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ENVIRONMENTAL HEALTH PERSPECTIVES
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LESSON 1
Taking Action on Passive Smoking

LESSON 2
Air Pollution Testing: New and Improved!

LESSON 3
Controlling Pollutants: A Look at California's Model

NIEHS
National Institute of Environmental Health Sciences

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STUDENT EDITION

Man always travels along precipices. His truest obligation is to keep his balance.
Pope John Paul II (1978–2005)

WARFARE

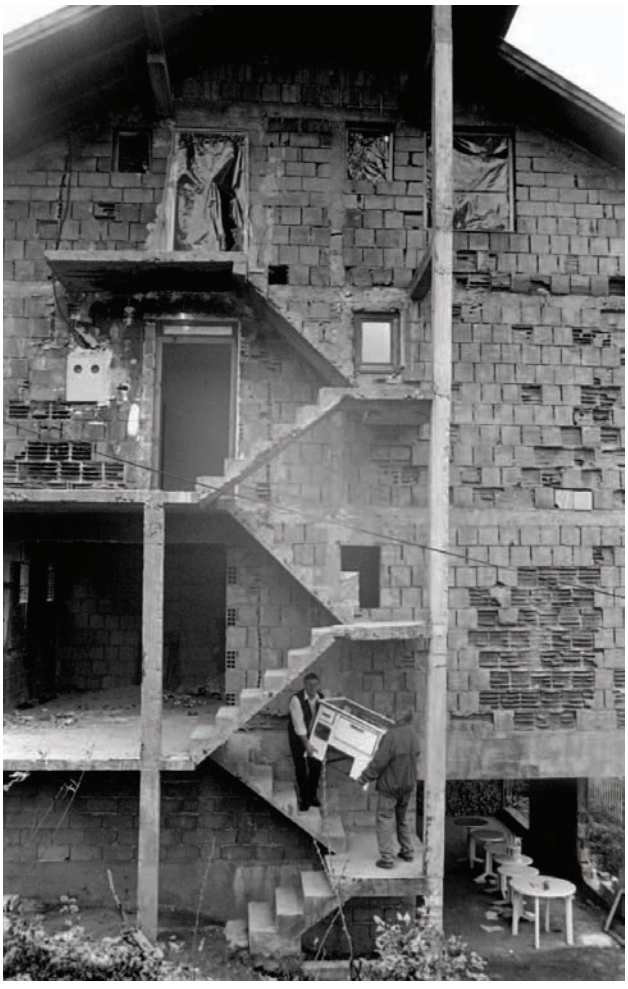
Rallying Around the Environmental Flag

Social scientists have long studied competition for natural resources as a source of conflict around the world, but they have paid little attention to the environment in postconflict societies. Must the environment invariably suffer in the wake of conflict? Can former combatants rally around the environment to help sustain peace? These were the types of questions asked at a November 2006 workshop convened by the Nicholas Institute for Environmental Policy Solutions at Duke University in conjunction with the Environmental Change and Security Program of the Woodrow Wilson International Center for Scholars, the Harrison Program on the Future Global Agenda at the University of Maryland, and the Center for Unconventional Security Affairs at the University of California, Irvine.

Noting that half of all peace agreements collapse within five years, Erika Weinthal, an associate professor of environmental policy at the Nicholas School, asked whether the environment is being addressed in these agreements and whether it can be used to help people think and act "beyond borders." Ken Conca, director of the Harrison Program on the Future Global Agenda, commented that the UN is involved in 30 peacekeeping missions around the world. One common element of the conflicts he has analyzed is a high environmental toll. "The environmental dimension of peace is ignored at great peril, especially in poor countries, in rural areas, and among disenfranchised people," Conca said. "Do not look at the environment as a secondary issue to be dealt with later."

Richard Matthew, director of the Center for Unconventional Security Affairs, cautioned that many factors rule against addressing environmental issues in the immediate wake of conflict. He said that governing bodies charged with protecting the environment typically do not function well or face large budget cuts, while NGOs find it difficult to operate. Infrastructure is often damaged, and criminal activity and profiteering (such as illegal logging) proliferate. Large numbers of refugees and/or internally displaced persons tax natural resources, and traumatized citizenry lack trust in the motives of outside organizations.

As a consultant to the European Commission on peace settlements in Eastern Europe, Alexander Carius of Adelphi Research agreed with Matthew's summation. "It's difficult to push transboundary environmental programs when the institutions don't exist to carry them out," he said.



Recovering worlds torn apart. Bosnian Muslims return to their homes in Foca, the first to return to the village eight years after an ethnic cleansing campaign by Serbian forces in 1992.

Paul Lowe/Panos Pictures

At the same time, people in postconflict societies are desperate for water, fuel, shelter, and food. Matthew said every effort should be made to meet these needs in ways that are sustainable from the outset in order to avoid long-term problems that might undermine reconstruction efforts. One example of this is a program sponsored by the Belgian government that supplies gas cookstoves in Rwanda to limit illicit woodcutting.

Liz McBride, director of the Post-Conflict Development Initiative of the International Rescue Committee, said her organization traditionally provided direct relief for victims of war, but eventually recognized the need to be more involved in contributing to durable solutions from the start. "We were good at saving lives, but we needed to strengthen institutions in a way that would bring people back together," she said. "For us, that now involves building governance systems, which is a movement beyond traditional humanitarian action." Several speakers acknowledged that in postconflict societies, environmental problems will not be solved unless and until basic governance systems are in place.

Judy Oglethorpe, director of community conservation at the World Wildlife Fund, said her organization recognizes the importance of maintaining a presence throughout a conflict, especially during periods of political transition. "Great windows of opportunity open up when a new government comes in," Oglethorpe said. "If you are there, you can have an influence on new policies." She cited as two examples Nepal, where conservation measures have been incorporated into a new draft constitution, and Mozambique, where demobilized soldiers were employed as park guards following that country's peace agreement in 1994.

Weinthal says the event cosponsors are now trying to come up with a research design to address a range of questions that arose in the wake of the panel and workshop. "We need to come up with recommendations for research that are both theoretical with respect to the environment and have direct policy relevance," she said. **–John Manuel**

Secondhand Smoke

Displaced Enthusiasm?

As evidence mounts that secondhand smoke (SHS) can harm human health, an increasing number of U.S. and Canadian cities are passing bans on smoking in restaurants and bars. Proposed bans have been opposed by a few commercial establishments and their respective trade associations, who fear they may lose clients as a result. Some heating, ventilating, and air-conditioning (HVAC) contractors have suggested that a method called "displacement ventilation" can effectively control SHS, making it unnecessary to impose smoking bans. But a recent study indicates these systems cannot be depended upon to bring SHS down to safe levels.

Displacement ventilation systems typically introduce fresh air at or near floor level at a temperature slightly below the desired room temperature. This cooler fresh air displaces the warmer room air at the occupied level; heat and pollutants rise to the ceiling and are drawn out by an exhaust fan. (In comparison, traditional HVAC systems supply air through ceiling vents and recirculate it after diluting it with outdoor air.) A study in the December 2001 issue of *Regulatory Toxicology and Pharmacology* reported that displacement ventilation can control SHS in smoking areas of restaurants. That study has been used to justify opposing local and provincial smoking ban proposals.

Citing various flaws in that study, James Repace, an adjunct professor of public health at Tufts University School of Medicine, and Kenneth Johnson, a research scientist with the Public Health Agency of Canada, undertook their own study of displacement ventilation, which was published in the fall 2006 issue of *IAQ Applications*. They selected the same establishment used in the 2001 study. The Black Dog Pub housed a smoking bar connected by two pass-through windows and two open doorways to a nonsmoking dining room. Ventilation air was drawn into the nonsmoking area and exhausted out the far corner of the smoking area.

Repace and Johnson conducted real-time measurements of particulate polycyclic aromatic hydrocarbons (PPAH), a tobacco smoke carcinogen, and respirable suspended particles (RSP), known to contribute to a variety of respiratory problems. The tests measured PPAH levels of 152 ng/m^3 in the Black Dog's smoking section and 16 ng/m^3 in the nonsmoking section. RSP levels of 199 µg/m^3 and 40 µg/m^3 were recorded in the smoking and nonsmoking areas, respectively. Measurements taken later, after a smoking ban was implemented, showed that levels of RSP and PPAH dropped by 80% and 96%, respectively, in the smoking area, and by 60% and 80% in the nonsmoking area. According to Repace, *de minimis* (i.e., negligible) risk levels of SHS would occur at average RSP concentrations of 0.075 ng/m^3 for persons exposed to an average of 8 hours a day over 40 years (PPAH is not regulated).

The following year, Repace and Johnson conducted similar tests in two restaurants in Mesa, Arizona. The restaurants were exempt from the city's nonsmoking ordinance based on their managers' claims that they could meet smoke-free standards by using displacement ventilation. At Romano's Macaroni Grill, RSP levels averaged 80 µg/m^3 in the smoking bar and 229 µg/m^3 in the adjacent nonsmoking restaurant. PPAH levels averaged 304 ng/m^3 in the bar and 451 ng/m^3 in the restaurant. At T.G.I. Friday's, RSP levels averaged 205 µg/m^3 in the smoking bar and 306 µg/m^3 in the nonsmoking restaurant. PPAH levels averaged 13 ng/m^3 in the bar and 2 ng/m^3 in the restaurant (the latter reflects in part a period during which an outside door was propped open).

Based on the nonsmoking sections' having higher levels of pollutants than the smoking sections, the authors concluded that the ventilation systems in both restaurants were seriously out of balance. However, the Black Dog system, though properly designed and operated, still could not prevent all workers and patrons from being exposed to hazardous levels of SHS.

David Sutton, a spokesman for Phillip Morris USA, says he can't comment on displacement ventilation in particular, but maintains that "in many indoor public places, reasonable ways exist to respect the comfort and choices of both the smoking and nonsmoking adults." Sutton says establishment owners "should have the flexibility to address the preferences of nonsmokers and smokers through separation, separate rooms, and/or high-quality ventilation."

However, Repace and Johnson concluded that banning smoking is the only way to guarantee a smoke-free indoor environment. "The 2006 Surgeon General's report states flatly that there is no safe level of SHS exposure," Repace says. "Displacement ventilation is not a viable substitute for smoking bans in controlling SHS exposure in either designated smoking areas or in contiguous designated nonsmoking areas." Repace says studies indicate that if you can't smell tobacco smoke, you are probably not being exposed to a dangerous amount. However, he adds, people with heart conditions or asthma should avoid any place where people are smoking. **–John Manuel**

Top to bottom: philpell/iStockphoto; Karin Lau/Shutterstock

Défense de Fumer

February 2007 saw something many believed could never happen: the banning of public smoking in France, a country often seen as staunchly pro-smoking. Public places as defined by the law include metro stations, museums, government offices, and stores, but not streets. Cafés, nightclubs, and restaurants have until January 2008 to comply with the ban. Individuals found lighting up will be fined about US$97, while the establishments where the person is found breaking the rules will be fined US$195. The French government will partially subsidize smoking cessation treatments to help residents quit smoking. In France, 60,000 deaths each year are directly linked to tobacco use, and 5,000 are attributed to secondhand smoke.

Green Building Comes to DC

The District of Columbia passed legislation in December 2006 that makes it the first major city to require private developers to follow the Leadership in Energy and Environmental Design (LEED) standards of the U.S. Green Building Council. Under the law, district-funded commercial and housing projects beginning in 2008 must meet LEED standards. All commercial structures of 50,000 square feet or more must meet the standards by 2012. Separate standards for schools, still being developed by the council, are also to be adopted. Washington's new baseball stadium is already being built in compliance with LEED standards.

Will WIC Can Tuna?

A number of health advocacy groups have urged the USDA to remove canned tuna from its Special Supplemental Nutrition Program for Women, Infants, and Children, also known as WIC, saying the inclusion of tuna exposes breastfeeding mothers and their nursing infants to methylmercury when safer fish options exist. Though the agency plans to end an allowance for canned albacore tuna under the program, it may still offer light tuna, which critics say also can contain enough mercury to cause health effects. A 2005 Institute of Medicine review of the WIC program recommends offering canned salmon, which has far less mercury than tuna and costs only about 2¢ more per ounce. Over 250,000 women exclusively breastfeed as part of WIC, and canned tuna is offered as an incentive to those mothers who make this commitment. More than 8 million low-income mothers and their children receive WIC assistance each month. A final decision on tuna's inclusion is expected in September 2007.

ENVIRONMENTAL JUSTICE

The Tuskegee Legacy Project

Medical research studies often do not include ethnic and racial minorities as study participants in numbers that are representative of their populations. A study published in the November 2006 issue of the *Journal of Health Care for the Poor and Underserved* aimed to determine whether the paucity of minorities included in research could be explained by differences in willingness and misgivings related to participation in health research.

