ehp ENVIRONMENTAL HEALTH PERSPECTIVES

SEPTEMBER 2006
ehponline.org/science-ed

Soy: Filling in the Gaps

Mothers' Milk—Unleaded, Please

Cross Out Secondhand Smoke

NIEHS
National Institute of Environmental Health Sciences

PUBLIC HEALTH SERVICE
U.S. DEPARTMENT OF HEALTH & HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES
NIH PUBLICATION 2006-218

STUDENT EDITION

Our minds are like our stomachs; they are whetted by the change of their food, and variety supplies both with fresh appetites.
Quintilian, Roman rhetorician

HERBAL MEDICINE

High-Test Mothers' Milk

A lack of regulation means that herbal remedies can be ineffective or, conversely, far more potent than users may suspect. Researchers in Taiwan have found a new reason for consumers to be wary of certain herbal remedies: some herbs used in traditional Chinese medicine contain lead, which mothers can pass to their infants through breast milk. The study, published in the 1 February 2006 issue of *Science of the Total Environment*, adds to the growing evidence that infants can be exposed to potentially dangerous lead levels *in utero* and through breast milk.

Bans on leaded paint and gasoline have caused lead exposure during childhood to plummet. Now scientists are focusing more attention on perinatal exposure to lead via maternal circulating blood levels and breast milk. The neurotoxicant is associated with behavioral problems and diminished intelligence. Although lead has declined in the environment in a number of countries, it may still persist in soil, dust, and water in many parts of the world. Maternal bone lead accumulated during earlier exposure is released during pregnancy and lactation as the body redistributes its calcium stores.

Principal investigator Bor-Cheng Han and colleagues originally recruited 72 pregnant women, but only 16 completed the study. The women were interviewed during pregnancy and lactation to collect information on residential and occupational lead exposures, sociodemographic characteristics, and consumption of nutritional supplements, traditional Chinese herbs, alcohol, and tobacco. The women provided breast milk samples weekly from 1 to 60 days postpartum. Nine of the women took traditional herbs, while seven did not.

The researchers purchased samples of herbs that the mothers reported taking regularly—*Angelicae sinensis radix*, *Lycii fructus*, *Zizyphi fructus*, and a preparation known as Shy-Wuh-Tang. Then they tested the samples for lead content. All the samples contained lead; Shy-Wuh-Tang, used to treat menstrual and circulatory problems, had the highest levels, at 322.31 micrograms per kilogram (µg/kg).

The nine herb users had a mean lead concentration of 9.94 µg per liter (L) in colostrum, the form of milk produced just after delivery. Lead levels in their breast milk dropped with most weekly samplings, to a mean concentration at the final sampling of 2.34 µg/L. Lead levels also declined in the seven mothers not using herbs, from 8.11 µg/L in colostrum (likely reflecting occupational or pollution exposures) to 2.36 µg/L in mature milk.

Bad medicine? Testing revealed elevated lead in the breast milk of mothers who took four traditional Chinese herbal remedies.

The finding that Chinese herbs were contaminated with lead comes as no surprise to research scientist Richard Ko of the California Department of Health Services. In the 17 September 1998 issue of the *New England Journal of Medicine*, Ko reported high lead levels in 24 of 260 Chinese patent medicines sold in California. Tainted products slip easily into the hands of consumers because the FDA does not have enough resources to inspect all imported herbs, nor does it regulate herbs, which it considers dietary supplements rather than drugs. The Taiwanese researchers speculate that the herbs their subjects took were grown in contaminated soil.

In spite of the risks, the worldwide market for herbal treatments is estimated to be more than $60 billion and growing fast, according to the UN Conference on Trade and Development. Some 30–50% of all medicines consumed in China are traditional herbs.

It is unclear how much risk the lead-contaminated herbs posed to the babies in the Taiwan study. Jenny Pronczuk de Garbino, a physician with the WHO Department of Public Health and the Environment, says that "only if the doses were extremely high would they outweigh the benefits of breastfeeding," but that "prevention of exposure is paramount." The FAO/WHO Joint Expert Committee on Food Additives and Contaminants, an independent scientific expert body regularly convened by the FAO and the WHO, last assessed the risk of lead exposure to human health in 1999, and established a provisional tolerable weekly intake of 25 µg/kg body weight as a value that would not lead to any appreciable health risk. The WHO maintains in its 2003 document *Global Strategy for Infant and Young Child Feeding* that "[b]reastfeeding is an unequalled way of providing ideal food for the healthy growth and development of infants."

Han admits his study of just 16 mothers is too small to draw conclusions from. But it adds to the growing information about how mothers' exposure may influence their infants' lead levels. The Lead and Pregnancy Work Group organized by the CDC is reviewing such studies in an effort to develop national guidelines on assessing and managing risk of lead exposure during pregnancy and lactation.

"We need a better understanding of neonatal exposure from breastfeeding," says Adrienne Ettinger, a Harvard School of Public Health researcher and member of the CDC work group. "We don't have all the scientific data yet." **–Cynthia Washam**

Photodisc

Occupational Health

Will Work for Air

Indoor air specialists Olli Seppänen of the Helsinki University of Technology and William Fisk and Q.H. Lei of the Lawrence Berkeley National Laboratory are not the first to establish a link between work performance and ventilation—for several decades, researchers have seen an association between an inadequate supply of outdoor air and discomfort and illness among building occupants. But Seppänen and colleagues, in a meta-analysis published in the February 2006 issue of *Indoor Air*, are the first to present a model showing the quantitative relationship between these two variables. Their findings are simple: if you want your workers to perform, you have to let them breathe fresh air.

