epigenetic changes can be reversed). There likely will continue to be debate over exactly what the term means and what it covers.

Many types of epigenetic processes have been identified—they include methylation, acetylation, phosphorylation, ubiquitylation, and sumoylation. Other epigenetic mechanisms and considerations are likely to surface as work proceeds. Epigenetic processes are natural and essential to many organism functions, but if they occur improperly, there can be major adverse health and behavioral effects.

Perhaps the best known epigenetic process, in part because it has been easiest to study with existing technology, is DNA methylation. This is the addition or removal of a methyl group (CH₃), predominantly where cytosine bases occur consecutively. DNA methylation was first confirmed to occur in human cancer in 1983, and has since been observed in many other illnesses and health conditions.

Another significant epigenetic process is chromatin modification. Chromatin is the complex of proteins (histones) and DNA that is tightly bundled to fit into the nucleus. The complex can be modified by substances such as acetyl groups (the process called acetylation), enzymes, and some forms of RNA such as microRNAs and small interfering RNAs. This modification alters chromatin structure to influence gene expression. In general, tightly folded chromatin tends to be shut down, or not expressed, while more open chromatin is functional, or expressed.

One effect of such processes is imprinting. In genetics, imprinting describes the condition where one of the two alleles of a typical gene pair is silenced by an epigenetic process such as methylation or acetylation. This becomes a problem if the expressed allele is damaged or contains a variant that increases the organism's vulnerability to microbes, toxic agents, or other harmful substances. Imprinting was first identified in 1910 in corn, and first confirmed in mammals in 1991.

Researchers have identified about 80 human genes that can be imprinted, although that number is subject to debate since the strength of the evidence varies. That approximate number isn't likely to

rise much in years to come, writes a team including Ian Morison, a senior research fellow in the Cancer Genetics Laboratory at New Zealand's University of Otago, in the August 2005 *Trends in Genetics*. Others in the field disagree. Randy Jirtle, a professor of radiation oncology at Duke University Medical Center, and his colleagues estimated in the June 2005 issue of *Genome Research* that there could be about 600 imprinted genes in mice; in an October 2005 interview Jirtle said he's anticipating a similar tally for humans, even though the known imprintable genes of mice and people have an overlap of only about 35%.

Links to Disease

Among all the epigenetics research conducted so far, the most extensively studied disease is cancer, and the evidence linking epigenetic processes with cancer is becoming “extremely compelling,” says Peter Jones, director of the University of Southern California's Norris Comprehensive Cancer Center. Halfway around the world, Toshikazu Ushijima is of the same mind. The chief of the Carcinogenesis Division of Japan's National Cancer Center Research Institute says epigenetic mechanisms are one of the five most important considerations in the cancer field, and they account for one-third to one-half of known genetic alterations.

Many other health issues have drawn attention. Epigenetic immune system effects occur, and can be reversed, according to research published in the November–December 2005 issue of the *Journal of Proteome Research* by Nilamadhab Mishra, an assistant professor of rheumatology at the Wake Forest University School of Medicine, and his colleagues. The team says it's the first to establish a specific link between aberrant histone modification and mechanisms underlying lupus-like symptoms in mice, and they confirmed that a drug in the research stage, trichostatin A, could reverse the modifications. The drug appears to reset the aberrant histone modification by correcting hypoacetylation at two histone sites.

Lupus has also been a focus of Bruce Richardson, chief of the Rheumatology Section at the Ann Arbor Veterans Affairs Medical Center and a professor at the University of Michigan Medical School. In studies published in the May–August 2004 issue of *International Reviews of Immunology* and the October 2003 issue of *Clinical Immunology*, he noted that pharmaceuticals such as the heart drug procainamide and the antihypertensive agent

hydralazine cause lupus in some people, and demonstrated that lupus-like disease in mice exposed to these drugs is linked with DNA methylation alterations and interruption of signaling pathways similar to those in people.

Substantial Changes

Most epigenetic modification, by whatever mechanism, is believed to be erased with each new generation, during gametogenesis and after fertilization. However, one of the more startling reports published in 2005 challenges this belief and suggests that epigenetic changes may endure in at least four subsequent generations of organisms.

Michael Skinner, a professor of molecular biosciences and director of the Center for Reproductive Biology at Washington State University, and his team described in the 3 June 2005 issue of *Science* how they briefly exposed pregnant rats to individual relatively high levels of the insecticide methoxychlor and the fungicide vinclozolin, and documented effects such as decreased sperm production and increased male infertility in the male pups. Digging for more information, they found altered DNA methylation of two genes. As they continued the experiment, they discovered the adverse effects lasted in about 90% of the males in all four subsequent generations they followed, with no additional pesticide exposures.

The findings are not known to have been reproduced. If they are reproducible, however, it could “provide a new paradigm for disease etiology and basic mechanisms in toxicology and evolution not previously appreciated,” says Skinner. He and his colleagues are conducting follow-up studies, assessing many other genes and looking at other effects such as breast and skin tumors, kidney degeneration, and blood defects.

Other studies have found that epigenetic effects occur not just in the womb, but over the full course of a human life span. Manel Esteller, director of the Cancer Epigenetics Laboratory at the Spanish National Cancer Center in Madrid, and his colleagues evaluated 40 pairs of identical twins, ranging in age from 3 to 74, and found a striking trend, described in the 26 July 2005 issue of *Proceedings of the National Academy of Sciences*. Younger twin pairs and those who shared similar lifestyles and spent more years together had very similar DNA methylation and histone acetylation patterns. But older twins, especially those who had different lifestyles and had spent fewer years of their lives

together, had much different patterns in many different tissues, such as lymphocytes, epithelial mouth cells, intra-abdominal fat, and selected muscles.

As one example, the researchers found four times as many differentially expressed genes between a pair of 50-year-old twins compared to 3-year-old twins, and the 50-year-old twin with more DNA hypomethylation and histone hyperacetylation (the epigenetic changes usually associated with transcriptional activity) had the higher number of overexpressed genes. The degree of epigenetic change therefore was

The methylation changes furthermore appeared to protect the mouse offspring against obesity in adulthood, although there are hints that genistein may also cause health problems, via additive or synergistic effects on DNA methylation, when it interacts with other substances such as folic acid.

Other Drivers of Change

Substances aren't the only sources of epigenetic changes. The licking, grooming, and nursing methods that mother rats use with their pups can affect the long-term

pups could negate the benefits of high-quality maternal care received when they were younger.

Along with behavior, mental health may be affected by epigenetic changes, says Arturas Petronis, head of the Krembil Family Epigenetics Laboratory at the Centre for Addiction and Mental Health in Toronto. His lab is among the first in the world, and still one of only a few, to study links between epigenetics and psychiatry. He and his colleagues are conducting large-scale studies investigating links between schizophrenia and aberrant methylation, and he says understanding epigenetic mechanisms is one of the highest priorities in human disease biology research. "We really need some radical revision of key principles of the traditional genetic research program," he says. "Epigenetics brings a new perspective on the old problem and new analytical tools that will help to test the epigenetic theory." He suggests that more emphasis is needed on studying non-Mendelian processes in diseases such as schizophrenia, asthma, multiple sclerosis, and diabetes.