The study, funded by the National Institute of Dental and Craniofacial Surgery, was conducted by a research team within the Tuskegee Legacy Project (TLP), which was inspired by a 1994 bioethics conference at the University of Virginia. The research team was created to assess how the infamous Tuskegee Syphilis Study affected the attitudes of black Americans toward health research. From 1932 to 1972, 399 black men with syphilis were studied to observe the effects of untreated syphilis, even though effective treatment was already available. This unethical study has often been used to explain the assumption that blacks may be more prone than whites to distrust research and refuse to participate.

To test this assumption, the research team developed the TLP Questionnaire, which contained two scales: the Likelihood of Participation Scale and the Guinea Pig Fear Factor Scale, which measured self-reported general willingness to participate in research and fear of participating in research, respectively. The TLP Questionnaire was taken by more than 1,000 black, white, and Hispanic residents of four U.S. cities (two in Alabama including Tuskegee, one in Texas, and one in Connecticut).

Significantly, the results showed that only about 30% of all people surveyed expressed a willingness to participate in research studies. Blacks were 1.8 times as likely as whites to fear participating in biomedical research. Still, they were equally as willing to participate in research as whites. "Given the history of the treatment of African-Americans in our country, it makes sense that blacks would have a heightened awareness of potential dangers," says lead author Ralph V. Katz, chairman of the Department of Epidemiology and Health Promotion at the New York University College of Dentistry.

These findings are consistent with the few other studies in which racial/ethnic differences in research participation have been assessed. They also are particularly important in studies addressing health disparities and those that aim to study environmental justice issues in minority populations. "African Americans come from varied experiences in the health care system. As such, there is no monolithic response to health-seeking behaviors, including participation in health research," says Ruth Browne, CEO of the Arthur Ashe Institute for Urban Health in Brooklyn, New York. "This really points to the importance of culturally appropriate outreach efforts."
–Luz Claudio

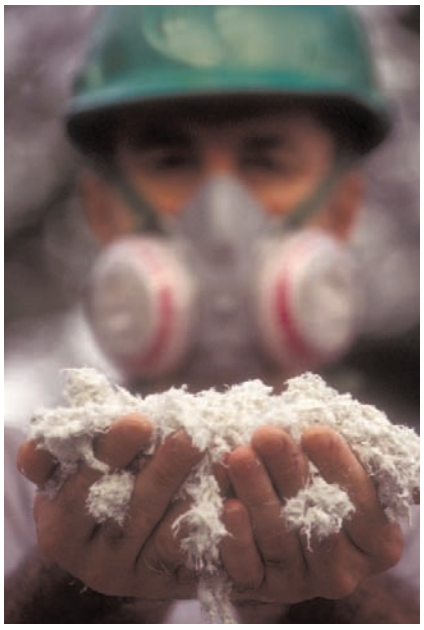
POLICY

Chrysotile on Ice

Parties to the Rotterdam Convention, a group of more than 100 countries that have agreed to share information about hazardous chemicals, elected in October 2006 not to add chrysotile asbestos to the list of hazardous chemicals subject to right-to-know export controls. The 1998 Rotterdam Convention currently requires prior informed consent (PIC) for more than 30 chemicals, meaning an exporting nation must ensure that the substances do not leave its territory without the informed consent of recipient countries.

This is the second time the Convention has declined to list chrysotile, a track record that raises serious concerns about the future of the agreement, according to Carl Smith, vice president of the nonprofit Foundation for Advancements in Science and Education. "Listing isn't a ban," he says. "It's just an agreement to share information." He adds, "Chrysotile would be on the PIC list already if the member countries would just follow through on the agreement they made when they joined the convention. If parties are going to start ignoring the Convention text, the train is off the tracks."

Chrysotile fulfills all the requirements for listing, Smith says, but unlike many of the other chemicals on the list—such as polychlorinated biphenyls, lindane, and all other types of asbestos—chrysotile is still economically important. Use of asbestos in Western countries has declined due to health concerns, but chrysotile-based products such as pipes and roof shingles are still widely used in the developing world. Trade in chrysotile is worth $600 million a year, according to a 7 November 2005 *Wall Street Journal* estimate.



The stuff of controversy. Chrysotile asbestos was not added to the Rotterdam Convention despite concerns about its health effects.

Brasil2/iStockphoto

Prior to the October meeting, a panel of 31 experts on the convention's Chemical Review Committee determined that chrysotile met the criteria for listing. Most of the meeting participants supported the proposal to list chrysotile. But Canada, Ukraine, Russia, Kyrgyzstan, India, Iran, and Peru objected and blocked action, citing either scientific uncertainty about chrysotile's health effects or the material's usefulness.

The Chrysotile Institute, a nonprofit organization funded by the Canadian government, maintains that chrysotile is not as toxic as amphibole asbestos. Institute president Clément Godbout says the high rates of respiratory disease and cancer associated with asbestos stem from exposure to the amphibole form and to high exposures from dangerous past practices such as blowing asbestos mixtures onto walls for insulation and fireproofing. Used properly, chrysotile is a cost-effective ingredient for the cement that is often used in water pipes in underdeveloped countries, he contends.

But many groups say that the concept of controlled use, particularly in developing countries, is a fallacy. Chrysotile is classified as a human carcinogen by the WHO, the Collegium Ramazzini, the World Trade Organization, and other groups. In the meantime, the parties to the Convention have deferred further consideration of the issue until their next meeting in 2008.
–Rebecca Renner

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California Environmental Protection Agency

Sizewise, California is the third largest state in the United States, it has the largest population, and its economy ranks among the top ten in the world. Because of its economic clout, laws that are made in the state can have a ripple effect throughout the country and even the world. One area of law in which California is making an impact is environmental issues. The California Environmental Protection Agency (Cal/EPA) website at http://www.calepa.ca.gov/ provides information on the state's many initiatives and programs.

At the center of the homepage is a Topics of Interest section, currently headed by information on the Cal/EPA Climate Action Team. This team was established by a June 2005 executive order signed by governor Arnold Schwarzenegger that also created greenhouse gas targets for the state. The team, which submitted its first biannual report to Schwarzenegger and the state legislature in April 2006, is composed of members from several state agencies and charged with implementing and monitoring programs for reducing emissions that contribute to global warming. The Climate Action Team section of the site contains the 2006 report, public comments on the draft of the report, and fact sheets on California's climate change activities and policies.

Four of the nation's busiest 20 ports are in California. The concentration of diesel emissions in these areas, where ships, trucks, and trains converge, contributes to a toxic mix of air pollutants that threatens the health of nearby residents. According to a September 2005 article in the *Los Angeles Times*, the port complex in that city has become the single largest air polluter in the Los Angeles Basin. The Cal/EPA Topics of Interest section has a link to the state's recently unveiled Goods Movement Action Plan, which includes approximately 200 potential projects in areas including public health and environmental impact mitigation and community impact mitigation.

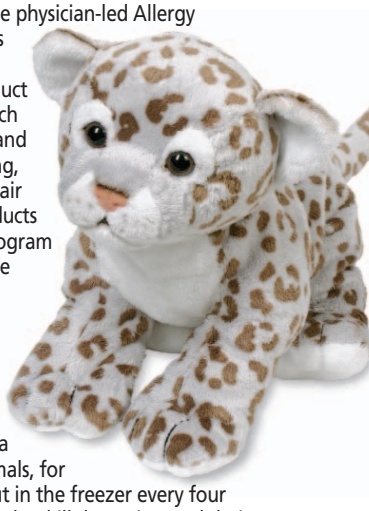
Another Topic of Interest centers on California's efforts to develop hydrogen as an alternative fuel. Within the Hydrogen Highway Initiative section is information on pertinent laws passed by the state. The latest of these, Senate Bill 76, provides funding for state-funded hydrogen demonstration projects including fueling stations and the purchase of hydrogen-fueled vehicles. Also available are fact sheets, brochures, and other documents about these fuels.

Waste disposal is the fourth Topic of Interest currently featured on the website. According to the Cal/EPA, Californians have cut their amount of trash in half since 1989. Among other initiatives that have facilitated this progress is the California Take-It-Back Partnership, a project between the state government and the business sector to provide convenient drop-off points for toxic trash such as used batteries, fluorescent lamps, and electronic devices. Also in this section are pages for consumers that answer the questions of why, what, how, and where they can recycle, what "zero waste" is, and where all of California's trash goes.

The Cal/EPA homepage also offers links to information on children's environmental health, environmental justice, environmental sustainability, and the Education and the Environment initiative, which mandates a broad-ranging strategy to bring education about the environment into the state's K–12 schools. **–Erin E. Dooley**

A Friend Indeed

The Asthma and Allergy Foundation of America, in conjunction with the physician-led Allergy Standards Limited, has developed the first asthma friendly® product standards for items such as plush toys, pillows and other bedding, flooring, vacuum cleaners, and air filtration devices. Products certified under the program are less likely to expose asthma and allergy sufferers to allergenic materials or chemical irritants. Certified items also come with instructions for keeping them "asthma friendly." Stuffed animals, for instance, should be put in the freezer every four weeks and then washed to kill dust mites and their eggs. A list of certified products is available at http://www.asthmafriendly.com/.



Backyard Boiler Risk

In many areas of the United States, residents are using outdoor wood boilers to save money on heating oil and natural gas. These units are not equipped with air pollution controls, nor are they regulated, and owners are free to fuel them with anything that will burn, including painted wood and garbage. According to a study slated for the February 2007 issue of *Human and Ecological Risk Management*, the emissions from these units may significantly increase the risk of cancer, heart attack, and heart disease. People breathing the smoke from these boilers have a lifetime cancer risk of 1 in 1,000—practically the same odds faced by a cigarette smoker. According to estimates by the Michigan Department of Environmental Quality, in one hour of use a typical outdoor wood boiler may emit 160 g of toxics including benzene, dioxins, and polycyclic aromatic hydrocarbons.

Young Lungs in China

As China's economy booms, so does its air pollution. This, according to a November 2006 Chinese health report, is a main reason why increasing numbers of Chinese people in their 30s are now beset with chronic lung diseases that traditionally have affected mostly elderly people. The report says about 43 million people in China are affected by chronic lung diseases such as emphysema and chronic bronchitis, with about 1 million of these dying each year. Smoking was also named as a culprit in the rise of these diseases.


Datong, China

Top to bottom: Allergy Standards Ltd.; P_Wei/iStockphoto; J.B. Russell/Panos Pictures

Centered on Breast Cancer

A relationship between early menarche in girls and later development of breast cancer has long been observed. Some environmental factors, such as diet and exposure to endocrine disruptors and other chemicals, could affect children's timing and pace of puberty and development. This leads to the question of whether exposure to these agents may also lead to breast cancer later in life. That is the scientific premise for the establishment of the Breast Cancer and the Environment Research Centers (BCERCs), which presented results of ongoing studies at their third annual meeting on 2–3 November 2006.

The centers are co-funded by the NIEHS and the National Cancer Institute (NCI) Epidemiology and Genetics Research Program. They work together to integrate community outreach and two lines of research: 1) basic biology of the mammary gland and its development using animal models, and 2) epidemiological studies of how environmental factors affect puberty in girls. Four centers were established across the nation: the Fox Chase Cancer Center in Philadelphia (collaborating with Mount Sinai School of Medicine in New York and the University of Alabama at Birmingham), the University of Cincinnati in Ohio (collaborating with Cincinnati Children's Hospital Medical Center), the University of California, San Francisco (collaborating with numerous partners including Lawrence Berkeley National Laboratory), and Michigan State University in East Lansing.

"Using a transdisciplinary scientific approach, the centers are able to investigate possible windows of susceptibility during pubertal development more comprehensively and on a larger scale," said Shannon Lynch, a program analyst at the NCI. The BCERCs' ability to work closely together, share resources, compare findings, and establish common research protocols is especially important in epidemiological studies. With each center recruiting a number of girls from diverse racial/ethnic, geographical, and socioeconomic backgrounds, the data that are ultimately generated should be more robust due to a greater number of samples that are more representative of different populations.

Research Update

One example of the kind of multidisciplinary data that can be generated by this collaboration can be found in a paper presented at the meeting, which was later published in the January 2007 issue of *EHP*. Center researchers collaborated with the CDC to analyze urine samples collected from 6- to 8-year-old girls to assess the levels of biomarkers of exposure to a number of phytoestrogens, phthalates, and phenols. Many of these compounds are known or suspected to cause endocrine disruption.

Window to the future. The Breast Cancer and the Environment Research Centers are looking at ways that early environmental exposures may influence later breast cancer risk.