No air, no work. Reducing ventilation to save money ends up costing more in lost productivity.

Ventilation rates vary considerably within and among commercial buildings due to such factors as equipment design and operation. Experts say these rates are often below levels recommended by groups such as the American Society of Heating, Refrigerating, and Air-Conditioning Engineers (ASHRAE). However, says Charlene Bayer, a principal research scientist at Georgia Tech Research Institute, the results seen by Seppänen and others suggest that even the ASHRAE standard is probably not high enough. Further, with the recent spike in oil and natural gas prices, building managers may be keeping ventilation rates intentionally low to save on energy bills—a practice that robs Peter to save Paul, as worker productivity can end up dropping.

The researchers subjected the data from nine earlier studies to statistical analysis to compare the results across studies. Five studies collected data from call centers, one was conducted in school classrooms, and three were conducted in a controlled simulated office setting. Each study compared performance at a minimum of two different ventilation rates.

From each study, Seppänen and colleagues calculated a "performance change" parameter by subtracting performance at the lower ventilation rate (expressed in liters of air per second [L/s]) from performance at the higher ventilation rate and dividing the difference by performance at the lower ventilation rate. (The performance figures were expressed in terms of speed of work, and the change in speed was expressed as a percentage.) The resulting parameter was further normalized by dividing by the difference between the two ventilation rates and multiplying by 10.

Results typically showed increases in average work performance in the range of 1–3% for each 10 L/s-per-person increase in outdoor ventilation rate. The performance increase was greater when ventilation rates were initially low (below 20 L/s per person, which is twice the ASHRAE standard) and almost negligible when ventilation rates were already high (above 45 L/s per person). The authors speculate that the improvement of performance was related to reducing levels of indoor air pollutants.

Will this analysis encourage those who design and manage office buildings to let more outside air flow to their occupants? Not in the short run, says Bayer. "Concerns are still primarily with energy conservation, and are increasing due to continually increasing energy costs." But, she says, this new analysis provides those who are interested with a tool to better balance the needs of energy conservation and worker health and performance. **–John Manuel**

New UNEP Leader Chosen

The UN General Assembly has unanimously selected Achim Steiner of Germany to succeed Klaus Töpfer as the fifth executive director of UNEP. Steiner is presently director-general of the World Conservation Union, the world's largest environmental network, and will begin his four-year UNEP term in June 2006. Of the selection, Töpfer said, "I am convinced that choosing Achim Steiner will prove to be a great decision, bringing youth, dynamism, intellect, and a deeply held commitment to environment and sustainable development issues." Steiner has degrees from Oxford University and the University of London, and has also studied at the German Development Institute and Harvard Business School.

China Approves Environment Plan

China is currently home to 16 of the world's smoggiest cities, many of the country's waterways are severely contaminated, and piles of construction refuse are being dumped in rural areas. Acid rain, industrial pollution, nuclear risks, and declining biodiversity also pose grave problems. In February 2006 China's State Council approved a plan to combat the country's pervasive pollution. A state media statement said, "The move is aimed at protecting the long-term interests of the Chinese nation and leaving a good living and development space for our offspring." The plan calls for regional governments to set environmental targets to be evaluated regularly, and for local officials to be assessed on their environmental performance, not just their success in promoting economic development. Poor environmental performance by officials will be punishable under the plan.

Safe Harbor for Fish Lovers

A number of recent public advisories have warned women of childbearing age to limit intake of swordfish, shark, tuna, and other fish with high levels of mercury, since studies show that brain development in young children is affected when their mothers consume such fish. Now northern Californian fish lovers who are concerned about mercury are in luck. Holiday Quality Foods markets and select Sam's Club stores in the region now stock Safe Harbor certified fresh fish, which uses a new technology to measure the fish's mercury content at the packaging plant in about one minute (conventional testing can take a week or more). Fish that register more than the median FDA level for that species are rejected. The certification is part of a test by the stores and Pacific Seafood Group, one of America's largest fish wholesalers, to see if consumers would buy more fresh fish if they knew it contained safe levels of mercury.

Safe Harbor™

Clockwise from top left: BananaStock; UN; Safe Harbor

CHILDREN'S HEALTH

Do Antibiotics Now Mean Asthma Later?

Asthma affects 1 in 8 school-aged children in industrialized countries, making it the most common chronic illness in this group. Now a meta-analysis of child asthma studies led by pharmaceutical scientist Fawziah Marra of the University of British Columbia shows that children diagnosed with asthma were twice as likely as nonasthmatics to have received antibiotics before age 1. The more courses of antibiotics a child received in the first year of life, the higher the risk for asthma.

Unpleasant side effect. Antibiotic use before age 1 could contribute to childhood asthma.

The meta-analysis, reported in the March 2006 issue of *Chest*, examined the link between antibiotic exposure in babies and subsequent development of asthma, as well as the dose–response relationship. Marra's team analyzed four prospective studies and four retrospective studies conducted between 1999 and 2004. Each study involved between 263 and 21,120 children, including cases who had been diagnosed with asthma between the ages of 1 and 18 years. The number of antibiotic courses taken ranged from one to seven, and averaged three.