The past decade has also been productive in developing strong links between aberrant DNA methylation and aging, says Jean-Pierre Issa, a professor of medicine at The University of Texas M.D. Anderson Cancer Center. He presented information on aging and epigenetic effects at a November 2005 conference titled "Environmental Epigenomics, Imprinting, and Disease Susceptibility," held in Durham, North Carolina, and sponsored in part by the NIEHS. Some of the strongest, decade-old evidence shows progressive increases in DNA methylation in aging colon tissues, and more recent evidence links hypermethylation with atherosclerosis. Altered, age-related methylation has also been found in tissues in the stomach, esophagus, liver, kidney, and bladder, as well as the tissue types studied by Esteller. Much of Issa's current work focuses on the links between epigenetic processes, aging, the environment, and cancer, and possible ways to therapeutically reverse methylation linked with cancer.

Current and Future Quandaries

The accumulated evidence indicates that many genes, diseases, and environmental substances are part of the epigenetics picture. However, the evidence is still far too thin to form a basis for any overarching theories about which substances and which target genes are most likely to mediate adverse effects of the environment on diseases, says Melanie Ehrlich, a



A pup of a different color. Supplementation of maternal diet with genistein and other compounds induced alterations in DNA methylation that were reflected in offspring coat color changes.

directly linked with the degree of change in genetic function.

Sometimes the effects of epigenetic mechanisms show up in living color. Changes in the pigmentation of mouse pup fur, ranging from yellow to brown, were directly tied to supplementation of the pregnant mother's diet with vitamin B₁₂, folic acid, choline, and betaine, according to studies by Jirtle and Robert Waterland published in August 2003 (issue 15) in *Molecular and Cellular Biology*. The color changes were directly linked to alterations in DNA methylation. In a study forthcoming in the April 2006 issue of *EHP*, Jirtle and his colleagues also induced these alterations through maternal ingestion of genistein, the major phytoestrogen in soy, at doses comparable to those a human might receive from a high-soy diet.

behavior of their offspring, and those results can be tied to changes in DNA methylation and histone acetylation at a glucocorticoid receptor gene promoter in the pup's hippocampus. This finding was published in the August 2004 issue of *Nature Neuroscience* by Moshe Szyf, a professor in McGill University's Department of Pharmacology and Therapeutics, and his colleagues. In the same study, the researchers found that the effects weren't written in stone; giving the drug trichostatin A to older pups could help reverse the effects of poor maternal care received when they were younger. In the 6 June 2003 *Journal of Biological Chemistry* and the 23 November 2005 *Journal of Neuroscience*, Szyf and many of the same colleagues also demonstrated that giving the amino acid L-methionine to older

biochemistry professor at the Tulane University School of Medicine and Tulane Cancer Center who has been conducting research on the topic for more than two decades.

That sense of uncertainty generally leaves epigenetics out of the regulatory picture. “It’s [too early] to actually use it at the moment,” says Julian Preston, acting associate director for health at the EPA’s National Health and Environmental Effects Research Laboratory. But Preston says the agency already relies more on its improving understanding of mechanistic processes, including epigenetics, and there is a clear effort within the EPA to expand genomics efforts both within the agency and with others with whom the agency works.

At the FDA, scientists are investigating many drugs that function through epigenetic mechanisms (although as spokeswoman Christine Parker notes, the agency bases its approvals on results of clinical trials, not consideration of the mechanism by which a drug works). One such drug, azacitidine, has been approved for use in the United States to treat myelodysplastic syndrome, a blood disease that can progress to leukemia. The drug turns on genes that had been shut off by methylation. The drug’s epigenetic function doesn’t make it a “miracle drug,” however. Trials indicate it benefits only 15% of those who take it, and a high percentage of people suffer serious side effects, including nausea (71%), anemia (70%), vomiting (54%), and fever (52%).

Ehrlich points out that azacitidine also has effects at the molecular level—such as inhibiting DNA replication and apoptosis—that may be part of its therapeutic benefits. The drug’s mixed results might also be explained in part by a study published in the October 2004 issue of *Cancer Cell* by Andrew Feinberg, director of the Johns Hopkins University Center for Epigenetics in Common Human Disease, and his colleagues. They found that each of two tested drugs, trichostatin A and 5-aza-2'-deoxycytidine (which is related to azacitidine), can turn on hundreds of genes while also turning off hundreds of others. If that finding holds in other studies, it suggests one key reason why it is so difficult to create a drug that doesn’t cause unintended side effects.

Public and Private

Despite the potentially huge role that epigenetics may play in human disease, investment in this area of study remains tiny compared to that devoted to traditional

U.S. Human Epigenome Project

In December 2005 a group of 40 international scientists publicly proposed a U.S. Human Epigenome Project to complement a European project of the same name launched in 2003. Group member Andrew Feinberg, a geneticist at the Johns Hopkins University School of Medicine, says, “We’re hoping to see how this idea takes hold. There is this ocean of information that is largely unexplored.”

The goal of the U.S. project will be to comprehensively map methylation and histone modifications—the two main classes of epigenetic modifications—in a diverse set of normal tissues. These epigenomes would then serve as a reference for comparison with diseased tissues, revealing epigenetic causes of disease. Project organizers are now compiling a detailed proposal, with budget estimates and a timeline.

Although both the U.S. and European projects ultimately aim to map all genes, the U.S. effort will look at different tissue and cell types than the European effort, and will also look at model organisms like yeast and the fly. The two groups are already working closely together in planning their projects to avoid redundancies, and this cooperation will likely continue.

Understanding cancer would be one long-term goal for the U.S. project, but epigenetics—changes in gene expression heritable from cell to daughter cell without changes in DNA sequence—transcends any one disease. “It has profound implications in aging, neurological disorders, and child development,” says Peter Jones, another group member and director of the Norris Comprehensive Cancer Center at the University of Southern California. Jones and his colleagues argue that the importance of epigenetics in human disease, together with the maturing of technologies for mapping epigenetic changes, make a human epigenome project both critical and feasible.

Epigenetics, says cancer biologist Jean-Pierre Issa of The University of Texas M.D. Anderson Cancer Center, could prove more important than genetics for understanding environmental causes of disease. “Cancer, atherosclerosis, Alzheimer’s disease [are all] acquired diseases where the environment very likely plays an important role,” he points out. “And there’s much more potential for the epigenome to be affected . . . than the genome itself. It’s just more fluid and more easy to be the culprit.” – Ken Garber



genetics work. Several efforts to change that are under way.

In Europe, the Human Epigenome Project was officially launched in 2003 by the Wellcome Trust Sanger Institute, Epigenomics AG, and the Centre National de Génotypage. The group's focus is on DNA methylation research tied to chromosomes 6, 13, 20, and 22. They may be joined soon by organizations in Germany and India, where scientists plan to work on chromosomes 21 and X, respectively, says Sanger senior investigator Stephan Beck.

But comprehensively studying all the epigenetic and epigenomic factors related to a multitude of diseases and health conditions will take much more work. "A [comprehensive] Human Epigenome Project is a lot more complicated than a Human Genome Project," Jones says. "There's only one genome, [but] an epigenome varies in each and every tissue." The Human Genome Project was a worldwide effort that took more than a decade and billions of dollars to complete.