The authors found that these potentially hormonally active compounds were widely detectable in the girls studied. For several of the compounds, concentrations varied by geographic location, body size, or race/ethnicity such that meaningful comparisons may become possible. It is still too early to tell whether exposure to these compounds will be associated with puberty, but so far the preliminary data show that exposures can be substantial and perhaps biologically relevant.

Each center is also producing new research results within its own populations of study. The University of California center, for example, working together with the Kaiser Permanente Division of Research, has recruited 444 girls, along with their families, to study how environmental exposures may affect growth and development. The study participants are members of Kaiser Permanente of Northern California, an integrated prepaid health care system, so researchers have access to early medical information for the girls including birthweight.

In preliminary analyses, Larry Kushi, associate director of the Kaiser Permanente Division of Research, and colleagues found that higher birthweight predicted risk of overweight in 6- to 7-year-old girls. It is important to assess peripubertal obesity in girls enrolled in the study because obesity is a strong predictor of breast development; how obesity may affect timing and degree of puberty development is one of the subjects of this investigation. A significant proportion (29%) of girls enrolled in this study were classified as overweight, defined as being at or above the 85th percentile for body mass index. Ongoing analyses are assessing the levels of pubertal development in the cohort. The preliminary findings suggest that the black and Latina girls are having a higher prevalence of early puberty onset.

Investigators at the meeting also presented results of studies conducted in the basic science components of their centers. Deborah J. Clegg, an assistant professor in the Department of Psychiatry at the University of Cincinnati College of Medicine, conducted a yet-unpublished study to assess how caloric content and type of dietary fat eaten may affect obesity and carcinogenesis susceptibility in laboratory rats. Her team tested high- and low-fat diets rich in olive oil, fish oil, safflower oil, or butter (a "1950s diet"). They found that the high-fat olive oil diet accelerated puberty and increased propensity to carcinogenesis in the exposed rats. Additionally, they found that the high-fat diets enriched with safflower oil and fish oil increased body weight and susceptibility to carcinogenesis. Interestingly, however, the high-fat butter diet did not increase body weight or carcinogenicity.

Defining Messages

In addition to the basic and epidemiological research being conducted, each center

Ramona Helm/Shutterstock

also has a Community Outreach and Translation Core (COTC). The COTCs have different functions within each center, including sharing information with the community, conducting research on translating science findings, and studying recruitment and retention strategies.

The COTC at Michigan State University presented data on the types of meaningful messages about breast cancer that women remember for long periods of time and the sources of such messages. Based on responses from 137 women who completed an online questionnaire, researchers concluded that most messages were recalled from the mass media. Most messages were about the detection of breast cancer rather than its prevention. These findings demonstrate that the media has a strong influence on the messages received by women, perhaps more influence than the medical community.

The issue of what health messages can impact the community at large resonated with many participants present at the meeting, which included leaders of the breast cancer control advocacy community, who are active participants in the centers. Beth Hartung, a member of the Young Survival Coalition (a network of breast cancer survivors who are 40 or younger), expressed this succinctly as she asked a panel of center scientists, "What do we tell our daughters [about their risk of breast cancer]?" Program administrator Les Reinlib of the NIEHS Division of Extramural Research and Training, himself the father of two daughters and husband to a breast cancer survivor, answered, "We tell them to make lifestyle choices that improve general health."

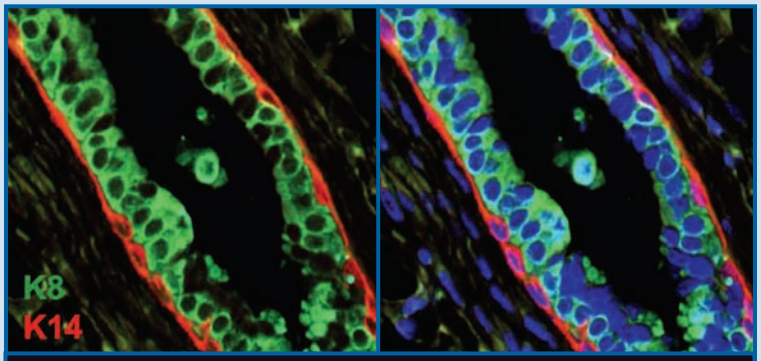
For now, until more definitive research on specific environmental exposures has been completed, exercise and good nutrition are the two main lifestyle choices that can help in the prevention of disease. And despite the uncertainty that remains, there have been many scientific advances that have extended the lives of women diagnosed with breast cancer.

Andrea Ice, cofounder of the Sisters Network (a national breast cancer survivorship organization for black women), has seen this first-hand. "Since my initial diagnosis in 1989, the biggest change has been that women see other women who have survived the disease for many years," she says. "It is no longer a death sentence." The hope is that the BCERCs will be able to add prevention to the progress made in breast cancer research. **–Luz Claudio**

Zena Werb

Headliners | Breast Cancer

NIEHS-Supported Research



Loss of *GATA-3* leads to expansion of undifferentiated luminal cell population

GATA-3 Maintains Differentiation of Mammary Ductal Cells

Kouros-Mehr H, Slorach EM, Sternlicht MD, Werb Z. 2006. GATA-3 maintains the differentiation of the luminal cell fate in the mammary gland. Cell 127:1041–1055.

GATA-3 is one of a family of genes responsible for driving the processes that turn undifferentiated stem cells into specialized mature cells. Now NIEHS grantee Zena Werb of the University of California, San Francisco, and colleagues have determined that the GATA-3 protein is also required for the maintenance of differentiation in ductal cells of the mammary gland. This new finding suggests that *GATA-3* may play a key role in the development of breast cancer.

Mammary ductal cells, also known as luminal cells, line the mammary ducts that carry milk during lactation. Although not much is known about the differentiation of luminal cells, they are implicated as a primary site in the mammary gland for cancers to form. Cancer researchers know that breast tumors with high GATA-3 expression have a good prognosis. These cancers tend to be well-differentiated, and the cells maintain many characteristics of normal mammary cells, including high numbers of estrogen receptors. However, cancers with low expression of the protein tend to be diffuse and poorly differentiated, and lead to poor prognosis for the patient.

Upon devising a microarray strategy to identify novel regulators of mammary development, the investigators observed the mammary epithelium *in vivo* of laboratory mice genetically altered to lack *GATA-3*. They found that mature cells reverted to the less specialized, undifferentiated state, which is characteristic of aggressive cancer cells. The research team also found GATA-3 in all mammary duct luminal cells in normal mice at puberty and into adulthood.

The results suggest that the loss of functioning genes and the subsequent failure to maintain the mature state of the cells is what leads to the loss of differentiation and uncontrollable proliferation during cancer progression. Prior to this finding, it was unclear that maintaining differentiation of mammary cells was an active process and that the GATA-3 protein was responsible for that maintenance. The team is now studying how GATA-3 controls cell fate and its role in breast cancer. This research could also have implications in other cancer types. **–Jerry Phelps**

BEYOND THE BENCH

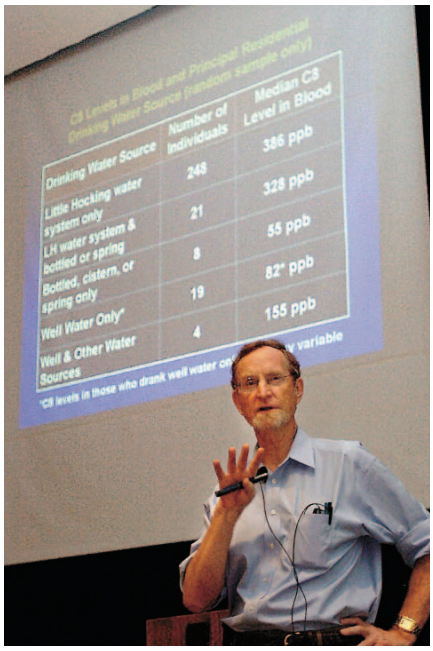
Research Helps Clean Up A Water Supply

Many of the conveniences of modern life are made possible with man-made compounds. One such chemical, perfluorooctanoic acid (PFOA), has a broad spectrum of use, from the manufacture of non-stick cookware to aerospace technology. PFOA's persistence in the environment is troubling, especially given studies demonstrating that exposure to the compound can cause developmental delays and cancer in lab animals. Thus, when PFOA was detected in the water supply of Little Hocking, a village located across the Ohio River from and downwind of a Washington, West Virginia, fluoropolymer manufacturing facility, researchers at the University of Pennsylvania NIEHS Center of Excellence in Environmental Toxicology (CEET) felt compelled to investigate. The contamination was first reported to Hong Zhang, a local doctor enrolled in a practicum residency for physicians in occupational and environmental medicine at the university.

According to CEET deputy director Edward Emmett, who also directs the center's Community Outreach and Education Core, the research team's immediate focus was on determining whether, how, and to what extent Little Hocking residents were being exposed to PFOA. The CEET investigators joined with community partners Grand Central Family Medicine in Parkersburg, West Virginia, and the Decatur Community Association in Cutler, Ohio, to design a study, recruit study participants, and collect data. The group applied for and received an environmental justice grant from the NIEHS, and began work in July 2004.

The investigators distributed questionnaires to a random sampling of residents who used either private or public drinking water sources, and examined blood serum samples to assess PFOA concentrations. PFOA water concentrations were obtained from the Ohio EPA. Levels averaged 3.55 ng/mL in 2002–2005, some of the highest ever reported in public water supplies in the United States.

Overall, blood serum analysis showed that the residents' levels were 60–75 times higher than in the general U.S. population. The investigators found that serum PFOA was especially high in those who ate more home-grown fruits and vegetables. Emmett says it is unclear if this was due to PFOA making its way into the fruits and vegetables themselves, or to PFOA in water used for cooking, canning, and cleaning.



Town meeting. Edward Emmett presents results of the CEET's findings to the Little Hocking community.

An air dispersion model based on estimated emissions from the Washington plant revealed that serum PFOA levels were no different for those people living in areas with higher air concentrations than for those living where there was minimal PFOA in the air. Regardless of location, higher concentrations were found in young children and older adults, as well as in people who worked directly with PFOA in production areas of the Washington plant (all three groups' serum levels were almost twice as high as other residents accessing the Little Hocking water supply).

The research team examined all blood samples for biomarkers indicating DNA damage, but found no sign of adverse health effects. However, given what is known about the chemical's effects in lab animals, Emmett says that lowering the Little Hocking residents' exposure was prudent, and the independent research conducted by the partnership helped empower the community to secure a cleaner drinking water supply. "What has been so compelling and gratifying about this work has been witnessing how powerful credible, nonbiased information collected without conflict of interest can be in altering peoples' behavior voluntarily," says Emmett.

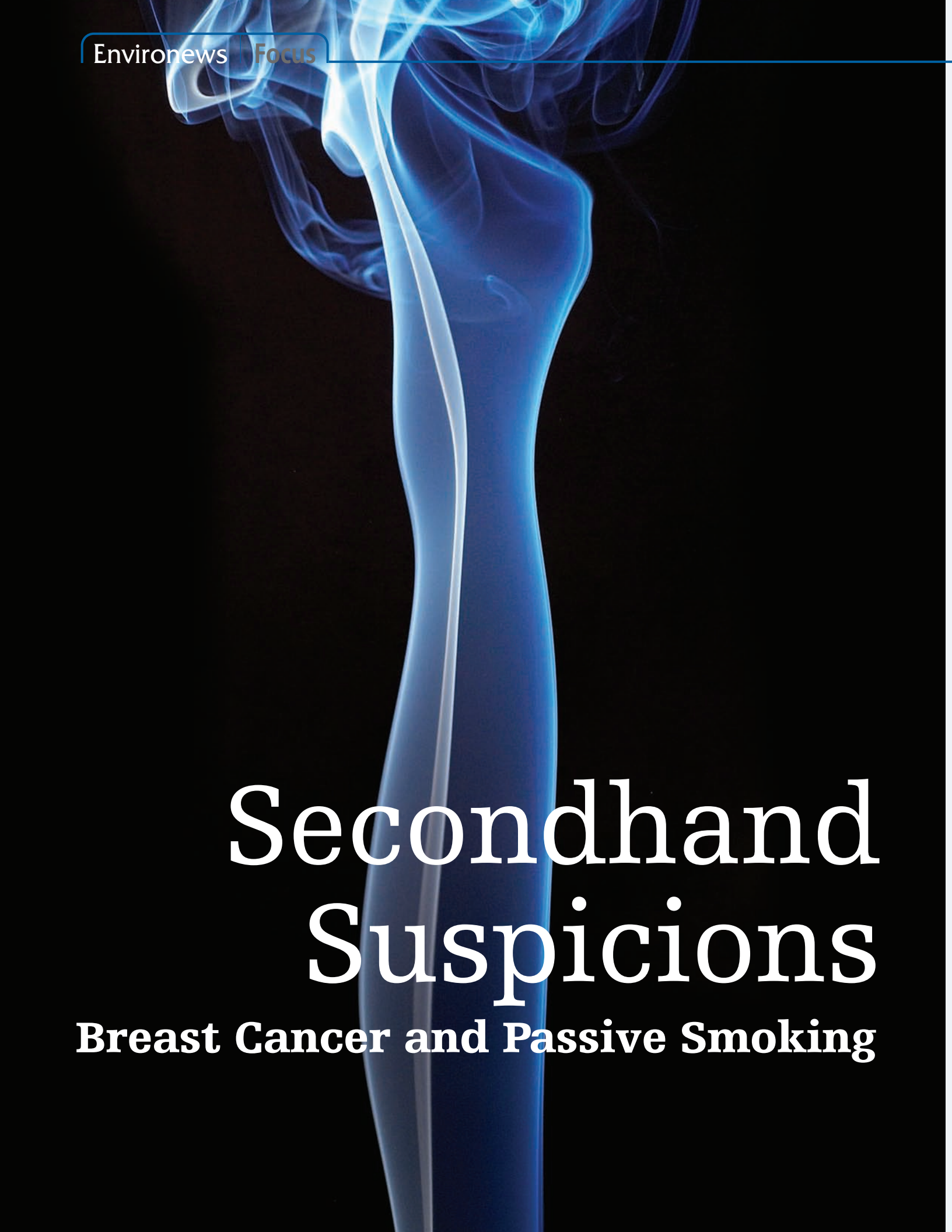
The Washington plant began offering bottled water to all residents being serviced in the Little Hocking Water District within days of an October 2005 community meeting where study results were presented. Other findings from the study suggested that carbon filters in the home could help to remove some PFOA from the water. Still, these were not considered viable long-term solutions, so a new filtration facility is being created to remove all PFOA from the water supply. The facility should be functioning in a few months.