Pooling the data from all eight studies revealed a twofold risk of developing asthma with at least one course of antibiotics. Each additional course raised asthma risk 1.16 times. Information about the antibiotics prescribed could not be obtained from the studies.

The findings support the "hygiene hypothesis," which proposes that an immune system that doesn't get enough practice killing germs (due to either an excessively clean environment or overuse of antibiotics) will become overly sensitized and overreact to normally harmless environmental agents such as pollen and dust.

Marra and her colleagues recently launched a community education campaign in British Columbia called "Do Bugs Need Drugs?" The program uses media ads, classroom visits, and educational materials to teach health professionals and the general public about the overuse of antibiotics. The campaign emphasizes the difference between bacterial and viral infections, useful preventive measures such as hand washing, and the need to use antibiotics wisely. "In children, antibiotics are commonly used to treat ear infections, upper respiratory tract infections, and bronchitis," says Marra, even though many such infections are viral and don't respond to antibiotics. Some parents may refuse to leave a doctor's office without a prescription.

The information gained from the meta-analysis is valuable for physicians who are striving to cut back on prescribing antibiotics, says W. Michael Alberts, president of the American College of Chest Physicians: "It can help to convince parents of young children to hold off on giving antibiotics unless absolutely necessary." **–Carol Potera**

POLICY

Live from Dubai: A New Chemical Agreement

After late-night, last-minute negotiations, a voluntary international agreement to protect humans and the environment against harmful chemicals was adopted on 6 February 2006. Representatives from 140 countries, environmental advocacy groups, industry associations, and UN agencies attended the three-day International Conference on Chemicals Management (ICCM) in Dubai, United Arab Emirates. The agreement establishes the Strategic Approach to International Chemicals Management (SAICM), which gives nations a framework for fulfilling the 2002 World Summit on Sustainable Development goal of ensuring that chemicals are produced and used in ways that minimize significant adverse effects.

Implementation of SAICM will be supported by a new chemicals secretariat within the UN Environment Programme that will carry out the "Overarching Policy Strategy." This strategy provides countries, especially economies in transition, with templates to begin coping with issues such as remediating contamination, using safer substitutes, and creating toxic release inventories. The agreement offers broad suggestions such as reducing exposures by improving occupational safety, developing better responses to spills and accidents, and eliminating child labor involving chemicals. Some EU countries offered modest funding for a "Quick Start Programme" to help developing countries move ahead in the near term.

Many participants saw the meeting as polarized into EU and U.S. camps on some of the most contentious issues, including precaution—regulating or banning chemicals suspected of harm without complete certainty of their effects. In a February 7 statement on behalf of the EU presidency, Austrian minister Josef Pröll said, "We don't need to see a tragedy happen to put safety systems in place." The same day, U.S. assistant secretary of state Claudia McMurray told an AP reporter, "We have a different approach to the way we regulate chemicals in our country. We may not know everything now, but let's move forward anyhow."

The agreement incorporates wording from the 1992 Rio Declaration on Environment and Development stating that precaution "shall be widely applied by States according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation." The EU pushed to elaborate on this statement with a clearer connection between chemicals and human health—a push the U.S. delegation opposed.

Disagreement also arose over whether the agreement should invoke specific international bodies such as the World Bank or the Global Environment Facility as potential funding sources. The United States failed to win language that would have made SAICM irrelevant to multinational regulations such as those of the World Trade Organization, a position some say was aimed at keeping environmental and human health values from challenging trade practices.

In a February 27 press release McMurray said, "SAICM recognizes that while we all share the goal of minimizing the risks presented by some chemicals, there are many valid ways to achieve that goal." But others saw the meeting as lacking political will. Daryl Ditz, senior policy adviser for chemicals at the Washington, DC–based NGO Center for International Environmental Law, says, "Regrettably, the United States was the number-one obstacle to a coordinated global response to the problems posed by chemicals."

The ICCM next revisits the Dubai agreement in 2009 to assess progress and identify problems. **–Valerie J. Brown**

ehpnet

The World of Food Science

The International Union of Food Science and Technology (IUFoST) is a nonprofit network of national food science organizations and is the only global food science and technology group, while the Institute of Food Technologists (IFT) is a U.S. scientific society for food scientists, technologists, and related professionals in industry, academia, and government. These two groups have come together to produce an online magazine, *The World of Food Science*, located at http://www.worldfoodscience.org/. With *The World of Food Science*, the IUFoST and the IFT work together to promote the scientific and technical aspects of international food production, distribution, preparation, and marketing, as well as provide current information to all food science professionals and others involved in the industry.

The homepage for *The World of Food Science* provides in-depth Focus and Feature articles collected from a variety of sources, including IUFoST correspondents and food scientists from around the world. Recent articles have covered modern biotechnology in food production, obesity, and how food packaging has evolved to ensure the safety of the food supply. Select articles are available in French and/or Spanish as well as English. Past articles are available by clicking the Archive link at the top of the homepage. The site also includes two Spanish-language sections: the full text of a report titled *Biotecnología y Alimentos* ["Biotechnology and Food"] and IFT Scientific Status Summaries that have been translated from English to Spanish.