Jones and Robert Martienssen addressed some of the complexities of a comprehensive, worldwide Human Epigenome Project in the 15 December 2005 issue of

Cancer Research. Reporting on a June 2005 workshop convened by the American Association for Cancer Research, they concluded that, despite all the looming difficulties, such a project is essential, and the technology is sufficiently advanced to begin.

"I think it's going to happen a lot sooner than I thought just a year or so ago," Jirtle says. A group of researchers has already started the footwork to launch a U.S. complement to the European Human Epigenome Project effort [see box, p. A165].

Other efforts are gaining ground. Another European group, the Epigenome Network of Excellence, took off in June 2004. This information exchange network includes members in the public and private sectors spread throughout ten Western European countries. Their objectives are to coordinate research, provide mentors, and encourage dialogue via their website. And in Asia, a conference held 7–10 November 2005 in Tokyo, "Genome-Wide Epigenetics 2005," was dedicated in large part to facilitating a coordinated epigenomics research effort in Japan and possibly all of Asia, says Ushijima, one of the conference's organizers.

In the United States, the National Cancer Institute and the National Human Genome Research Institute formally kicked off a major effort 13 December 2005 that will include epigenomic work. The pilot project of The Cancer Genome Atlas, funded by \$50 million each from the two institutes, is designed to lay the groundwork for comprehensive study of genomic factors related to human cancer. The initial three-year effort is expected to focus on just two or three of the more than 200 cancers known to exist, but if it's successful in developing methods and technologies, the number of cancers evaluated could then expand. If a high number of cancer genes are eventually scrutinized, the effort would be the equivalent of thousands of Human Genome Projects.

To help push the boundaries further, the NIEHS and the National Cancer Institute are in the midst of awarding grants totaling \$3.75 million to study a wide range of epigenetic topics, such as identification of high-risk populations, dietary influences on cancer, and detailed study of numerous specific mechanisms linking environmental agents with epigenetic mechanisms and resulting disease. The dozen or so recipients are expected to launch their projects by fall 2006.

The NIEHS has also begun to integrate epigenomics projects into its research portfolio over the past five to six years. "It's an emerging area that's very important," says Frederick Tyson, a program administrator in the NIEHS Division of Extramural Research and Training. And epigenetics is likely to be one of the half dozen or so most important considerations as NIEHS proceeds with its Environmental Genome Project, according to institute director David Schwartz.

The DNA Methylation Society, a professional group, has been growing slowly but steadily over the past decade, says founder and current vice president Ehrlich. As part of its efforts, the society launched a journal, *Epigenetics*, in January 2006 with the goal of covering a full spectrum of epigenetic considerations—medical, nutritional, psychological, behavioral—in any organism. Such groups are a valuable rallying point for this field, Jirtle says. He himself slowly worked his way into epigenetics from an initial cancer focus, and his segue is typical of many. "If you study epigenetics, you don't have a home; we come from all different fields," he says.

Interest in the private sector is also picking up. For instance, Epigenomics AG, with offices in Berlin and Seattle, is working on early detection and diagnosis of cancer and

Resources

Professional Organizations and Projects

- DNA Methylation Society (international)
<http://www.dnamethsoc.com/main.htm>
- Epigenome Network of Excellence (Europe)
<http://www.epigenome-noe.net>
- Human Epigenome Project (Europe)
<http://www.epigenome.org>

Journal

Epigenetics

<http://www.landesbioscience.com/journals/epigenetics/>

DNA Methylation Database

<http://www.methdb.de/front.html>

Imprinted Gene Databases

- <http://igc.otago.ac.nz/home.html>
- <http://www.geneimprint.com/databases/?c=clist>
- <http://www.mgu.har.mrc.ac.uk/research/imprinting/>

endometriosis (for which there is limited evidence of an epigenetic component), as well as development of products to predict effectiveness of drugs to treat these diseases. Founded in 1998, and now with about 150 employees, the company is focusing on DNA methylation mechanisms, and is working with companies such as Abbott Laboratories, Johnson & Johnson, Philip Morris, Roche Diagnostics, Pfizer, and AstraZeneca. CEO Oliver Schacht says the surging interest in this field is typified by the difference between the 2004 American Association for Cancer Research conference, which had half a dozen or so talks or posters on epigenetics, and the 2005 event, which had about 200.

Tool Time

If epigenetic work is to continue breaking new ground, many observers say technology will need to continue advancing. Jones and Martienssen note in their paper that there must be additional improvements in high-throughput technologies, analytical techniques, computational capability,

mechanistic studies, and bioinformatic strategies. They also say there is a need for basics such as standardized reagents and a consistent supply of antibodies for testing.

Preston agrees with many of these ideas, and says there is also a need to develop a comprehensive tally of all proteins in the cell and to get better protein modification information. He says universities are recognizing the demand for the talents needed to solve epigenomics problems, and are increasing their efforts to cover these topics in various ways, especially at the graduate school level.

Other groups are doing their part by creating tools to further the field. All the imprinted genes identified so far are tracked in complementary efforts by Morison's and Jirtle's groups and the Mammalian Genetics Unit of the U.K. Medical Research Council. The European managers of the DNA Methylation Database have assembled a compendium of known DNA methylations that, although not comprehensive, still provides a useful tool for researchers investigating the roughly 22,000 human genes.

Kunio Shiota, a professor of cellular biochemistry at the University of Tokyo and one of the co-organizers of the November 2005 Tokyo conference, says epigenetic advances will rely in part on a range of processes that are slowly becoming familiar to more researchers—massively parallel signature sequencing (MPSS), chromatin immunoprecipitation microarray analysis (ChIP-chip), DNA adenine methyltransferase identification (Dam-ID), protein binding microarrays (PBM), DNA immunoprecipitation microarray analysis (DIP-chip), and more. Someday, he says, these terms could become fully as familiar as MRI and EKG.

The rapidly growing acceptance of epigenetics, a century after it first surfaced, is a huge step forward, in Jirtle's opinion. "We've done virtually nothing so far," he says. "I'm biased, but the tip of the iceberg is genomics and single-nucleotide polymorphisms. The bottom of the iceberg is epigenetics."

Bob Weinhold

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Open House: The Ethics of



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Home is where the heart is, but for children, home all too often can be where the danger is—danger of exposure to lead, asthma triggers, pesticides, safety hazards, and other potential sources of harm. Those risks are disproportionately common among poor and minority children, whose families more often lack access to decent, affordable housing.

In recent years, environmental health scientists have increasingly sought to identify and ameliorate risk factors affecting such children. Research into housing-related health hazards involving children has proven to be an area of investigation both rich in potential for discovery of effective intervention methods and fraught with opportunities for ethical lapses. A recent report by the National Academies now provides researchers and their sponsors clear guidelines to avoid ethical pitfalls while aggressively pursuing new and beneficial knowledge.

Studying Children at Home

A 2001 decision by the Maryland Court of Appeals cast a spotlight on the ethics issue. In the case *Grimes v. Kennedy Krieger Institute*, two mothers sued researchers, claiming they had placed child subjects in a lead abatement study at an unacceptable level of risk. In determining that the case should proceed to trial, the court included comments in which it scolded the research community for what it perceived to be shortcomings in the approach to conducting research involving low-income populations, particularly with regard to obtaining truly informed consent from the parents of children participating in such studies.