Emmett says it is also gratifying to know that the partnership's research has had a positive impact on the community. One resident remarked, "There was a large fine from EPA. There was a lawsuit, and a lot of money changed hands. But it's [the CEET] study that has changed the water I drink."

The study, which won first prize in the May 2006 EPA Science Forum, was described in two articles in the August 2006 *Journal of Occupational and Environmental Medicine*. A follow-up study is now under way to measure community members' current PFOA blood levels. More information is available at http://lhwc8study.org/.

–Tanya Tillett

CEET



Secondhand Suspicions

Breast Cancer and Passive Smoking

Does a young woman living with a smoker or taking a job working in a smoky bar have a greater chance of developing breast cancer? Some scientists believe that such situations can indeed raise a woman's risk of developing breast cancer before the age of 50. Because epidemiological and toxicological studies show that women's breast tissue may be especially sensitive to exposure to carcinogens prior to first pregnancy, these researchers contend that public education should be directed at alerting adolescents and young women to the potential risk. However, not everyone in the international public health community agrees that the evidence to date supports a link between passive smoking and breast cancer, and some say that women are being alarmed unnecessarily. This disagreement has sparked debate that is sometimes heated.

The stakes are high because breast cancer is the most common cancer in women in industrialized countries, according to the WHO. It is the leading cancer killer of nonsmoking women, and second only to lung cancer deaths among women who smoke.

Among the researchers interviewed for this article who disagree that there is enough evidence to link secondhand smoke (SHS) with breast cancer, the majority call the evidence to date "suggestive but not sufficient," as the Surgeon General's 2006 report, *The Health Consequences of Involuntary Exposure to Tobacco Smoke*, put it. That characterization is based largely on the fact that the research considered when the Surgeon General's report was being amassed did not clearly link even active smoking to breast cancer. Researchers in this camp do, however, stress that ongoing campaigns to prohibit smoking in public will protect the whole of society against the wide variety of ills proven to be caused by SHS. These include lung cancer, cardiovascular disease, and sudden infant death syndrome, among others.

A smaller group contends that the question of whether or not SHS causes breast cancer is a political issue with the potential to compromise the scientific process. "A premature decision about causality could jeopardize the credibility of the entire review process and all of the other, established effects of secondhand smoke," says Michael Thun, national vice president of epidemiology and surveillance research for the American Cancer Society. Adds Valerie Beral, director of the University of Oxford Cancer Research UK Epidemiology Unit, "To prematurely come to conclusions about the causation when there is a big division in the scientific community . . . is bad science."

Thun debated the subject in a series of public forums held in conjunction with scientific meetings. Taking the opposing view was Kenneth C. Johnson, a research scientist with the Public Health Agency of Canada, who was one of the first scientists to discern a potential link. During the debates, Johnson pointed out there are about the same number of studies linking breast cancer to passive smoking as there were linking lung cancer to SHS in 1986, when the Surgeon General concluded that

Mariusz Szachowski/Shutterstock

passive smoking caused lung cancer. Johnson also says that more of the breast cancer studies are statistically significant, and that the estimated risk for breast cancer is higher.

The Importance of Carcinogens in Tobacco Smoke

The suspicion that exposure to SHS could cause breast cancer dates back more than two decades. Among the more than 50 carcinogens in tobacco smoke are approximately 20 substances listed as mammary carcinogens by the International Agency for Research on Cancer. These include compounds such as dibenzo[*a,l*]pyrene, which the research literature identifies as an extremely potent carcinogen in mammary tissue.

The chemicals in tobacco smoke are a mixed bag of directly genotoxic DNA-damaging compounds ("initiators"), compounds that enhance the action of these initiators ("promoters"), and compounds that do both, says Andrew Salmon, a toxicologist at the California EPA's Office of Environmental Health Hazard Assessment (Cal/EPA OEHHA). Some of these substances are more abundant in sidestream smoke, which comes off the tip of the cigarette, than the smoke inhaled by smokers themselves. This sidestream smoke is the major source of SHS.

Numerous studies have shown that toxicants from cigarette smoke reach rodent mammary tissue and can form the DNA adducts believed to represent the first step of carcinogenesis. If not repaired or if repaired incorrectly, these modifications may eventually lead to mutations and ultimately cancer. Irma Russo, a member of the Fox Chase Cancer Center's Medical Science Division, says, "We know that [tobacco smoke] is carcinogenic to the human breast [because we have] utilized some of the carcinogens, such as benzo[*a*]pyrene, that are present in tobacco smoke and induced tumor formation in breast epithelial cells." What is less clear, she says, is when and how this exposure causes cancer in women.

Jonathan Li, a professor in the University of Kansas Medical Center Department of Pharmacology, Toxicology, and Experimental Therapeutics, contends that, while it is true that some tobacco smoke components are strong carcinogens in rodent mammary tissue, the resulting tumors do not reflect the histopathology or molecular alterations seen in the vast majority of human pre-malignancies or in primary tumors except for their estrogen dependency. "A case in point is that carcinogen-induced mammary tumors in rodents are diploid, and human ductal breast cancers are seventy to ninety percent aneuploid," says Li. "Only breast tumors induced in rodents by estrogens are highly aneuploid. This would suggest why there is only a modest link between tobacco smoke and human breast cancer risk."

Salmon and colleagues at the Cal/EPA OEHHA dispute the validity of Li's observations. Salmon points to the differences not only between rodents and humans, but between different rodent species. "For instance," he explains, "most mammary cancers in mice are hormone-independent at the time of detection, whereas nearly all rat mammary tumors are hormone-dependent initially. Human mammary tumors are intermediate in this regard, although the balance varies with age at diagnosis. However . . . both rats and mice are susceptible to mammary carcinogenesis by tobacco smoke constituents." Salmon adds that karyotypic changes including aneuploidy are commonly seen at the later stages of tumor progression, but are not necessarily related to the initial cause of the tumor, being instead often dependent on host-related factors.

The data from epidemiological studies are even murkier. Taken as a whole, the 26 studies on the topic to date appear inconclusive, points out Alfredo Morabia, a professor of epidemiology at the City University of New York and lead author of a study in the 1 May 1996 issue of the *American Journal of Epidemiology* that did show a link. "There are large prospective studies that found no association, but some case–control studies find a strong association and others a weak association," he says. "Some studies indicate that the issue is with younger women, others with older women."

Others put it more bluntly. "If smoking was a major cause of breast cancer, we would have found it by now," says Dale Sandler, chief of the NIEHS Epidemiology Branch, the researcher who published an article linking SHS exposure with several kinds of cancers in the January 1985 *American Journal of Epidemiology*. Li adds that one would anticipate that if carcinogen–DNA adducts were an important end point in human breast cancer, this would eventually translate into mutations in human breast cells. "This is . . . not the case in sporadic ductal breast cancer, which comprises greater than ninety percent of all breast cancer cases," he says.

The researchers who are convinced that the data support a link between SHS and breast cancer counter that they do not claim that such exposure is the major cause of breast cancer, but simply one cause that many women can easily avoid. Mark Miller, a public health medical officer at the OEHHA, and Johnson were among the authors of *Proposed Identification of Environmental Tobacco Smoke as a Toxic Air Contaminant*, the Cal/EPA's first publication to claim a causative link between exposure to SHS and breast cancer. The 528-page report was published in 2005 as part of the evidence mustered by the agency in its ultimately successful bid to become the first state to identify SHS as an air pollutant that could be regulated by the state. The peer-reviewed report devoted 56 pages to the toxicological and epidemiological evidence for a link between breast cancer and passive smoking, including a meta-analysis of the 19 studies available at the time. The WHO is in the process of republishing the report in other languages for worldwide dissemination.

Miller, Johnson, and colleagues also presented their case in a review article published in the February 2007 issue of *Preventive Medicine* with the goal of providing additional information about how they made their determination for the medical and public health communities. The new paper reiterates that women regularly exposed to SHS increase their relative risk of developing breast cancer by age 50 between 68% and 120%. These estimates were first calculated and published in a

This page: Arnold Greenwell/EHP; background: Mladen Mitrinovic; opposite: Eric Audras/PhotoAlto

meta-analysis by Johnson that appeared in the 20 November 2005 issue of the *International Journal of Cancer*.

Exposure Assessment and Age

There are two important reasons why the breast cancer risk from passive smoking can be difficult to tease out from earlier studies: exposure assessment and age. Melanie Marty, chief of the Air Toxicology and Epidemiology Branch of the OEHHA and a coauthor of the *Preventive Medicine* paper, explains, "Exposure assessment is always an issue in epidemiology studies, unless they are lucky and have lots of exposure data. In the case of secondhand tobacco smoke, many of these studies didn't do a very thorough job of determining how long people were exposed, when in their lifetimes they were exposed, how much they were exposed." Further, she continues, if exposure to SHS is not assessed carefully, "you are going to mix up the group that you think is unexposed with the group that you think is exposed."

To address the issue of accurate exposure reporting, the 2005 Cal/EPA report identified a subgroup of studies that did a better job of assessing exposure, which were weighed more heavily in the final evaluation. The report also broke out younger, primarily premenopausal women—which it defined as under the age of 50—as the most vulnerable. This subgroup was first highlighted by Johnson in 2000, and the idea has been reaffirmed in recent findings. For instance, although researchers from the M.D. Anderson Cancer Center recently reported a dramatic 7% overall drop in breast cancer rates between 2002 and 2003, the decline was mainly observed in women aged 50 and older. The drop among younger women was much lower—only 1% for women aged 40 to 49, for example. These data were presented in December 2006 at the San Antonio Breast Cancer Symposium.

Only fourteen of the studies looking at passive smoking and breast cancer allowed analysis by menopausal status, according to the 2005 Cal/EPA report. These included ten case–control studies and four prospective cohort studies that began following a large group of women before any had the disease. Thirteen of these studies reported elevated risk estimates for breast cancer in premenopausal women, and the risk was statistically significant in seven of the studies.

The preponderance of case–control studies is a weakness of the case for linking breast cancer with passive smoking, an issue that Thun stressed in his debates with Johnson last summer. All things being equal, epidemiologists generally consider the findings of cohort studies to be more persuasive than those of case–control studies, because exposure information is ascertained before the development of disease and because both cases and noncases arise from the same study

A matter of age. Much of the discrepancy in findings related to passive smoking and breast cancer may relate to differences between younger and older women. Premenopausal women are believed to be more vulnerable to breast cancer.

population. Beral points out that "every textbook of epidemiology says that once someone has a disease they might remember things differently."

In response, Johnson says he doubts that recall bias explains premenopausal risk, pointing out that one would expect to observe similarly increased risk for pre- and postmenopausal women, which has not been seen. He says it is very difficult to collect good information about passive smoking in prospective study questionnaires.