At the bottom of the homepage are links to documents and expert reports published by the IFT. Currently featured are annual buyer's guides for nutraceuticals (foods or food components that provide a medical or health benefit) and food industry services. Visitors will also find links to the expert panel reports *Functional Foods: Opportunities and Challenges* ("functional foods" are formulated with ingredients believed to impart health benefits) and *Emerging Microbiological Food Safety Issues: Implications for Control in the 21st Century*. Clicking on the links for the expert reports takes visitors to webpages devoted solely to those respective topics. Available resources include the full text of each expert panel review, one-page topic summaries, related news releases, and lists of frequently asked questions.

The homepage also includes a frequently updated collection of news coverage. Clicking on any headline takes the visitor to the Daily News page. A searchable library of past news articles is archived by month. Visitors can also choose from a menu of other news options: national food technology association news, regional reports on food issues, papers directed toward students in the field, international regulatory updates, and policy papers. The Events section has information on upcoming meetings related to food science and technology. **–Erin E. Dooley**

America's Best Development Projects

As part of its efforts to curb the expansion of low-density, vehicle-dependent communities, the Sierra Club has released its first *Guide to America's Best Development Projects*. The guide spotlights 12 U.S. projects that the group holds up as models for building healthier and more sustainable communities—and invites local governments to demand more of them. The featured projects boast access to a range of transportation choices, redevelopment of existing urban areas, proximity of homes to shops and offices, preservation of existing community assets such as older buildings and natural resources, minimization of stormwater pollution and runoff, input from the local citizenry in planning, and use of green building methods.

Building Eco-Cities in China

The London-based consulting firm Arup has signed a multibillion-dollar contract with Chinese officials to design and build up to five self-sustaining "eco-cities" around China. The first eco-city, Dongtan, on an island near Shanghai, is expected to be home to about 1.15 million people by 2040. The city's first phase, accommodating 50,000 residents, will be completed in time for the 2010 Shanghai Expo trade fair. Other locations have not yet been revealed. The cities, a magnet for investment funds, will feature the capture and purification of rainwater to support city life, use of organic waste materials as an energy source, and reduction of environmentally unfriendly landfills. Arup has also partnered with emissions brokerage CO2e to offset the carbon generated by personnel working on the initiative.

Maine Mandates e-Recycling

In January 2006, Maine became the first state to require that TV and computer monitor makers pay for the cost of recycling their products once they are discarded by consumers. The state has approved five waste services to gather and sort the electronic waste, ship it to recyclers, and bill manufacturers according to the amount of waste they originated. Municipalities may choose whether they will operate an ongoing collection center, do regular one-day collections, or have their residents deliver items directly to a nearby consolidator.

The law reflects similar initiatives in Europe and Japan to help keep toxic materials such as lead, mercury, cadmium, brominated flame retardants, and phosphorus coatings out of the environment. According to the EPA, e-waste is now the nation's fastest-growing category of solid waste.

Top to bottom: Chris Reuther/EHP; *The World of Food Science*; Chris Reuther/EHP

NTP Multigenerational Study of Environmental Estrogens

Nearly 10 years ago, researchers with the National Toxicology Program (NTP) and the National Center for Toxicological Research (NCTR) began a complex set of experiments in rats to determine whether exposure to estrogenic compounds throughout life and across generations could cause changes in development or patterns of endocrine-related cancers at doses that cause only subtle reproductive effects. Now, at last, specialists in the field of endocrine-active chemicals are close to getting a first look at the findings from these studies.

The three compounds chosen for study—genistein, ethinyl estradiol, and *p*-nonylphenol—represent a natural estrogenic substance, a drug, and an industrial chemical, respectively. The first experiments for all three compounds focused on determining appropriate dose ranges for later multigenerational studies. Additionally, studies were conducted with genistein and ethinyl estradiol to determine whether and how the carcinogenic potential of these substances changed across generations following long-term chronic exposure.

On 12 June 2006, the first reports based on these experiments will undergo peer review at a one-day meeting at the NIEHS, with final publication expected later this year and additional reports scheduled for review in 2007. The reports to be reviewed on June 12 center on genistein, an estrogen-like compound found in soy, and detail the results of dose range–finding studies and multigenerational reproductive and carcinogenesis experiments.

Years in the Making

According to John Bucher, deputy director of the NIEHS Environmental Toxicology Program and a member of the group that designed and monitored the studies, the potential for endocrine disruption affecting development has been a topic of interest at the NIEHS since the late 1970s, when the institute held its first conference to examine the matter. Through the 1980s and into the 1990s, accumulating research established solid biological plausibility for the idea that small perturbations in hormonal status triggered by environmental exposures could ultimately affect development.