Although *Grimes v. Kennedy Krieger Institute* was eventually settled out of court, the appeals court comments threw the field into a state of confusion as its ethical underpinnings were called into question. Ultimately, this uncertainty led the major federal funders of this type of research—HUD, the CDC, and the EPA—to ask the National Academies to appoint a committee to examine the issues in detail and recommend procedural and policy changes to clarify best practices and resolve ethical dilemmas.

The Committee on Ethical Issues in Housing-Related Health Hazard Research Involving Children, Youth, and Families, a project of the National Research Council and the Institute of Medicine, was carefully chosen to represent a variety of stakeholders in this

research enterprise. “We were diverse in terms of background and professional training,” says committee chairman Bernard Lo, a professor of medicine and director of the Program in Medical Ethics at the University of California, San Francisco. “We had scientists who do housing research, and we had several members who were very familiar with low-income housing and were advocates for people in low-income housing. Those are the sorts of viewpoints that needed to be included, supported, and recorded.”

Peeking into Scary Closets

A variety of ethical concerns coalesce to make housing-related health hazard research particularly challenging. It takes place in the home, with an inherent invasion of privacy. While in the home, researchers may notice hazardous conditions other than those under study—what is their responsibility in such a situation? The community may have expectations of and desires for ameliorative research outcomes that are vastly different from those of the investigators. Many health hazards (such as lead-based paint) occur disproportionately in poor-quality housing occupied by low-income, often ethnic minority families, so children in these families are the most likely candidates for study. This can lead to concerns about exploiting vulnerable groups of subjects for research that ultimately benefits others. Parents in these families are often poorly educated; this,

combined with the typical complexity of informed consent forms, makes it difficult for them to provide meaningful informed consent for their children’s participation. Also, inappropriate financial and other incentives may unduly influence parents’ decisions regarding their children’s participation.

The committee met five times over the course of 18 months to examine these issues. The panelists heard presentations from committee members and external experts, including parents, community leaders, researchers, government officials, and specialists in law and ethics. “Through those presentations, as well as their review of the literature and their deliberations, the committee came up with their consensus view on what ought to be done in this area,” says Mary Ellen O’Connell, a National Academies staff officer who was the committee’s study director. After the group’s initial draft was reviewed by an external slate of reviewers with similar expertise and revised based upon their comments, the final report, *Ethical Considerations for Research on Housing-Related Health Hazards Involving Children*, was released on 19 September 2005. Lo says the committee unanimously supported all of the recommendations put forth in the report.

The report addresses specific recommendations to the three main audiences in need of guidance:

researchers themselves, research institutions and institutional review boards, and the federal government and other research sponsors. The recommendations all revolve around two guiding themes: the need to involve community representatives at all stages of the research, from inception to follow-up, and the need to strengthen the process of informed consent so that parents fully understand the essential features of the research study. The report strikes an elegant balance, clarifying what have been ethical gray areas while still facilitating and encouraging housing-related health hazard research designed to improve the lives of the most vulnerable children and families.

Building a Firm Foundation

One key recommendation made by the committee is that all federal agencies sponsoring housing-related health hazard research should formally adopt the federal regulations addressing human subject research participation, particularly Subpart D of 45 CFR 46, which provides additional protections specifically for child participants. The CDC and the NIEHS, as agencies of the Department of Health and Human Services, are already governed by Subpart D. According to Lo, the EPA has committed to formally adopting it, while HUD has responded that it will follow the regulations and require their protections in their projects, but does not plan to make Subpart D part of its official policy, at least for now.

According to committee member Alan Fleischman, a senior advisor at the New York Academy of Medicine, the panel was particularly impressed with the amount of data supporting the value of community engagement in environmental health research—so-called community-based participatory research (CBPR). “It actually results in better, more focused research,” he says. “There is increasing evidence that engaging communities actually makes the research more powerful, more important, and more valid, and the potential to develop advocacy approaches to do public health intervention and change is more effective.” Further, subjects are better protected in that community involvement can act as kind of a buffer, ensuring that potential risks and benefits are well characterized and defined, and that there is far less potential for even inadvertent exploitation.

The committee recognized that in the real world the reforms they were recommending would translate into more time

and more money being required for housing-related health hazard research projects. The panel addressed this reality by recommending that research sponsors provide the additional funding and extended timelines necessary to support expanded community participation. The report also suggests that “researchers need to develop ongoing partnerships with their communities, which is of course complex, and takes time and effort,” says Fleischman. But in the long run, he adds, “individual research projects may not be slowed down, if in fact those



projects are part of a portfolio of research being done with relationship to the community as partners.”

Putting Out the Welcome Mat

By most accounts, the report has been received quite warmly. “This report was particularly important to us,” says Rebecca Morley, executive director of the nonprofit National Center for Healthy Housing, which both sponsors and conducts research. “In order to do our research, we were looking forward to having very clear guidelines, because what we were finding is that the current [oversight approaches] had the ironic and perverse effect of discouraging the study of the most serious health hazards, and prompted researchers to shy away from

studies that focus on communities at the highest risk, for fear of being seen as callous or discriminatory,” she says.

According to Lo, feedback from HUD, the EPA, and the CDC has been positive. “They’ve already adopted some of our recommendations,” he says. “They responded favorably, and they are obviously looking at the recommendations carefully, and hopefully they’ll encourage all of their researchers to adopt them.”

As an institute that has pioneered CBPR—in fact, often requiring community involvement in the research process—the NIEHS has also welcomed the refinements offered in the report. Children’s environmental health program administrator Kimberly Gray says that although the institute already practices much of what the report preaches, it’s good to get a wider outside perspective on the issues involved.

O’Connell thinks the tone of the responses to the report she’s been hearing bodes well for acceptance and active adoption of its recommendations. She says, “People have been asking me ‘how do we do this?’ rather than ‘why should we do this?’”

Rooms with a Long View

Of course, a committee report, however thorough and well-intentioned, cannot guarantee that ethical lapses will not still occur, that researchers might not still hesitate to pursue housing-related health hazard research projects for fear of litigation, or that its recommendations and suggestions will be universally followed. But committee members are optimistic that the report will be viewed as a milestone in efforts to support and encourage such research while putting it on a more solid ethical footing.

“We believe that these are feasible recommendations that are well within the ability of researchers and sponsors to carry out, and that they will improve trust within the communities that they serve, and also strengthen parents’ understanding of what this research is all about,” says Fleischman.

Morley is taking more of a wait-and-see approach. “The proof will be in the pudding in implementation,” she says. “As researchers actually start to apply this, I think we will see the practicality of the recommendations, and whether [the report] has delved into sufficient detail to enable researchers to operate with a clear conscience and also give participants peace of mind.”