"In a cohort study, you [might] have to interview a hundred thousand [people] in order to get a thousand cases. In a case–control study, you may have a thousand cases and a thousand controls, so you have to interview two thousand to find out the demographic and exposure information of interest," he explains. "Because you have to interview fifty times as many people [in a cohort study], there's a much higher price [in terms of administering the questionnaire] associated with every question you ask about exposure—the depth and quality of the exposure measures tend to be less unless it is a real focus of the cohort study."

Further, says Russo, a woman might not know whether her grandparents, parents, and other relatives living in the same household smoked, and how much and for how long, nor whether her mother smoked during pregnancy.

Another issue that continues to plague efforts to link passive smoking to breast cancer is the fact that many researchers feel that epidemiological studies have not conclusively linked even active smoking to the disease. This, says Jonathan Samet, chairman of the Johns Hopkins Bloomberg School of Public Health and the senior scientific editor for the Surgeon General's 2006 report, is one reason that report called the breast cancer–passive smoke evidence "suggestive but not sufficient."

Stanton Glantz, a professor of medicine at the University of California, San Francisco, Medical School, claims this is because many of the most recent reports on active smoking weren't considered when the Surgeon General's report was being produced. The *Preventive Medicine* paper says that six large prospective studies have now found a statistically significant elevated risk for breast cancer among smokers for at least some metrics of exposure.

Peggy Reynolds, a cancer epidemiologist at the Northern California Cancer Center, is the lead author of one of the case–control studies that does support a link between active smoking and breast cancer, published in the 7 January 2004 *Journal of the National Cancer Institute*. She and her colleagues have been following 116,544 California teachers since 1995, and reported that the incidence of breast cancer among the cohort's current smokers was higher than that for members who had never smoked. She said that the risk doubled for smokers with more than 31 pack-years—the equivalent of smoking one pack of cigarettes per day over the course of 31 years—compared with nonsmokers.

Although Reynolds did not report finding a link between passive smoking and breast cancer in that paper, she stresses that this could be because the questionnaire used to capture the data included information about whether women were exposed to smoking only in their homes. In a follow-up questionnaire, she and her colleagues asked more detailed questions to include other sources of passive exposure, particularly the workplace, and those data are currently being analyzed. These additional data are important because, although household exposures represented the major exposure source for women in this cohort during earlier decades, "following 1970, the workplace became women's most important source of exposure to secondhand smoke," she says.

This page: AP Photo/Herminio Rodriguez; background: Mladen Mitrinovic; opposite: AP Photo/Mary Godleski

In both their report and review article, Johnson and the Cal/EPA researchers also evaluated the biological plausibility of just how exposure to SHS might cause breast cancer, and concluded that "the chain of evidence indicates that a causal association is highly plausible." Glantz points out that this isn't always taken into consideration in epidemiological studies, which he argues gives the team's conclusion all that much more weight.

A Nonlinear Relationship

However, the chain of evidence regarding biological plausibility doesn't fit neatly with the fact that active smoking does not cause a significantly higher number of breast cancers compared with passive smoking exposure. "One reasonable biological explanation for the similarity in risk would be that the tobacco smoke exposure pathways might become saturated at levels of exposure associated with regular secondhand smoke exposure, so that the higher exposure [from active smoking] would not further elevate the risk," Johnson says.

It also appears that active smoking may partially mitigate effects of carcinogen exposure on the breast in smokers by reducing their estrogen levels. This fits with the M.D. Anderson study, which credited the declining popularity of hormone replacement therapy for the decreasing rates of breast cancer among older women. It might also explain why passive smoking, which is hypothesized to have less impact than active smoking on estrogen levels, could be associated with breast cancer. The problem with this, as Thun points out, is that no toxicological data exist to show there is a nonlinear relationship between the effects of low and high doses of smoke exposure.

Another potential explanation is that women may be especially susceptible during a key "window of exposure" that researchers have previously identified—namely, between puberty and when a woman bears her first child. Data from Hiroshima and Nagasaki, as well as from the treatment of young women with Hodgkin disease, show that the breast is not protected from potentially harmful environmental agents until it becomes fully differentiated in preparation for producing milk. This does not occur until the first full-term pregnancy. Pierre Band of Health Canada's Division of Epidemiology and Cancer Prevention led a case–control study that supports the "window of exposure" hypothesis. Published in the 5 October 2002 issue of *The Lancet*, it showed that women who started smoking within five years of menarche were around 70% more likely to develop breast cancer than nonsmokers.

A continuing revolution? As the female workforce grew at the end of the twentieth century, the job site became an important source of SHS exposure for many women. Today, however, health concerns and protests (like this one, right, at a January 2007 Atlantic City council meeting) have led to laws that protect such workers.

Many researchers interviewed for this article agreed that drinking alcoholic beverages does promote breast cancer, but not everyone felt that this factor has been adequately separated from tobacco exposure. "The breast cancer–alcohol association . . . is quite widely reported, but it is very hard to quantify and separate from the effects of concurrent active and passive smoking," Salmon points out. "There's also a complex multiway interaction between alcohol intake, smoking exposures, hormone levels, obesity, and breast cancer," he adds.

The evidence for a breast cancer–alcohol link was presented in a large meta-analysis by Beral and colleagues that included over 50 studies and data from more than 150,000 women. That analysis, published in the 18 November 2002 *British Journal of Cancer*, found no link between active smoking and breast cancer, but Reynolds says her study showed otherwise; the data showed that the link between active smoking and breast cancer held whether or not alcohol consumption was considered. She says, "We did a second analysis . . .

Source: Miller et al. Prev Med 44:93–106 (2007).

limited to nondrinkers, and we found the same elevated risk association."

Johnson suggests that because passive smoking was not considered in Beral's meta-analysis of alcohol risk, it is possible that it could have confounded the alcohol association results. He adds that the study simply compared women who said they currently smoked to women who reported smoking previously and those who said they never smoked, with no consideration of how much the women had smoked over their lifetimes.

Salmon says that if there is in fact an increase in breast cancer incidence directly caused by alcohol—as opposed to an association related to co-exposures—then promotional effects on cell growth might be involved. "At least," he says, "there is a contrast with tobacco smoke, where genotoxic carcinogens are clearly present and involved along with other types of effects, and carcinogen–DNA adducts have been observed in the breast tissue of exposed women."

However, Li notes there are numerous studies that show that women metabolize alcohol much more slowly than men, and that serum concentrations of 17β-estradiol are elevated after alcohol ingestion. "This," he says, "is likely the reason why alcohol ingestion increases breast cancer risk."

Although it had yet to be published when this article was being written, a new analysis by Beral and colleagues that linked alcohol to breast cancer is already having an effect on this debate. Thun said that after being briefed on the new data, he decided to hold off on moving forward with a workshop that the American Cancer Society planned to fund to identify the outstanding questions in the breast cancer–passive smoking debate. Beral would not comment on the data other than to say that the "findings are essentially null." She said the analysis included 22 studies as well as new data and "several measures of exposure to secondhand smoke." An article in the 1 January 2007 *Boston Globe* said the study focused on 1.3 million women aged 50 to 64, a different population than what the Cal/EPA scientists say is at greatest risk.

What Next?

While some members of the research community are perturbed by the disagreement regarding the strength of the evidence connecting passive smoking and breast cancer, Samet says he thinks it is to be expected. "People should not be surprised if review groups don't exactly come into complete alignment. Different groups bring expert judgment to bear in somewhat parallel but not definitely overlapping processes. I don't think that the way we approach evidence review and synthesis is leading us astray," he says.

Samet says that he would like to see the biological understanding advanced on ways that SHS could cause breast cancer. "Of course the simple story is that there are carcinogens in tobacco smoke, and they reach breast tissue, which is true," he says. "[What makes the issue so complex is that] we would expect that far greater doses of these carcinogens would reach the breast tissue of women who actively smoke. We have not seen a clear signal showing that this is the case. I would like to see the biological framework laid out and better understood, as well as watch the epidemiological evidence grow." Both the National Cancer Institute (NCI) and the NIEHS are funding research aimed at providing additional evidence on the topic. [For more information on this research, see "Centered on Breast Cancer," p. A132 this issue.]

The Cal/EPA researchers contend it is likely that there are a number of subgroups

This page: Jim Arbogast/Photodisc; background: Mladen Mitrinovic; opposite: Mladen Mitrinovic

Noisy environment. Drinking adds another dimension to the uncertainty about the effects of SHS. Breast cancer studies published to date have been unable to tease out the effects of concurrent active and passive smoking from alcohol intake.

genetically susceptible to breast cancer who could be especially sensitive to tobacco smoke exposure depending upon the polymorphisms of several genes. This is plausible, says Deborah Winn, acting associate director of the NCI's Epidemiology and Genetics Research Program. "To actually look at gene–environment interactions and then try to look at breast cancer subgroups—you run out of numbers very quickly," she says.

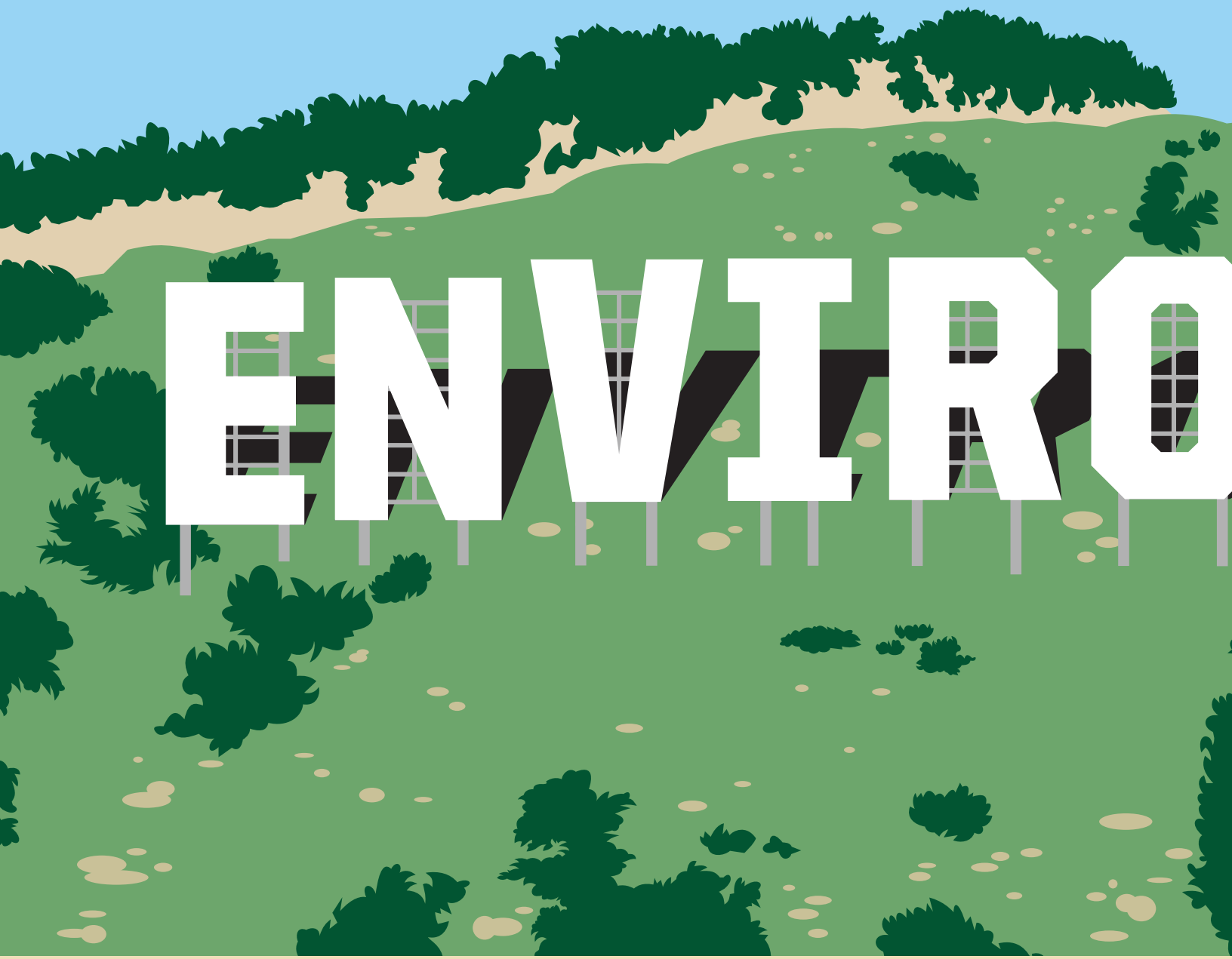
For that reason, researchers with the NCI Cancer Genetic Markers of Susceptibility initiative are doing genome-wide scans on more than 500,000 single-nucleotide polymorphisms and looking for changes between breast cancer patients and controls in a hypothesis-free approach. "Eventually, you might find things that map up to candidate genes that you already thought—on the basis of function or their role in estrogen metabolism—might be involved," Winn says. Other research is focused on more detailed looks at promising candidate genes, she says.