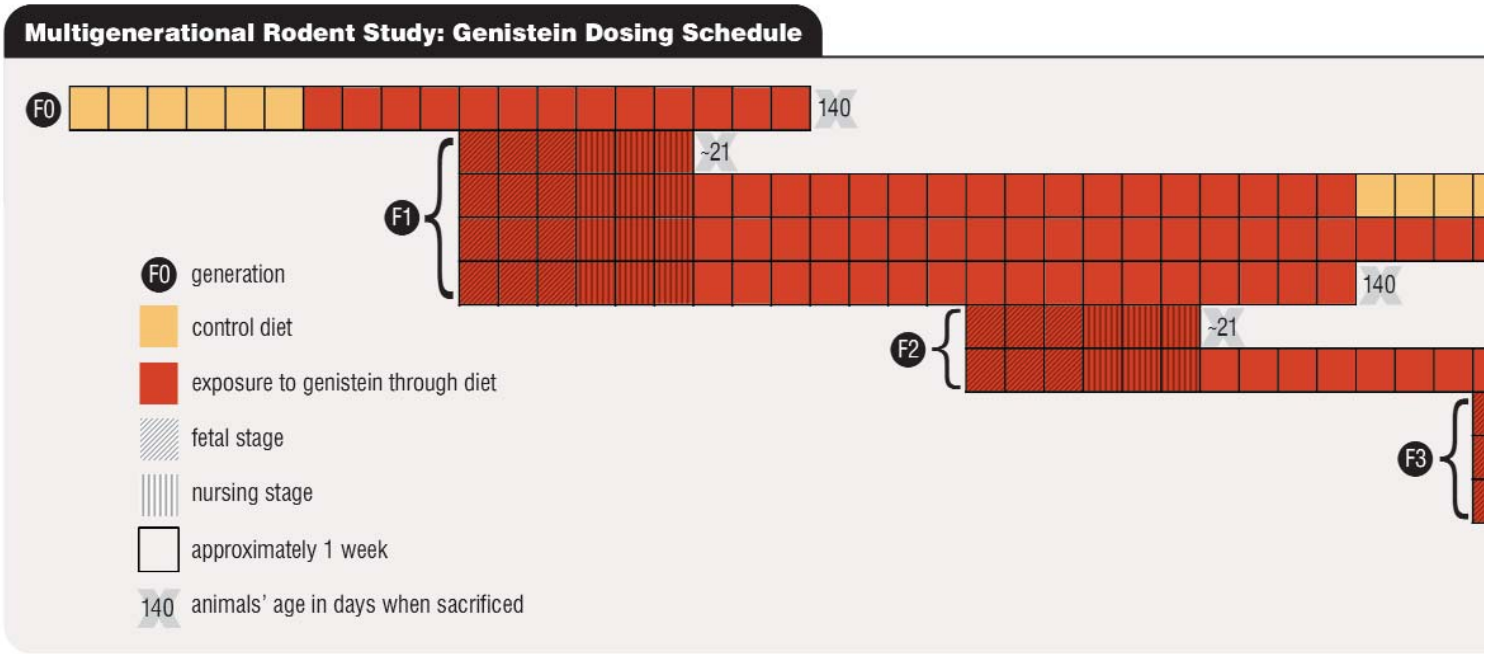
There were still many unknowns, however, according to Robert Chapin, a former NIEHS reproductive toxicologist now at Pfizer. "As is most often the case in science," he says, "there was a whole lot more that was unknown than was known about low-dose exposure to estrogenically active chemicals. There were lots of claims being made about these [chemicals] that were not biologically plausible."

Following the 1994 NIEHS-sponsored meeting "Estrogens in the Environment III," Bucher, Suzanne Snedeker (a former NIEHS scientist now at Cornell University), Chapin, and others at the NTP decided to put together a new series of experiments. The goal: to evaluate the potential of estrogenic influences during development to change developmental patterns for sexually related characteristics or hormonally mediated tumor patterns in animals as they aged. "We thought the NTP would be able to do this in a way that few other groups could, in a very comprehensive and thorough manner," says Bucher.

The NCTR's expertise in such large-scale studies and its interest in the area made it the ideal partner. This branch of the FDA conducts toxicological research to determine the human exposure to and risks of products that are regulated by that agency. With that, the NTP entered into an interagency study with K. Barry Delclos, the principal investigator at the NCTR, and Retha R. Newbold, the principal investigator at the NIEHS.

Selecting the Candidates

The researchers originally selected five chemicals for study: methoxychlor, genistein, ethinyl estradiol, *p*-nonylphenol, and vinclozolin. The first four seemed to have estrogenic properties in addition to other, unique characteristics, and their inclusion was expected to provide the opportunity



to tease out which effects could be related to estrogenicity versus the responses specific to the individual chemical.

After dose range–finding studies were completed in 2001, the researchers decided against conducting multigenerational studies on methoxychlor and vinclozolin. There were several reasons for this decision, including the fact that methoxychlor didn't exhibit enough of an estrogenic effect to justify doing the additional studies, and that vinclozolin was the only antiandrogen, with no comparison compounds being tested.

The doses of 5, 100, and 500 milligrams of genistein per kilogram per day were selected very carefully. "What we were interested in was studying a wide range of concentrations," says Bucher. "We wanted to select a top dose for the multigenerational studies that had a clear biological effect but didn't affect the animals to the extent that reproduction would be inhibited. We wanted to put the lower doses in the range of human exposures."

Studies Begin

Exposure for the parental (F_0) generation began when the animals were weaned to feed supplemented with the test compound. The feed did not contain alfalfa or soy, because both contain naturally occurring estrogenic compounds. The subsequent F_1, F_2, and F_3 generations of offspring experienced exposure to the chemicals prenatally but much less so through lactation; subsets of the F_1 and F_2 generations consumed supplemented feed upon weaning, but exposure ceased for the F_3 generation at weaning. The F_4 generation was unexposed. Subsets of animals were examined at each stage of development, and additional subsets of the F_1 and F_3 generations were used for the chronic exposure assays. "It was a long, complicated series of studies," says Bucher.