Ernie Hood



Linking

Lessons

Learning

Tomorrow's Scientists

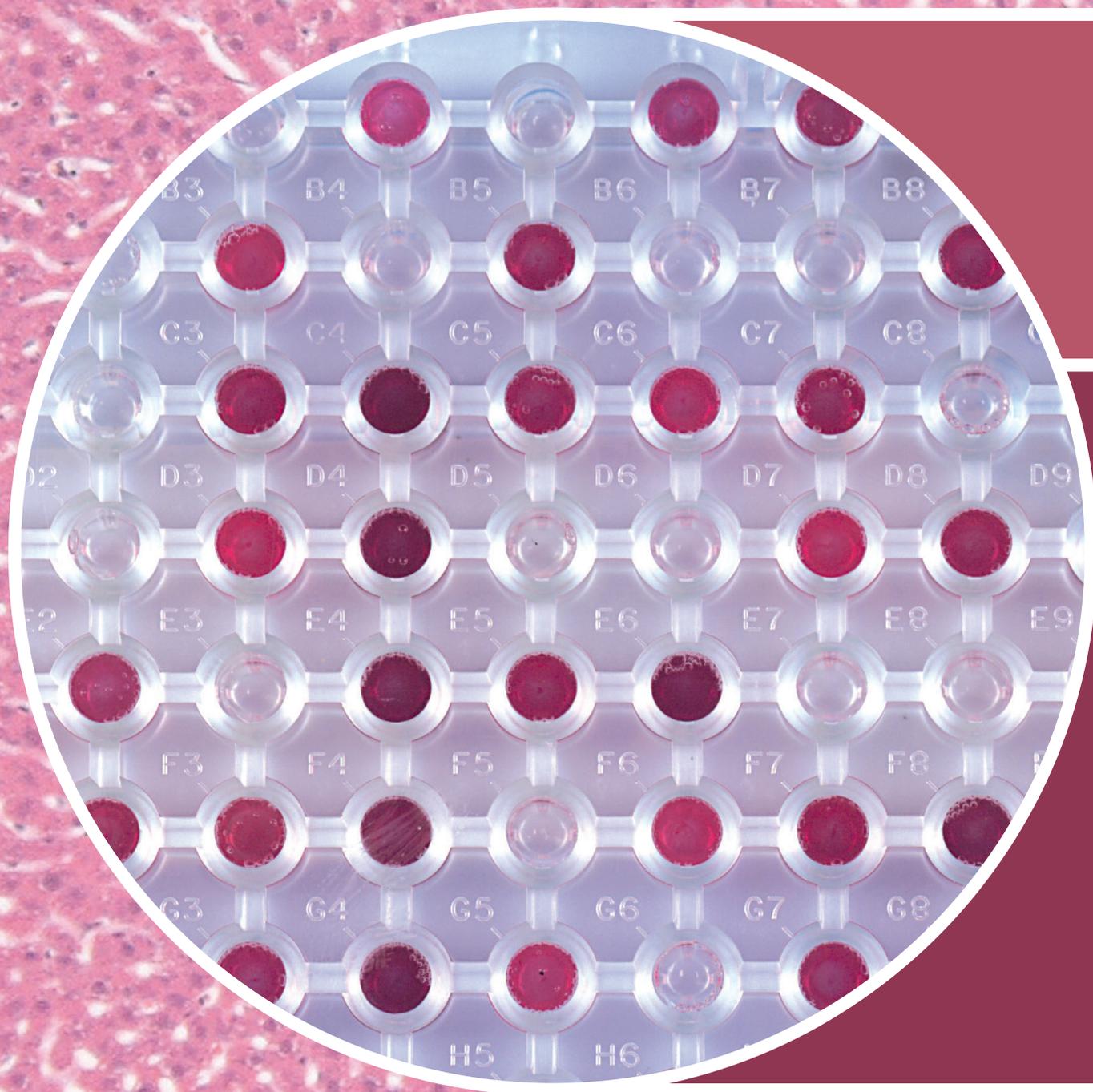
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Cell Scenario

A New Look at Microarrays

The field generally known as cell-based analysis started in the 1970s with two-dimensional gel electrophoresis, which tracked levels of proteins in cells. Next came DNA microarrays that measured thousands of genes simultaneously. Today researchers are exploring the potential of a new tool, the Phenotype MicroArray™ (PM), which offers a panoramic view of cellular events. With the recent introduction of a mammalian version of the PM, the tool is poised to provide even more insight into how cells behave when affected by environmental agents.

Inset: Biolog; Background: David Malarkey/NIH5

Just like a battery of tests on a person's blood can scan the health of vital organs, the PM can scan the physiology of cells, yielding data on hundreds of traits at once. Typical cell-based assays measure only one trait at a time (for example, cell death or DNA synthesis), but the PM can measure up to 2,000 traits—or phenotypes—under hundreds of growth conditions. The PM can be used to fingerprint cell lines used in research or biomanufacturing to ensure stability, or monitor the effects of drugs or toxicants on cells. Researchers can also compare normal and diseased cells to see how cell functions are altered.

Building on Success

PM developer Barry Bochner, a bioengineer, patented a simple dye method to measure cellular respiration while a graduate student in the 1970s. Ten years later, he started operations at Biolog Incorporated in Hayward, California, to commercialize the technology as a tool to identify more than 1,900 species of bacteria and fungi based on patterns of carbon metabolism. These first products, a series of five kits that each can identify about 300–600 species, have been a chief tool of microbiologists since 1988.

Microbes grow on many carbon sources, such as glucose and other sugars, amino acids, and carboxylic acids. The original microbial identification kits were based on redox reactions that produce a color change in microwells; that is, when cells utilize carbon for energy, they turn a colorless dye purple. Each well of the microbial identification kits contains a different assay—a different carbon fuel and a tetrazolium dye—dried on the bottom. When a microorganism metabolizes a carbon source, an irreversible chemical reaction occurs, and the intensity of the purple color formed in each well over time is analyzed and compared to a database for identification.

Tetrazolium dye has long been used by toxicologists to measure cell viability. Bochner's team improved the older dye chemistry by making it water-soluble and less toxic to cells. In addition, they eliminated high background color that results from serum in culture media reacting with older tetrazolium dyes. With the improved dye, researchers can measure as few as 100 or up to 20,000 cells in one well.

Bochner's next invention came during the genomics era, when DNA microarrays allowed scientists to measure the expression of thousands of genes simultaneously. "I had this idea that we could go beyond carbon metabolism," says Bochner, now chairman and vice president of research and development at Biolog. So he created the PM.

The PM uses the same technology as the microbial identification kits, except that the PM measures nearly 2,000 chemical reactions related to carbon, nitrogen, phosphorus, and sulphur metabolism, as well as pH, growth range, and sensitivity to antibiotics and stress factors. The reactions reflect key cell pathways, including cell surface binding, biosynthesis of molecules, stress and repair processes, and the metabolism of carbon and

nitrogen. "With two thousand phenotypes we can detect most of the important changes in cellular physiology," says Bochner.

Few technical skills are required to run any of the Biolog kits. A researcher simply adds a cell suspension to the wells to start the reactions. The data generated are captured and interpreted by Biolog's OmniLog® system, a combination incubator and scanner that monitors, analyzes, records, and graphs changes in each well with proprietary bioinformatic software. Data are collected in 15-minute intervals for up to 48 hours.