Says Russo, "The fact that carcinogen-metabolizing enzymes such as CYP1A1 are increased in both lung and breast cancers, but that the death rate from lung cancer in American women has increased six hundred percent from 1930 to 1997, whereas breast cancer has remained stationary during the same period, might suggest that women carriers of susceptibility genes would be more prone to develop both breast and lung cancer. Unfortunately, statistics on the incidence of breast lesions in women diagnosed with lung cancer are almost nonexistent."

Sandler is currently in the process of recruiting women for an NIEHS-funded prospective study of sisters of women who have or had breast cancer; this group is twice as likely as other women to develop breast cancer. She has already enrolled 30,000 such women who don't have breast cancer, and she aims to sign up 20,000 more. "The purpose is to look at environmental and genetic risk factors for breast cancer—certainly . . . we'll be looking at their life history of exposure to cigarette smoke, their own and through their parents and their spouses and their roommates and their jobs," she says. "The questionnaires were designed to do a thorough job of looking at it."

Just what all this research will show is unclear. The only certainty, Samet says, is that "scientific evidence will continue to accumulate on this topic."

Kellyn S. Betts



California Out in Front

Matt Ray/EHP

When it comes to ecological diversity, California has it all: snow-capped mountains, wide deserts, scenic beaches, and some of the worst environmental problems in the country. Six of the country's ten most polluted cities—Los Angeles, Bakersfield, Fresno–Madera, Visalia–Porterville, Merced, and Sacramento—are found in California, where children face fivefold greater risks of reduced lung function compared with children who live in less-polluted areas. Beyond its air pollution problems, California could also face catastrophic consequences from climate change. Assuming warming trends continue at their present rates, experts generally agree that the Sierra snowpack—which is crucial

to the state's drinking water supply—could decline by 50–90% by the century's end.

With statistics like that, environmentalism has become a powerful force in California. According to a 2006 survey conducted by the Public Policy Institute of California (PPIC), a San Francisco–based research organization, 65% of Californians don't think the federal government is doing enough to combat global warming. Two-thirds of the population support state efforts to address climate change, while an equal number support tougher air pollution standards on new vehicles, even if it makes vehicles more expensive.

California legislators have responded with some of the strongest environmental laws ever passed. Whereas the U.S. government has yet to regulate carbon dioxide, California recently passed AB 32, a groundbreaking law signed by governor Arnold Schwarzenegger in September 2006 that directs industries to reduce all greenhouse gas emissions by 25% over the next 13 years. Another law—AB 1493, which was enacted in 2002—directs automakers to reduce greenhouse gases emitted by passenger vehicles sold in California after 2009, with a 30% reduction in statewide vehicular emissions by 2016. (That law is currently being challenged by a lawsuit from the automotive industry.)

This year, California will consider a statewide green chemistry policy that could exceed the scope of the federal Toxic Substances Control Act (TSCA), which sets national policy on chemicals used in products and industrial processes. Local governments have also tightened environmental controls. San Francisco, for instance, recently passed the country's first ban on baby products containing bisphenol A and has also regulated levels of phthalates in these products. Bisphenol A and phthalates are both suspected endocrine disruptors.

Coming from one of the world's largest economies, these preemptive legislative efforts have impressive clout. "California provides an example [for other states]," says Cympie Payne, associate director of the California Center for Law and Policy at the University of California (UC), Berkeley. "Other states find it easier to model their own laws on those that another state has already put into effect."

Clearing the Air

California's aggressive environmental policies build on a long history. In 1965, the state became the first to regulate vehicle exhaust by setting limits on hydrocarbons and carbon monoxide emissions. Two years later, the newly formed California Air Resources Board (ARB)—now part of the California EPA—set the nation's first air quality standards for total suspended particulates, photochemical oxidants, sulfur dioxide, nitrogen dioxide, and other pollutants.

Early on, U.S. lawmakers recognized that California had a terrible problem with air pollution. Living in low-density sprawl, Southern Californians travel everywhere by car, generating exhaust plumes that get trapped at ground level in the area's low-lying valleys. Truck traffic across the Mexican border, in addition to emissions from the Los Angeles–Long Beach port complex—the largest man-made harbor in the western United States—also contribute to the region's poor air quality.

To give the state more leverage on pollution control, Congress allowed California to enforce pollution standards that might be more stringent than those passed by the federal government. That allowance was first introduced in the Federal Air Quality Act of 1967, and later codified in Section 209 amendments to the Clean Air Act (CAA). California has since set the nation's tightest standards for ozone and particulate matter, according to ARB spokesman Jerry Martin. Other states, meanwhile, have no comparable authority when it comes to devising their own air quality standards. Rather, the CAA allows them to choose whether to adopt federal standards or the more stringent California standards.

If California's strict environmental policies were triggered by traditional air pollution, its current reputation as a green pioneer has more to do with recent initiatives on climate change. By signing AB 1493, governor Gray Davis put California at the leading edge of government efforts to regulate greenhouse gases. Reflecting California's legislative influence, ten other states—New York, Massachusetts, Connecticut, Maryland, Delaware, Rhode Island, Maine, Vermont, Washington, and Oregon—along with Canada have all adopted the same goal.

But AB 1493 has its detractors, particularly among the auto industry. The stakes are huge for U.S. automakers: California accounts for 10% of their total sales. Auto industry lobbyists have overcome every congressional attempt to improve fuel efficiency standards since 1990. But Martin stresses that although better fuel efficiency does advance AB 1493's goals, automakers have other alternatives for reducing emissions, such as cutting back on the use of halogenated refrigerants, which exceed carbon dioxide in terms of greenhouse potency. Automakers can also sell more "flex-fuel" vehicles that run on ethanol blends, he says.

According to Martin, this flexibility in options distinguishes AB 1493 from corporate average fuel economy (CAFE) standards, which dictate only the minimum average miles per gallon that cars of a particular class need to achieve. "Our standards are for greenhouse gases; we call them global warming standards," Martin says. "And they include not just carbon dioxide but other gases like methane and [halogenated refrigerants]."

But the auto industry sees things differently. Charlie Territo, a spokesman for the Alliance of Automobile Manufacturers, a national industry trade group, calls AB 1493 a thinly veiled attempt to regulate fuel economy. Moreover, he adds, California has no authority to impose higher fuel economy standards because its special state status on the environment applies only to the CAA. CAFE standards, on the other hand, are administered by the National Energy Policy and Conservation Act, under which California has no special status. Equally significant, California can set its own state standards only for criteria pollutants listed under the CAA, a list that doesn't yet include carbon dioxide, he says.

The U.S. Supreme Court will determine later this year if the U.S. EPA must regulate carbon dioxide as an air pollutant. At issue is *Massachusetts et al. v. Environmental Protection Agency et al.*, wherein Massachusetts represents a coalition of stakeholders who believe carbon dioxide should be regulated to limit global warming. The U.S. EPA doesn't want to regulate carbon dioxide without better knowledge of the gas's role in climate change, according to James R. Milkey, the counsel of record for the Supreme Court case. Meanwhile, the Alliance of Automobile Manufacturers and other auto industry groups have sued California, Rhode Island, and Vermont, arguing that the application of AB 1493 (and equivalent counterparts in other states) is illegal. The suit, originally scheduled for trial in California beginning 31 January 2007, has been postponed until the spring pending the Supreme Court's decision.

Meeting Legislative Goals

The upcoming Supreme Court decision could also be critical for AB 32's goal of reducing California's total greenhouse gas emissions to 25% below 1990 levels by 2020. A press release issued by California's Office of the Governor on 27 September 2006 stated that AB 32 is a "landmark bill that establishes a first-in-the-world, comprehensive program of regulatory and market mechanisms to achieve real, quantifiable, cost-effective reductions of greenhouse gases." Says Martin, "AB 1493 just focuses on cars, but AB 32 covers everything that uses energy in some way. And since California is the twelfth largest producer of greenhouse gases in the world, that's a big deal."

The law directs the ARB to determine how California can meet its emissions reduction goal. Toward that end, the board will

develop appropriate regulations (which it will also enforce) and create a reporting system to track and monitor greenhouse gas emissions. With ARB approval, the law could allow California industries to trade emissions on global markets. That effort would apply economic forces to the goal of limiting emissions below a statewide cap yet to be identified, which will be phased in starting in 2012.

As a first priority, Martin says the agency is producing an emissions inventory, to quantify how much carbon dioxide California industries and their suppliers produce. At the same time, the ARB is compiling a list of "discrete early actions"—simple measures—to limit greenhouse gases that can be phased in by 2010. Along those lines, Schwarzenegger recently ordered that the carbon content of all transportation-related fuels burned in California must be reduced by 10% by 2020. The ARB is currently reviewing the governor's order to see if it qualifies as a discrete early action under AB 32. Martin suggests that it might, and adds that fuel companies could meet the mandate in a number of ways, for instance, by selling more biofuels.

In contrast to AB 1493, lawsuits against AB 32 haven't been filed. That's because specific measures targeting individual industries aren't yet known, Martin explains. Once those measures are identified, affected industries will sue accordingly, he predicts.

Meanwhile, stakeholders everywhere are anxious to know if California can limit greenhouse gases without wrecking its economy. "It's going to be tricky," concedes Dominic DiMare, vice president of government relations at the California Chamber of Commerce, which opposed both AB 1493 and AB 32. "The law could harm the economy, but it could also help it, and that depends on how it's implemented, which is something we still don't know. My concern is that some companies might leave California rather than face those restrictions. Then they might wind up in other areas where they pollute even more than they do here."

Countering those concerns, UC Berkeley adjunct professor David Roland-Holst was quoted in the 15 September 2006 *New York Times* as estimating that AB 32 could pump $60 billion and 17,000 jobs into the California economy by 2020, by attracting investment in alternative energy. Roland-Holst could not be reached for comment.

Green Chemistry

Apart from global warming, California's next big effort on the environment could come from a burgeoning green chemistry policy—that is, one that identifies safer chemicals and processes. That pending effort responds to a 2006 UC Berkeley report titled *Green Chemistry in California: A Framework for Leadership in Chemicals Policy and Innovation*, which concluded that federal policies under TSCA don't do enough to protect public health. The 130-page document was drafted at the request of the California legislature.

According to lead author Michael P. Wilson, a research scientist at the UC Berkeley Center for Occupational and Environmental Health, TSCA's data requirements impede the transparency and oversight that are necessary to protect public health and allow proper function of the chemicals market. TSCA does not require producers to generate data on chemical toxicity, he says, and that produces uncertainty for companies that purchase chemicals. Moreover, he says, TSCA constrains the government's ability to control the sale of hazardous chemicals, which allows these substances to remain competitive in the market. The report concluded that these market conditions have dampened interest by industry in green chemistry.

"We have a failure in the U.S. chemicals market," Wilson stresses. "Chemicals are marketed on the basis of their function, price, and performance, but the hazard piece is still largely missing."

Responding to the report's message, state senator Joe Simitian, who chairs the California Senate Environmental Quality Committee, is investigating options for a new green chemistry policy that might address TSCA's shortcomings. Bruce Jennings, a senior advisor to the California legislature, with whom Simitian collaborates, says a number of green chemistry bills could go to the floor this year. One would create a clearinghouse on alternatives to hazardous chemicals, geared toward small companies that lack access to that type of information and patterned after a similar U.S. EPA program, Design for the Environment. Another would require the producers of high production volume chemicals to submit environmental health information to California, in addition to information about the use and disposal of such chemicals.

Simitian was quoted in the 2 November 2006 *Capitol Weekly* as saying that he wants to apply a precautionary approach to California's emerging chemical regulations. That approach—popular among European Union countries—shifts the burden of proof regarding chemical safety to manufacturers instead of regulators. The precautionary principle, as it is often called, drives some of the European Union's most sweeping—and controversial—environmental initiatives, particularly the REACH (Registration, Evaluation, and Authorisation of Chemicals) directive, which requires that chemicals manufactured or imported at volumes of greater than one metric ton be registered with the European Chemicals Agency. Under the REACH initiative, which goes into effect in June 2007, some toxic chemicals could be phased out in favor of less toxic alternatives.

U.S. industries have fought against REACH, which will affect their exports to Europe. Now some industry stakeholders worry that California's potential green chemistry policies could be a stepping stone toward REACH implementation in the United States.