The complexity was necessary, however, because a major thrust of the studies was to test whether effects worsened over succeeding generations and whether they were reversible if exposure ended. Chapin explains, "From the public policy exposure–decision point of view, if you could show that things started to get better once you stopped exposure, that would mean that . . . if we did something about it, it would reverse any endocrine disruptor–mediated reproductive compromise that might be happening in human populations."

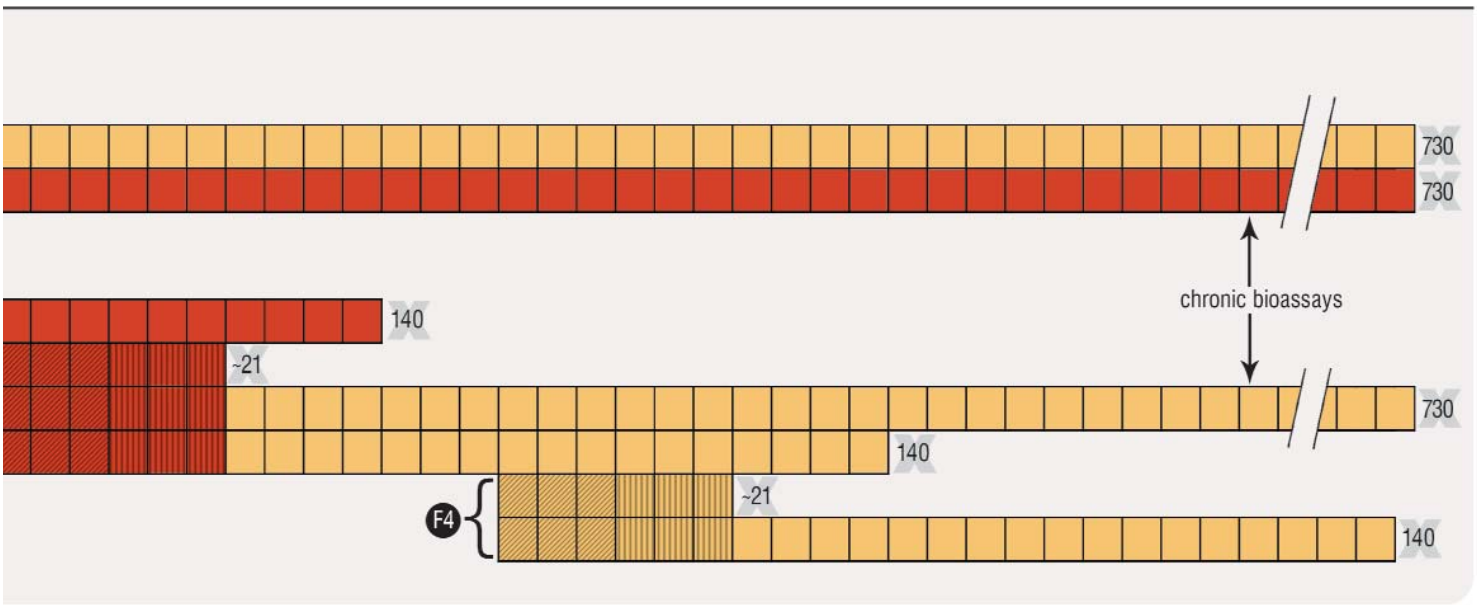
The studies could have been even more complex, according to Delclos. "I can think of a few things off the top of my head that would have been nice to do if resources were unlimited," he says. He speculates that including more dose groups would have allowed for better characterization of the dose–response curve, especially in the lower-dose region reflecting likely human exposure levels. Additionally, no one rodent model is ideal. Although the rats used in these experiments are well suited for multigenerational studies and have low background rates of some reproductive tumors, they do have high background rates of pituitary tumors and, in females, mammary tumors. This makes it more difficult to pick up on subtle changes that might be occurring.

With regard to genistein, Delclos indicates that the use of dosed feed led to very low exposure during the early neonatal period because the transfer of genistein through the milk was minimal; thus, the exposure was too low to have an effect. Overall, comparison of the pure compound with more complex soy extracts would have been of interest, to provide a better sense of the effects of real-life exposure.

Food for Thought

Nevertheless, the studies have yielded in-depth information on how mammals respond across developmental stages and through several generations to estrogenic compounds at exposures relevant to human health. Findings from different sets of experiments may ultimately be compared between test substances to elucidate common responses to estrogenic compounds.

These studies are a classic example of the value of the NTP, says Chapin. "They're much larger than any academic or even most contract research organizations would be able to accomplish, [and] they are clearly in the public interest," he says. "It's a perfect reason why we have an organization that does this kind of stuff—to do the kind of studies that are critically important for public health but don't get done anywhere else." **–Julia R. Barrett**



BEYOND THE BENCH

Bringing EXCITEment to the Classroom

Who are the scientists, public health officials, and policy makers who will monitor our relationship with the environment 20 years from now? Right now a lot of them are students in middle and high schools throughout the country. And it's a certainty that these future stakeholders will need to develop the diversity of skills required to tackle the complex issues that arise where environmental and human health intersect—skills that go beyond the practice of simple classroom science experiments. Answering this call to train is Project EXCITE (Environmental Health Science Explorations through Cross-Disciplinary and Investigative Team Experiences), an NIEHS-supported program at Bowling Green State University (BGSU) in Ohio.