PM Applications

Data from the PM and DNA microarrays complement each other and bridge the gap between molecular changes and biological outcomes. "Just because a gene is turned on or off doesn't mean that a biological pathway gets turned on," says Bochner. The PM gives a global view of cellular processes by detecting how gene changes alter one or many biological properties of cells.

The PM can therefore help researchers assess the effects of environmental toxicants on cells. Toxicants work by interfering with cellular respiration, damaging the pathways

New Applications for the Tried-and-True

Environmental scientists are finding new applications for Biolog's tried-and-true microbial identification kits. Ken Cullings, an evolutionary ecologist at NASA's Ames Research Center, used one kit to evaluate the effects of defoliation on soil fungal diversity. In a mixed lodgepole pine and Engelmann spruce forest in Yellowstone National Park, Cullings's team removed half the needles on naturally reseeded pine seedlings. A year later, soil samples showed a significant increase in the physiological diversity of soil fungi. "Fungi specific to pines jumped ship to the nondefoliated spruces when the pine was defoliated," says Cullings.

The results, reported in the April 2005 issue of *Applied and Environmental Microbiology*, translate directly to forestry practices: after harvesting old-growth forests, replant with mixed tree species to improve future productivity. The same advice holds for replanting after forest fire devastation. "We showed in a pine-spruce ecosystem that mixed species moderate detrimental effects," says Cullings.

Another NASA scientist, microbial ecologist Jay Garland of the Kennedy Space Center, is seeking beneficial microbes to prevent *Salmonella* contamination of alfalfa sprouts. NASA hopes to someday send alfalfa sprouts on space missions to produce oxygen and water and also serve as a food source for astronauts. But sprouts are vulnerable to *Salmonella* contamination. Since 1995, at least 21 food poisoning outbreaks due to eating contaminated sprouts have been reported in the United States, according to Garland.

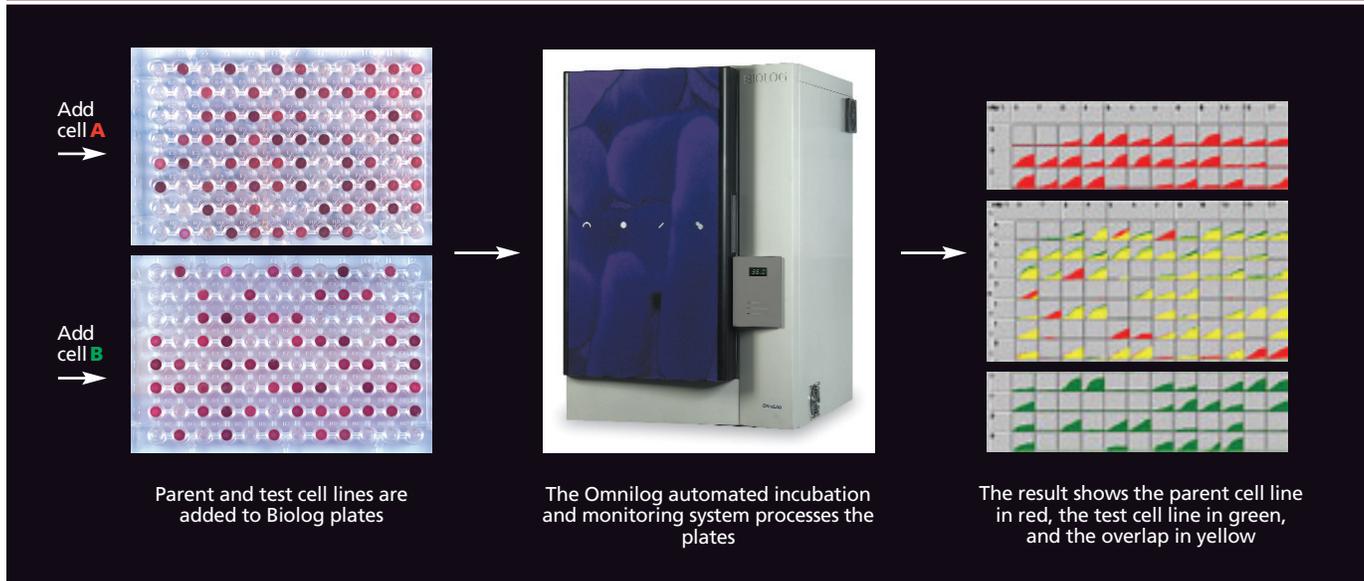
To prevent infection, seeds are soaked in bleach, but the practice is neither completely effective nor palatable to

health-conscious consumers. "An alternative is to manipulate the numbers of good microbes that live naturally on sprouts to stop *Salmonella* from growing," says Garland. He uses one of the microbial identification kits to screen bacteria growing naturally on sprouts to identify mixtures that rapidly block the growth of harmful *Salmonella* and other pathogens, as described in the January 2005 issue of *Journal of Food Protection*. This biocontrol strategy could protect both Earth-bound consumers and astronauts.

James Thomas, a molecular biologist at Canada's University of Lethbridge, performs source tracking of fecal indicator bacteria in southern Alberta, where an intense livestock industry generates enormous amounts of waste. During the summer, smaller communities often must boil water due to high levels of fecal bacteria. In collaboration with Health Canada, Thomas screens environmental water samples from the city of Lethbridge and surrounding rural communities with Biolog kits to find out how the contamination is happening, and why it especially occurs in the hot weather.

Thomas and his colleagues used a microbial identification kit to monitor 37 sites within the Oldman River Basin, where a network of irrigation canals and three reservoirs provide water to the agricultural region. The results showed that the lowest levels of the enteric bacteria *Escherichia coli* and *Enterococcus faecalis* are detected at the outflow of reservoirs. "Reservoirs remove microbial pollutants in agricultural wastewaters," says Thomas. The construction of more in-stream reservoirs or wetlands could significantly improve the water quality of rural watersheds, they concluded in the 15 September 2005 issue of *Science of the Total Environment*.
—Carol Potera

How the Phenotype MicroArray Works



that cells need to live and grow, so the formation of the purple dye color is either reduced or totally prevented. Similarly, pharmaceutical companies can use the PM to monitor toxicity of new drugs. “This is a general method for studying the effects of any chemical on cell pathways,” says Bochner.

The function of novel genes can be assessed with knockout experiments to see what cell phenotypes appear, disappear, strengthen, or weaken. In knockouts of *Pseudomonas aeruginosa*, which infects the lungs of cystic fibrosis patients, investigator Ian Paulsen of The Institute for Genomic Research uncovered unusual gene transport functions. “Biolog let us pin functions to novel genes,” says Paulsen, who is mapping the physiology of this pathogen. “There is no comparable product on the market that lets you do high-throughput physiological screening.”

The PM can also highlight pathways linked to a pathogen’s virulence. At Lawrence Livermore National Laboratory, Sandra McCutchen-Maloney studies *Yersinia pestis*, the cause of bubonic plague. *Y. pestis*, one of the most virulent bacteria known, is feared as a possible bioterrorism threat. The microbe infects rodents in North America, and fleas can transmit *Y. pestis* to humans.