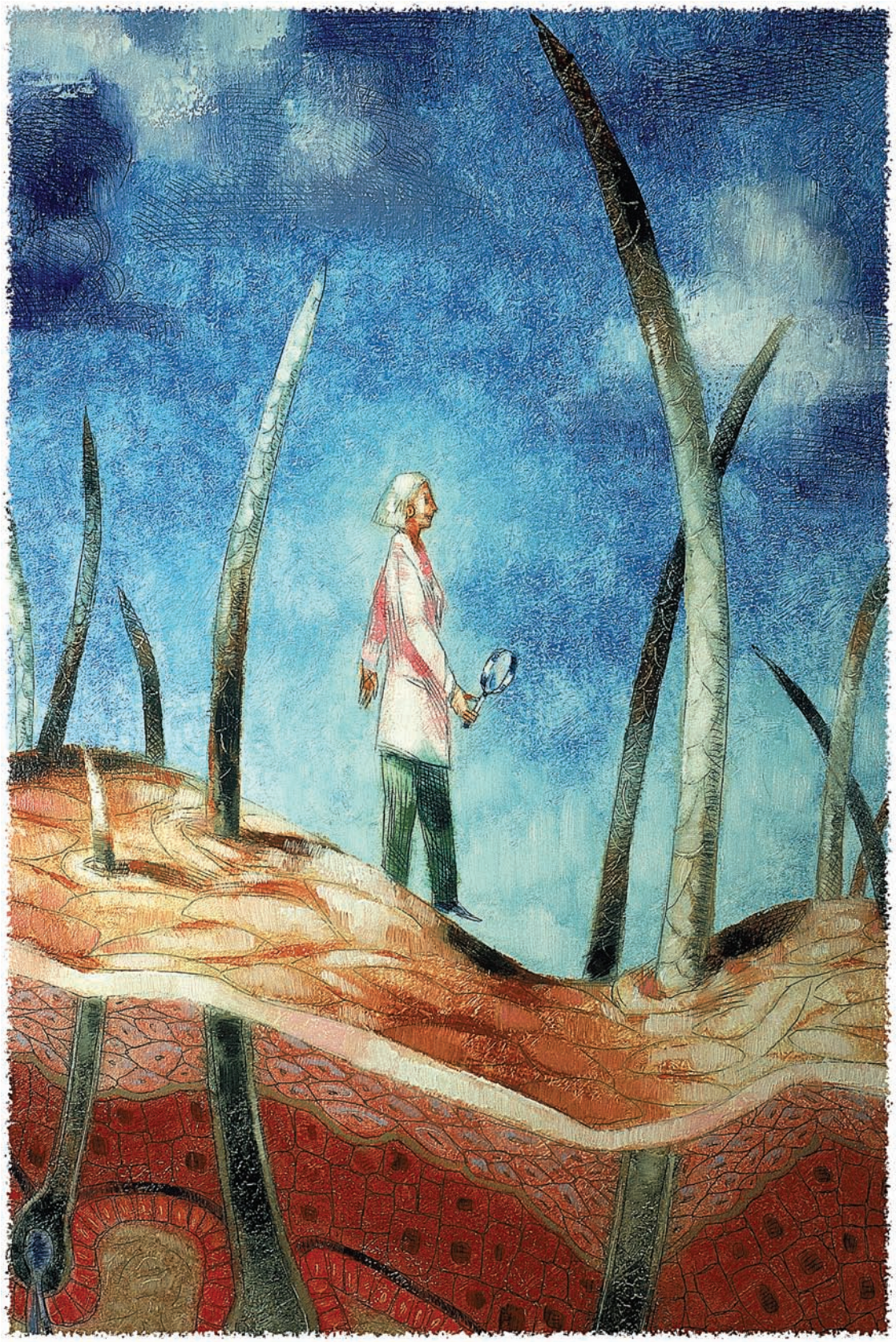
"We're concerned this could impose added costs on California businesses," says John Ulrich, executive director of the Chemical Industry Council of California, a trade group. "Anything that increases the cost of manufacturing across the board in California will discourage manufacturing here. I'm afraid a legislative package that claims to be green chemistry will go down a conventional route of legislating based on unfounded science, using timetables that aren't credible or achievable."

While claiming it's still too early to know what form the policy will take, Jennings stresses the goal isn't to replicate REACH or any other European initiative. "We want to complement what they're doing," he says. "And there are plenty of industry players who face challenges with operating in global markets when they lack information about the chemical content of their products. Chemical producers may be troubled by changes in the law, but we think downstream users will welcome efforts to give them more information."

In support of that view, Rachelle Reyes Wenger, who manages public policy and advocacy at Catholic Healthcare West, a San Francisco–based company that owns 42 hospitals and employs 44,000 people, says better information on chemical safety and alternative products can be good for business. She notes that her company recently awarded a multiyear $70 million contract to a company that supplies intravenous bags that do not contain polyvinyl chloride, phthalates, or other toxic chemicals. "With our purchasing power, we can really make a difference," she says. "A comprehensive chemical policy could hurt finances initially, but not in the long run. It's ultimately better not just for the financial bottom line, but also for the moral bottom line."

And of course, for the environmental bottom line. California's lawmakers have apparently decided that sacrifices made now to achieve environmental goals are worth the future benefits, not just for health and ecology, but for the long-term sustainability of the state's industries. Ultimately, California's paving a road forward on which others may inevitably follow.

Charles W. Schmidt



Todd Davidson/Brand X/Corbis

More Human, More Humane

A New Approach for Testing Airborne Pollutants

People not only inhale airborne contaminants but also absorb them through the skin. Both routes can set off localized toxic reactions or damage internal organs such as the liver, kidney, and brain. Conventional tests of the toxicity of gases and vapors, in which laboratory animals are exposed to lethal or sublethal doses of chemicals, have been criticized as expensive, unethical, inhumane, and time-consuming. Now researchers at the University of New South Wales (UNSW) in Sydney, Australia, have developed an animal-free alternative that uses human cells to test the effects of exposure to airborne toxicants.

The *in vitro* method "opens new possibilities for toxicity testing of industrial chemicals, occupational and environmental contaminants, and fire combustion products," says team leader Amanda Hayes, manager of the Chemical Safety and Applied Toxicology Laboratories in the UNSW School of Safety Science. In addition, the method could help researchers explore the health effects of nanoparticles, which increasingly are widely incorporated into cosmetics and pharmaceuticals even though "very little is known about their safety to human health," Hayes says. This project earned Hayes and colleagues Shahnaz Bakand and Chris Winder a 2006 Australian Museum Eureka Prize, which acknowledges outstanding achievements in Australian science.

A Cell-Based Model

In traditional *in vitro* testing, cells are grown in the bottom of a laboratory dish and covered with cell medium. Test contaminants are dissolved in the liquid medium that bathes the cells. However, this is a poor model for estimating damage from direct contact with airborne pollutants.

Hayes and coworkers improved on this method by growing human cells on Snapwell™ brand permeable polyester membranes. The cell types used, including A549 lung cells, HepG2 liver cells, and skin

fibroblasts, represent target organs that are likely to be affected by airborne toxicants. Once the cells attach to the membrane and begin to flourish, the upper layer of culture medium is drawn off to expose the cells directly to air contaminants at the air–liquid interface. Meanwhile, nutrients are fed from below to keep the cells healthy.

Next the cells are exposed to airborne pollutants in a diffusion chamber. Then routine laboratory tests measure changes in cell growth and energy metabolism, along with other end points. The researchers have found that toxic measurements obtained by their in vitro method, such as the amount of a chemical needed to inhibit cell growth, mirror lethal values reported from animal studies. "In vitro toxicity tests can improve the scientific, economic, and ethical value of research and play a significant role in the replacement of animals," Hayes says.

Testing the Concept

In a series of experiments, the Australian team demonstrated the feasibility of their in vitro technique by testing formaldehyde, an industrial chemical linked to human cancer; nitrogen dioxide, a lung irritant that causes inflammation, pulmonary edema, and pneumonia; fire combustion products including cyanide, hydrogen sulfide, and ammonia; and xylene and toluene, two volatile organic compounds (VOCs) found in solvents used by the printing, painting, and petrochemical industries. Environmental or occupational exposure to any of these chemicals causes local and systemic toxicity.

In the VOC study, all three cell types were treated with vapors from 0, 2.5, 5, 10, 15, 20, or 30 mL of xylene or toluene for one hour. Following exposure, cell cytotoxicity was measured with the MTS assay (which measures the number of viable cells) and the NRU assay (which measures cell membrane stability). In all three cell types, airborne toluene and xylene inhibited cell growth in a dose-dependent manner, and both the MTS and NRU tests yielded similar results.

Using these results, the researchers calculated airborne IC_{50} values, or the concentration of a chemical that blocks growth of half the cells. Xylene's IC_{50} values ranged from 5,350 to 8,200 ppm in the three cell types, making it roughly twice as toxic as toluene, with IC_{50} values of 10,500 to 16,600 ppm. These *in vitro* values correspond well to published acute inhalation data for animals. LC_{50} values (the concentration of a chemical that will kill half a group of test animals) were obtained from the NIOSH Registry of Toxic Effects of Chemical Substances for rats exposed to xylene or toluene for four hours. LC_{50} values of 5,000 ppm for xylene and 13,000 ppm for toluene correspond with IC_{50} values in the range calculated for human lung, liver, and skin cells by Hayes and her colleagues. These results appeared in the January 2006 issue of the *Journal of Environmental Monitoring*.

Now on the air. Human cells grown on ready-made culture inserts are placed in a horizontal diffusion chamber to test the effects of airborne chemicals.

In another study, described in the 1 August 2006 issue of *Toxicology Letters*, the researchers exposed A549 lung cells to nitrogen dioxide concentrations ranging from 2.5 to 10 ppm. Hayes found significant adverse effects on cells at the OSHA permissible exposure level of 5 ppm, suggesting that workplace exposure standards may need to be re-evaluated.

The researchers delivered nitrogen dioxide dynamically, meaning it was constantly exchanged during the one-hour test. "This is an important refinement that mimics actual life exposure," says epidemiologist William Lambert of Oregon Health & Science University. He explains that people are exposed to transient high levels of nitrogen dioxide from vehicle exhaust plumes while waiting at a bus stop, for example, or in the course of cooking meals on a gas stove. In contrast, traditional assays put animals in a chamber, and a known concentration of chemical is pumped in for a set amount of time.

In a third set of experiments, described in the August 2005 issue of *Toxicology in Vitro*, the researchers exposed all three cell types to 11 airborne pollutants commonly generated by fires. Fires release a variety of gases and organic vapors during their course, and 80% of fire-related deaths result from inhalation of toxic substances whose variation over the course of the fire is poorly defined. Of the compounds tested, sulfurous acid

Suggested Reading

Bakand S, Winder C, Khalil C, Hayes A. 2006. A novel *in vitro* exposure technique for toxicity testing of selected volatile organic compounds. J Environ Monit 8:100–105.

Bakand S, Winder C, Khalil C, Hayes A. 2006. An experimental *in vitro* model for dynamic direct exposure of human cells to airborne contaminants. Toxicol Lett 165(1):1–10.

Goldberg AM, Hartung T. 2006. Protecting more than animals. Sci Am 294:84–91.

Lestari F, Hayes AJ, Green AR, Markovic B. 2005. *In vitro* cytotoxicity of selected chemicals commonly produced during fire combustion using human cell lines. Toxicol In Vitro 19:653–663.

CSAT Labs/University of New South Wales

showed the greatest toxicity in all three types of cells, whereas sodium nitrate showed the least.

Several chemicals showed organ-specific action. For example, formaldehyde was twice as toxic to liver cells as it was to lung or skin cells. This highlights the importance of using a variety of target cells when testing toxic chemicals. *In vitro* tests could track the evolution of toxic substances as a fire grows, assess the safety of building materials, and provide more accurate safety information for fire professionals, the researchers concluded.

Future Promise

The team is still validating the method in real-life settings. According to Hayes, once *in vitro* tests have been validated, the cost for the new method will be considerably cheaper than animal experimentation and considerably faster, with assay times ranging from 4 to 24 hours. Assays are performed in 96-well plates, allowing for a number of test chemicals to be assessed at once.

Alan Goldberg, director of the Center for Alternatives to Animal Testing (CAAT) at The Johns Hopkins University, says the approach "is highly focused, has clear direction, and is good science." The new system fits the concept of the three Rs—reduction, refinement, and replacement—which are the guiding principles for scientists striving to find alternatives to animal testing. Reduction means designing experiments to use fewer animals, refinement refers to improving protocols to minimize the suffering of test animals, and replacement calls for entirely eliminating whole-animal tests. Goldberg says the alternative method being developed by Hayes and its successors "promise to reduce and possibly eliminate animals in the testing of airborne toxicants."