Project EXCITE was developed by the Environmental Health Program in the BGSU College of Health and Human Services and the School of Teaching and Learning in the College of Education and Human Development. Under the codirection of principal investigators Chris Keil and Jodi Haney, this seven-year program seeks to raise the bar on training for the next generation of environmental health stewards by focusing on problem-based learning techniques that encourage independent critical thinking skills—or "hands-on, minds-on" learning—for 4th- through 9th-grade students. Teacher and student participants come from schools across northwest Ohio.

The strength of Project EXCITE lies in its two-tiered approach of providing comprehensive training and education to both teachers and students. For teachers, professional development is offered in a two-year "cohort" program. In each cohort, teams of four or more teachers recruited from a variety of disciplines receive training in environmental health content and in research-based best practices for teaching. The teacher teams network with agencies and scientists in their communities as well as BGSU faculty, and spend the first year of the program creating their own "Odyssey"—an interdisciplinary, problem-based curricular unit based on an environmental health science topic—which is then implemented in the classroom the following school year. The teachers receive up to 10 graduate credit hours and a stipend.

For students, learning comes as they travel through the Odysseys their teachers create. Each Odyssey, lasting up to six weeks, is formatted into four steps: Meet the Problem, Investigate and Inquire, Build Solutions, and Take Action. As students follow the steps through an Odyssey, they learn to approach and examine a problem by identifying specific environmental agents and measuring their effects on health. Additionally, students begin to understand how environmental health science research can influence community policy decisions.

"One of the greatest things about Project EXCITE is the real-world context—students explore environmental health issues that are local and are important to them," says Project EXCITE program manager Jennifer Zoffel. "They learn not only that these problems exist, but also that they as students and as community members can build solutions and take actions to minimize the impacts of the issue or educate others about it."

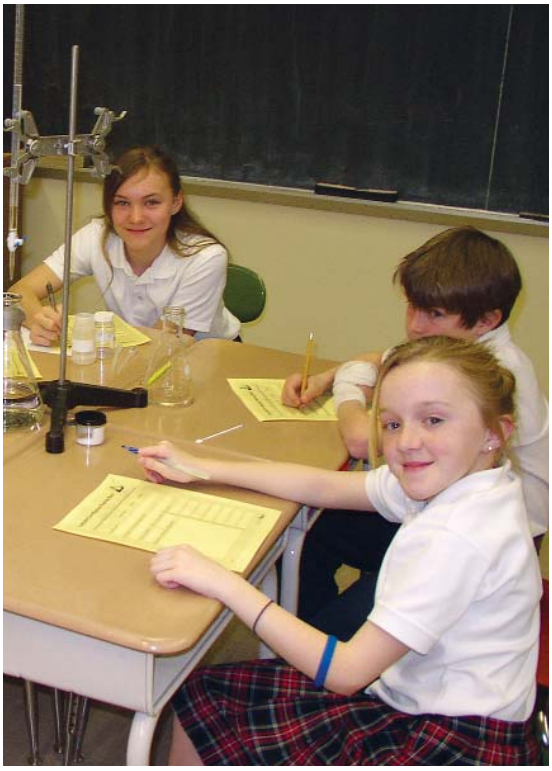
"Sick of School? Odyssey" was inspired by a group of middle school students who investigated the quality of their school's indoor environment as part of the 2001–2003 cohort. The students worked through the first three steps of the Odyssey by researching water damage, bioaerosols, drinking water quality, and elevated carbon dioxide levels in their school building. During the final Take Action step, they delivered recommendations for changes to the district principals and the school board. Two of their recommendations—to change room ventilator filters once per season rather than once per year, and to repair the leaking roof—were accepted.

An epic learning adventure. Project EXCITE offers science teachers the opportunity to craft interdisciplinary curricular units called "Odysseys," which they then carry back home to their students. Each Odyssey introduces students to a real-world environmental health issue. The students investigate the issue, devise solutions, and then take action, sometimes effecting actual changes in their own environments.

Odyssey programs created by previous cohorts are available for sale at the program website, http://www.bgsu.edu/colleges/edhd/programs/excite/. Besides "Sick of School? Odyssey," other programs currently available include "ZoOdyssey" (based on student illnesses that arise after a trip to the local zoo), "AgOdyssey" (which compares small- and large-scale farming), "Food Odyssey" (a study of food contamination in restaurants), and "ChemOdyssey" (which examines the safety of common chemical cleaners).

Educators who are unable to participate in a full two-year cohort can still take advantage of intensive two-day workshops, or "institutes." There they will receive one hour of graduate credit, funds to purchase classroom supplies, and a completed Project EXCITE Odyssey for classroom implementation.