When McCutchen-Maloney used the PM to test *Y. pestis* under biologically relevant conditions that occur in fleas and humans, the pathogen proved tougher than expected and less vulnerable to antibiotics. The data “uncovered new pathways involved in virulence that could be targets for future therapeutics,” says McCutchen-Maloney, who presented these findings at the 44th annual meeting of the American Society for Cell Biology in December 2004.

In another application, USDA veterinarian Jean Guard-Bouldin studied how eggs with uncracked shells become contaminated with *Salmonella*. The PM was used to “identify a number of physiological capabilities in *Salmonella* that we would not have otherwise predicted,” says Guard-Bouldin. It turned out the egg-contaminating strains had evolved metabolic capabilities that adapted the pathogen to grow in the reproductive tracts of hens that otherwise appeared healthy. The PM approach “accelerated our ability to locate the genes that were undergoing rapid evolution,” she says.

Like DNA microarrays, the PM generates massive amounts of data. “The challenge is to find ways to make sense of large data sets,” says Paulsen. It is up to bioinformatics experts to develop statistical methods to analyze data in a way that makes sense for their application.

With the new mammalian PM, introduced in September 2005 at the Society for Biomolecular Screening conference held in Geneva, Switzerland, researchers now have the ability to work with a variety of human cell lines, ranging from blood to liver cells, as well as primary rat hepatocytes, which toxicologists prefer.

The first mammalian PM contains 384 assays for energy-producing pathways shared by a range of cell types. “It was a big leap to mammalian cells,” says Bochner, who spent three years adapting the method to work in more complex mammalian cells. The goal is to expand the mammalian PM to 2,000 phenotypes as the methods are perfected.

Carol Potera

Suggested Reading

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Sour Finding on Popular Sweetener

Increased Cancer Incidence Associated with Low-Dose Aspartame Intake

More than 20 years have elapsed since aspartame was approved by regulatory agencies as an artificial sweetener. But scientists draw conclusions on carcinogenicity based on the evidence available at the time, and new research out of the European Ramazzini Foundation of Oncology and Environmental Sciences bolsters recent calls for reconsideration of regulations governing aspartame's widespread use in order to better protect public health, particularly that of children [*EHP* 114:379–385; Soffritti et al.].

The researchers added aspartame to the standard diet of Sprague-Dawley rats, using dosages designed to simulate a wide range of human intakes. Each rat was observed from 8 weeks of age until death. This is in contrast with earlier studies that typically sacrificed animals between 104 and 110 weeks of age, corresponding to about two-thirds of a rat's lifespan (in humans, approximately 80% of cancer diagnoses are made in the last third of life, after age 55). Deceased animals were examined for microscopic changes in various organs and tissues, enabling a comprehensive assessment of aspartame's carcinogenic potential. A total of 1,800 rodents were included, far more than in previous studies.

Aspartame-fed females showed significant evidence of lymphomas/leukemias and of carcinomas of the renal pelvis and ureter. The effect on the renal pelvis was much more evident when carcinomas were combined with atypical preneoplastic lesions. The researchers also observed an insignificant increase in incidence of malignant schwannomas of the peripheral nerves in males, as well as hyperplasia of the olfactory epithelium in males and females. Lesions of the kidney and olfactory epithelium are extremely rare in this strain of rats and therefore merit special attention.

The carcinogenic effects were evident at daily doses as low as 400 parts per million, equivalent to an assumed daily human intake of 20 milligrams per kilogram body weight (mg/kg). This dosage is much less than the acceptable daily intake for humans, with current limits set at 50 mg/kg in the United States and 40 mg/kg in Europe. Surveys of aspartame intake in the United States and Europe from 1984 to 1992 showed that consumers typically consumed 2–3 mg/kg daily, with small children and women of child-bearing age consuming slightly more, at 2–5 mg/kg daily.

The public health implications of these findings may be substantial, since aspartame is used in about 6,000 products, and more than 200 million people regularly consume aspartame through foods, beverages, drugs (such as chewable vitamins), and hygiene products (such as toothpaste). Because the study did not take into account *in utero* and perinatal exposures, the authors point to this as a salient direction for future research, given that children and pregnant and breastfeeding women are among the major consumers of aspartame. —M. Nathaniel Mead

The Cancer Differential

Minorities in Racially Segregated Urban Areas at Higher Risk than Whites

Nearly 80% of the U.S. population lives in metropolitan areas. With continued growth of urban centers has come increased study of the connection between the built environment, social inequality, and the health and well-being of inhabitants of these large cities. A number of factors related to neighborhood location and other area-level variables such as access to nutritious foods and health care can affect human health. Now a research team points to another health consideration, demonstrating that minority populations living in highly segregated metropolitan areas in the United States have higher estimated lifetime cancer risks from air toxics than whites [*EHP* 114:386–393; Morello-Frosch et al.].

The team analyzed more than 45,000 census tracts in 309 U.S. metropolitan areas for level of segregation. The metropolitan areas were classified as low-to-moderately segregated, highly segregated, or extremely segregated, based on the proportion of people who would have to move to achieve an even racial balance in every neighborhood of the city. They also used census data to divide racial and ethnic groups into six categories: Hispanics of any race, non-Hispanic whites, non-Hispanic blacks, non-Hispanic Asians and Pacific Islanders, non-Hispanic American Indians and Alaska Natives, and non-Hispanic persons of other race.

Then the team used federal air toxics data for 1996 to derive cancer risk estimates. Cancer risks were determined using inhalation unit risk estimates for each known, likely, or potential human carcinogen measured in the tracts' air. Inhalation unit risk estimates consider the individual lifetime excess risk resulting from chronic



Stirring debate. New rodent data on aspartame, an artificial sweetener used in a variety of consumer goods, suggest the chemical's potential cancer effects deserve more study.

lifetime exposure to one unit of pollutant concentration.

The researchers found a persistent relationship between increasing levels of racial/ethnic segregation and increased estimated cancer risk associated with ambient air toxics. Hispanics in extremely segregated areas were the most affected, with a 6.4-fold increased lifetime cancer risk compared to Hispanics in low-to-moderately segregated areas. Non-Hispanic American Indians and Alaska Natives in highly segregated areas were the least affected, with a 1.39-fold increased risk over their counterparts in low-to-moderately segregated areas. The influence of racial segregation on cancer risk appeared independent of the effect of poverty across racial categories. The most significant contributors to cancer risk were mobile sources such as on-road vehicles, airplanes, and trains, with diesel emissions an overwhelming source of pollution.