After Hayes validates the *in vitro* method, she plans to develop a portable on-site test for environmental sampling and toxicity monitoring. Indeed, says Lambert, "There is a need for *in vitro* exposure systems for studying the effects of air pollutants on cells of the respiratory tract." This type of on-site test could have helped with rescue efforts after the September 11 attack on the World Trade Center. "The complex and unique mixture of smoke and pyrolytic products could not be simulated in the laboratory, and a device like this could provide quick assessment of toxic potential," says Lambert.

Taking such a system onsite has merit, says JeanClare Seagrave, an associate scientist at the Lovelace Respiratory Research Institute in Albuquerque, New Mexico, as long as the *in vitro* method is thoroughly validated to get reproducible responses for known exposures and includes appropriate positive and negative control treatments.

However, there are limitations to the use of such a system; these include the lack of interactions of cultured cell with the immune system or detoxification mechanisms that occur in the body. Moreover, the air is full of biological agents such as bacteria, mold, and viruses, which may deposit on cells and culture media and proliferate. Such contamination could skew results if cells react with the microbes instead of, or in addition to, toxicants. Nonetheless, says Seagrave, "Air–liquid interface exposures are clearly more physiologically relevant for lung and skin cells than conventional submersion culture systems."

Carol Potera

Breaking In through Critical Windows

p,p´-DDE May Alter Fetal Neurodevelopment

DDT has been widely used to control mosquito-borne malaria since the late 1940s. The compound and metabolites such as *p,p´*-DDE linger in the environment for decades; even in areas where DDT has been banned, these neurotoxic chemicals are still detected in human blood, fat, breast milk, and umbilical cord blood. Researchers examined the possibility that prenatal exposure to *p,p´*-DDE damages early neurodevelopment, and present the first evidence that exposure during a critical window of development adversely affects infant psychomotor development.[***EHP* 115:435–439; Torres-Sánchez et al.**].

Back tracks. Long-ago pesticide spraying can still affect today's children.

From January 2001 to June 2005, 1,585 reproductive-age women in the State of Morelos, Mexico, where DDT had been used for malaria control until 1998, were invited to join the prospective cohort study. Each woman choosing to participate provided a blood sample and information about sociodemographic characteristics, obstetric and gynecologic history, alcohol and tobacco use, occupation, and previous pesticide use.

Once a woman became pregnant, the researchers conducted in-home visits each trimester to collect a blood sample and data on her pregnancy, weight, and diet. After the woman gave birth, they evaluated her child at 1, 3, 6, and 12 months of age, focusing on health, feeding, growth, and cognitive and psychomotor development. The researchers also tested maternal intelligence and assessed the home environment by observing factors such as parent–child interaction and available toys. Data were available for 244 mother–child pairs.

p,p´-DDE was detected in all maternal blood samples. Concentrations were the highest in the third trimester, but analyses revealed that only first-trimester concentrations were associated with impaired psychomotor development. This association remained after controlling for maternal intelligence and the home environment; breastfeeding appeared to have a slight protective effect.

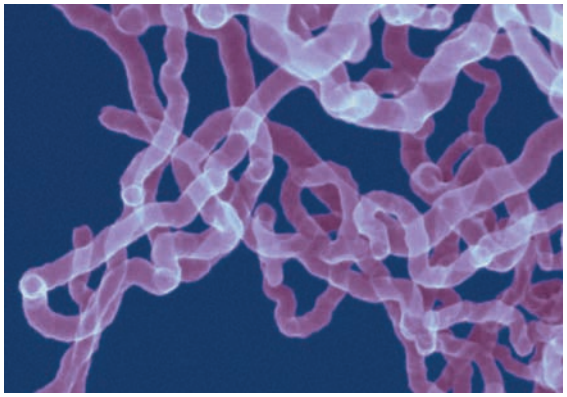
A subset of 105 maternal blood samples were also tested for lead. Because maternal lead concentrations were not available for all infants, lead exposure could not be completely excluded as contributing to effects correlated with first-trimester *p,p´*-DDE exposure. However, the low negative correlation between the two neurotoxicants made it unlikely that the effects observed were either amplified or masked by lead. There did not appear to be an association between prenatal *p,p´*-DDE exposure and cognitive development.

These findings add to the growing evidence that DDT metabolites in a mother affect her child's psychomotor development during infancy. The researchers suggest that prenatal *p,p´*-DDE exposure needs further attention, even in countries where DDT has not been used for decades. **–Julia R. Barrett**

Carbon Concerns

Nanotubes Cause Cardiovascular Damage

Lung deposition of single-wall carbon nanotubes (SWCNTs), one of the most commonly used materials in nanotechnology, is already known to cause localized toxic effects. Now scientists have demonstrated that such deposition also leads to cardiovascular damage in mice, including accelerated formation of atherosclerotic plaques [***EHP* 115:377–382; Li et al.**]. The findings add to concerns that exposure to SWCNTs could result in systemic toxic effects.



Worming their way in? SWCNTs may cause systemic toxicity.

The team conducted a series of experiments, instilling SWCNTs into the lungs of mice. In an initial screen for extrapulmonary effects, *Ho1-luc* reporter transgenic mice were exposed to single SWCNT doses of 10 or 40 µg. Heme oxygenase-1 (HO-1) gene expression, a biomarker of oxidative stress, was activated in the animals' lung, aorta, and heart tissue at 7 days post-exposure, declining to control levels by day 28. This held with pulmonary toxicity studies showing an early, transitory inflammatory response.

The same dosing scheme was used in experiments with the commonly used C57BL/6 mouse, which showed dose-dependent aortic mitochondrial DNA (mtDNA) damage at 7, 28, and 60 days post-exposure. mtDNA is highly susceptible to oxidative damage, considered to be an initiating event in atherogenesis. Among the treatment groups, glutathione and protein carbonyl levels—two other indicators of oxidative stress—were also significantly reduced and increased, respectively, adding to the evidence that exposure to SWCNTs can lead to oxidative insult. Exposure to comparable doses of ultrafine carbon black particles in a control group produced no such damage to aortic mtDNA.

The group then tested the effects of SWCNT exposure in ApoE$^{-/-}$ mice, a widely used model of human atherosclerosis. They exposed the mice to 20 µg of SWCNTs once every other week for 8 weeks. Then the mice were fed either a regular chow diet or a high-fat diet for the first half of that period to induce the elevated lipid concentrations that often precede atherosclerosis. Although SWCNT exposure was not associated with changes in the animals' lipid profiles, the exposed mice on the high-fat regimen did exhibit accelerated plaque formation in the aorta and brachiocephalic arteries compared with controls.

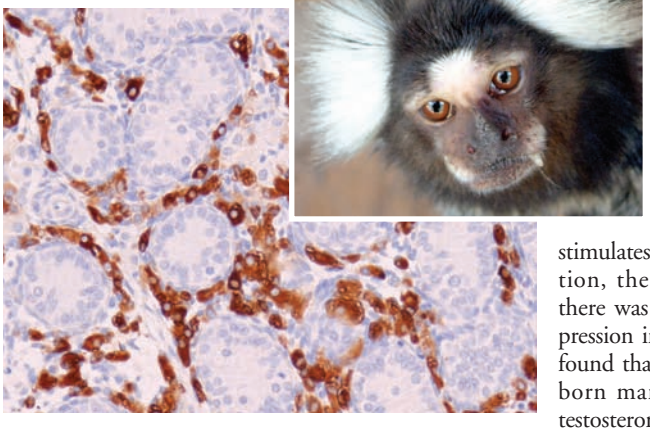
The researchers note that the cardiovascular effects resulting from SWCNT exposure could be either direct, as a result of translocation of particles from the lung into the systemic circulation, or indirect, caused by the release of inflammatory mediators in the lung or by altered pulmonary function (although no increase in several measured inflammatory mediators was detected in the exposed animals). Whichever mechanism may be at work, these data show that lung deposition of SWCNTs, a possible workplace exposure scenario, can cause systemic damage and may contribute to cardiovascular disease. **–Ernie Hood**

Top to bottom: Aubrey Wade/Panos Pictures; Dennis Kunkel

The Testosterone Test

Phthalate Inhibits Leydig Cell Aggregation

Testicular cancer and low sperm count are adult disorders, but evidence increasingly suggests they have a fetal origin. Cryptorchidism and hypospadias, apparent at birth, also appear linked to prebirth events. According to the testicular dysgenesis syndrome (TDS) hypothesis, all four disorders, which by some reports have become more common in recent decades, partially stem from fetal abnormalities in testosterone-producing Leydig cells. An investigation now reveals that di(*n*-butyl) phthalate (DBP) and its metabolite monobutyl phthalate (MBP) suppress testosterone production in rats and primates [***EHP* 115:390–396; Hallmark et al.**]. Attempts to establish *in vitro* models were unsuccessful, however.



Testicular effect. The numbers and size of Leydig cells (in brown, above) increased in MBP-treated marmosets.

In rats, prenatal exposure to DBP can induce Leydig cell changes and TDS-like effects. Chronic, low-level exposure to DBP and other phthalates, widely used as plasticizers, is common among humans, but it is unknown if it causes the same effects. The primary goal of the current study was to determine whether effects seen in rats could be replicated *in vitro* with fetal rat and human testis explants (extracted tissue maintained in culture). The team also conducted experiments in male infant marmosets, whose neonatal testosterone production mirrors that of human males.

Preliminary work revealed that rats with prenatal DBP exposure produced significantly less testosterone and had more medium or large Leydig cell clusters. This is notable because larger clusters are associated with defective testicular development. Rat fetal testis explants, however, showed only minor MBP-related effects, and results from comparable human explants were even less conclusive.

Because known *in vivo* reactions could not be replicated *in vitro*—indicating either a problem with the method or misidentification of the active metabolite—the team tested MBP in marmosets. In five sets of marmoset twins, one twin was exposed to MBP for two weeks while the other served as a control. Blood testosterone levels did not differ significantly, but Leydig cell numbers and size were consistently increased in the MBP group.

Because low testosterone triggers increased secretion of luteinizing hormone, which stimulates Leydig cell testosterone production, the researchers checked whether there was an initial MBP-associated suppression in testosterone production. They found that a single dose of MBP in newborn marmosets significantly reduced testosterone levels within hours. This finding led to the hypothesis that increased luteinizing hormone secretion compensates for an initial MBP-associated inhibition of testosterone production, which the researchers conclude should be considered in future animal studies. They also conclude that *in vivo* marmoset research represents the best current means for investigating the steroidogenic effects of DBP relevant to humans. **–Julia R. Barrett**

Metal Duo Damages Lungs

Lead and Manganese in Fine Particulates

Extensive evidence indicates that fine particulates can damage human lungs. But much remains unknown about exactly which components of these particulates are to blame. In a small study of Korean children, researchers have found that two metals, lead and manganese, are among the substances likely at fault [***EHP* 115:430–434; Hong et al.**].

To pin down the particulate culprits, the researchers evaluated 43 children who attended school on an island near Incheon City. The island has low traffic density and industrial emissions, but concentrations of fine particulates 2.5 μm in diameter or smaller were relatively high by U.S. standards, perhaps owing to natural sources or dust from China or Mongolia. The mean of 20.27 μg/m^3 measured during the six-week study period was about one-third higher than the U.S. annual standard.

After an introductory period during which the children (median age 10) learned how to use a peak expiratory flow meter to measure their lung function, each child used the device at three fixed times every day. Meanwhile, the researchers sampled fine particulates every day on the roof of a building 2 km from the school and analyzed the concentration of five metals: aluminum, iron, lead, manganese, and zinc. Previous studies have shown these metals might play either beneficial or harmful roles when present in particulates.

Gotcha! In a study of Korean children scientists identified some of the health-damaging components of fine particulates.

The researchers also tested the children for polymorphisms of *GSTM1* and *GSTT1*. These two genes play a role in the function of the enzyme glutathione *S*-transferase, which scavenges the damaging reactive oxygen species created by some metals. In addition, they took into account many other factors, including weather, day of the week, sex, age, height, weight, asthma history, passive smoking exposure at home, and socioeconomic status. They did not test for other metals, acquire data on other lung-damaging pollutants (such as nitrogen dioxide or ozone), or measure personal fine particulate exposures.

Typical of Asian populations, roughly half the children did not have one gene or the other due to deletion. The team found that lead and manganese were linked with significant reductions in peak expiratory flow rate, regardless of whether a child had either of the tested gene polymorphisms. The three other metals had no significant effects, even though they sometimes were present at much higher concentrations than lead and manganese. The team acknowledges that additional studies are needed to comprehensively determine the impact of metals on the respiratory system. **–Bob Weinhold**

Top to bottom: Marion Walker/Medical Research Council; inset: Alan Wesley/Shutterstock; Photodisc