The authors note that these results are consistent with findings from a previous national study that analyzed the relationship between black/white residential segregation and ambient air toxic exposure in U.S. metropolitan areas. They believe this study to be the first examination of environmental health disparities to use a generalized multiethnic segregation measure. They assert that future research on this issue that incorporates new and better models of exposure should include segregation as a key factor in analysis.
—Tanya Tillett

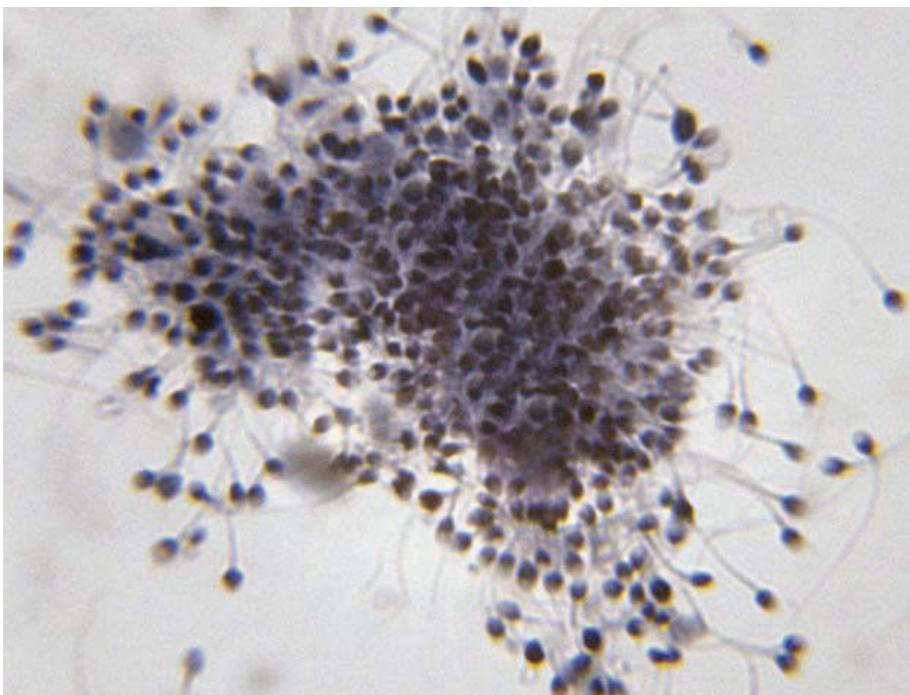
Sperm Alert

Semen Quality Decline Linked to Ozone Pollution

Some recent studies examining the effect of environmental hazards on fertility claim that sperm counts are declining in certain industrialized countries. Although the validity of these findings is uncertain, most researchers agree that if there is in fact a decline in semen quality, it's probably linked to geographic location. Now a team of California researchers has examined how exposure to specific air pollutants—ozone, nitrogen dioxide, carbon monoxide, and particulate matter smaller than 10 micrograms in diameter (PM₁₀)—affects semen quality and reports a direct connection between ozone exposure and reduced sperm count [*EHP* 114:360–365; Sokol et al.].

It is estimated that at least 2.1 million couples in the United States have difficulty achieving pregnancy, with male infertility responsible for 40–50% of infertility cases. Exposure to environmental toxicants that disrupt sperm production (spermatogenesis) or the function of reproductive hormones or sperm may increase the risk of male infertility.

The investigators analyzed semen samples collected from 48 men who regularly donated to a Los Angeles sperm bank between January 1996 and December 1998. Subjects were healthy, educated males between the ages of 19 and 35 who had



Gametes and gray skies. A new study shows significant declines in semen quality associated with exposure to ozone air pollution.

abstained from sex for two to three days before sample collection. Also available were data on each donor's age, date of birth, race, dates of collection, and zip code of residence at the time of first donation.

The researchers also collected air quality data gathered for ten-kilometer grid areas during the same two-year period, and assigned subjects a grid location based on their zip code at the time of first donation. Ozone, nitrogen dioxide, and carbon monoxide were measured daily, and PM₁₀ was measured once every six days. Then the researchers examined the relationship between each semen sample and the air quality at 0–9, 10–14, and 70–90 days prior to its collection (human spermatogenesis is a 72-day process). They assessed semen volume, sperm concentration, and sperm motility within one hour of collection and compared it against air quality data specific to the donor.

Ozone was the only pollutant associated with changes in sperm quality. The analysis showed an inverse relationship between ozone exposure and sperm density at all points in spermatogenesis. The results remained significant after adjusting for donor age, season, and temperature.

It is known that ozone and its reaction products can cross the blood–gas barrier and enter the bloodstream, and exposure to ozone can cause oxidative stress, which has been shown to disrupt testicular and sperm function. As with smoking, ozone exposure may trigger an inflammatory reaction in the male genital tract or the formation of circulating toxic species. Both events could cause a decline in sperm concentration.

These findings support an earlier study conducted in the Czech Republic by scientists from the U.S. EPA. Young men who were exposed to elevated air pollution were more likely to have altered sperm quality than those who lived in areas with less air pollution. The authors note that the current study controlled well for potential confounders, and the connection between ozone and sperm quality is consistent across several models.

—Tanya Tillett



Unhealthy home fires. An assessment of solid fuel use reveals that continued widespread global dependence on such fuels for household needs will impede success in meeting the UN Millennium Development Goals.

Keeping the Home Fires Burning Cleaner

Solid Fuel Use, Health, and the Millennium Development Goals

Many people in industrialized nations give little thought to central heating, electric lighting, and flick-of-a-switch cooking. But more than half of the people in the world rely on solid fuels to heat and light their homes and cook their food. After assessing global solid fuel use, researchers estimate that 52% of the world's people burn solid fuels such as wood, coal, peat, and dung [*EHP* 114:373–378; Rehfuess et al.]. Burning these fuels, they say, can profoundly harm the health of the people exposed to them as well as damage regional environments.

The researchers set out to assess household solid fuel use on a country-by-country basis. In this report the researchers describe the impact that increases in worldwide dependence on solid fuels would have on meeting the UN Millennium Development Goals.

These eight goals set in 2000 aim to reduce poverty, hunger, disease, illiteracy, environmental degradation, child mortality, and gender inequality, and improve maternal health.

For 52 wealthier countries (in which per-capita income is more than US\$10,500), the researchers assumed that fewer than 5% of the population depended on household solid fuels. For 147 poorer countries, the researchers melded surveys and modeling where possible. They collected national census or household survey data on solid fuel use—often for cooking only, the fate of most household solid fuel—for 93 countries. For 36 countries that had no such data available, the researchers modeled solid fuel use based on factors such as gross national income and each country's proportion of rural dwellers. Finally, for 18 countries, many of them small island states such as the Cook Islands, the Maldives, and Tuvalu, there were not enough data available to feed the models. These 18 countries were excluded from the study.

Solid fuel use varied widely among the low-income regions, from 77% in sub-Saharan Africa to 16% in Latin America. According to the authors, 3.2 billion people depended on solid fuels as of 2003, not many fewer than the estimated 3.4 billion using such fuels three years earlier. About 75% of these people burned biomass fuels, which can lead to depletion of natural resources when harvested, and which typically are burned in crude stoves or open fires, resulting in incomplete combustion and releasing high levels of greenhouse gases.

Significantly reducing global dependence on solid fuels is necessary if the Millennium Development Goals—in particular reducing child mortality and improving maternal health—are to be met, the researchers explain. Burning solid fuels in cooking rooms, where women and their children typically spend much of their time, fills homes with pollutants such as carbon monoxide, particulate matter, and other carcinogens that penetrate deep into the lungs.

The researchers believe global society must embrace safe alternatives to solid fuels if the Millennium Development Goals are to be achieved, and they point to examples of interventions already in place that have rapidly bettered the lot of solid fuel users. It is essential, they write, that nations work together to make the necessary policy changes and implement technical solutions. —**Scott Fields**



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