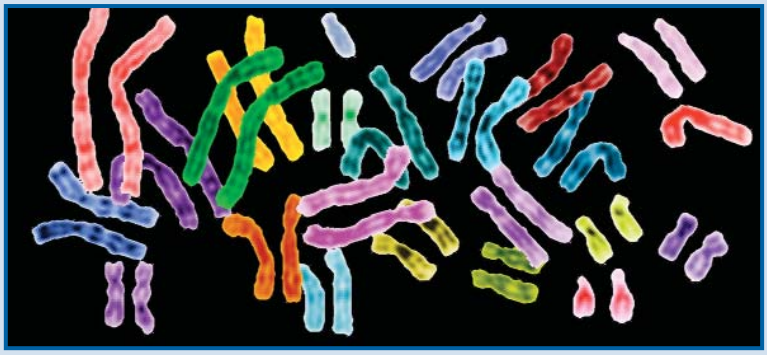
The program, now in its sixth year, recently received the U.S. EPA's 2006 Children's Environmental Health Recognition Award—one of 30 given, and the only one awarded in the state of Ohio. New Odysseys are also in the works: among others, "GermOdyssey" will allow students to become "disease detectives" by learning about different pathogens and how they infect the body, as well as the mechanisms that the body uses to fight off these illnesses, and "Sick Ship Odyssey" will look at illnesses aboard cruise liners. **–Tanya Tillett**



Left to right: Project EXCITE; National Human Genome Research Institute

Headliners | Neurodevelopment

NIEHS-Supported Research



Genomewide Screen Reveals Candidate Genes for Neural Tube Defects

Rampersaud E, Bassuk AG, Enterline DS, George TM, Siegel DG, Melvin EC, et al. 2005. Whole genomewide linkage screen for neural tube defects reveals regions of interest on chromosomes 7 and 10. J Med Genet 42:940–946.

Neural tube defects are among the most serious and most common severely disabling forms of human birth defects. These defects—which arise from failure of the neural tube to close, an event that usually happens around day 28 after conception—are thought to be caused by a complex interaction between a person's genetic makeup and environmental factors. Now NIEHS grantee Marcy C. Speer of Duke University Medical Center and colleagues from 14 research facilities across the United States report on a nationwide collaborative effort to gain new insights into the possible sites of a neural tube defect gene or genes.

There are three major types of neural tube defects, all with devastating consequences. Nearly all children with anencephaly (the absence of a major portion of the brain, skull, and scalp) die *in utero* or shortly after birth. Children with encephalocele (in which the brain protrudes through an opening in the skull) may survive but are almost always mentally retarded. And children with spina bifida (in which the spine fails to close properly) have varying degrees of muscle weakness and sensory disorders.

The most important environmental risk factor for neural tube defects is insufficient folate consumption by the mother around the time of conception. Folate supplementation reduces the risk of neural tube defect recurrence by 50–70%, but it does not entirely eliminate the risk. This suggests underlying genetic factors, a supposition bolstered by the increased rate of recurrence in siblings and the increased risk of defects in the offspring of a person with a neural tube defect. However, studies of folate-related and other developmental genes in humans have failed to definitively identify a gene causing neural tube defects.

In the current study, the researchers identified 44 families with more than one occurrence of a neural tube defect. They extracted DNA from whole blood samples of the affected individuals and related family members, for a total of 292 samples. Then they performed both parametric and nonparametric genomic linkage analyses. The results pointed to two candidate genes on human chromosome 7 and three on chromosome 10.

The researchers expect these results will help to prioritize future studies of neural tube defect candidate genes, and they plan to add additional families to their analyses. They also want to expand the phenotypic classifications to allow for a greater sample size and integrate other data such as those from mouse models of neural tube defects. The data in the present study bring the medical community closer to the day when individual-level prediction of the risk of a neural tube defect may be possible. **–Jerry Phelps**

Eddie Lepp/ShutterStock

the SCIENCE of SOY

What Do We Really Know?

A stroll through nearly any American grocery store or pharmacy yields ample proof of the soybean's increasing role in the U.S. diet. Food packaging offers statements about products' soy content and the purported associated health benefits. Products such as tofu, soy milk, soy-based infant formula, and meatless "texturized vegetable protein" burgers are widely available. Shelves of dietary supplements and nutraceuticals are stocked with isoflavones, naturally occurring estrogenic compounds found in soy. The general impression is one of certainty that both soy and soy isoflavones deliver many health benefits, including prevention of cardiovascular disease, cancer, and osteoporosis, as well as treatment of menopausal symptoms. The science is less absolute, however, and still evolving.

Soy provides a complete source of dietary protein, meaning that, unlike most plant proteins, it contains all the essential amino acids. According to the American Soybean Association, 3.14 billion bushels (85.5 million metric tons) of soybeans were harvested in the United States in 2004. Approximately half of the harvest was exported, and most of the remainder was crushed to produce oil and protein meal for domestic use. An April 2006 report from the USDA Economic Research Service indicates that only a small amount of whole soybeans are used to produce soy foods, and just 2% of soy protein meal is used for human consumption; the rest is used for animal feed.

The Soyfoods Association of North America reports that U.S. sales of soy foods reached $3.9 billion in 2003, continuing an 11-year trend of 15% average annual increases. According to the United Soybean Board's 2004–2005 *Consumer Attitudes About Nutrition* report, 25% of Americans consume soy foods or beverages at least once per week, and 74% view soy products as healthy.

Nevertheless, Americans as a whole still consume very little soy protein. Based on 2003 data from the UN Food and Agriculture Organization, per-capita soy protein consumption is less than 1 gram (g) per day in most European and North American countries, although certain subpopulations such as vegetarians,

Asian immigrants, and infants fed soy-based formula consume more. The Japanese, on the other hand, consume an average 8.7 g of soy protein per day; Koreans, 6.2–9.6 g; Indonesians, 7.4 g; and the Chinese, 3.4 g.

Traditional soy foods include tofu, which is produced by puréeing cooked soybeans and precipitating the solids, and miso and tempeh, which are made by fermenting soybeans with grains. "Second generation" soy products involve chemical extractions and other processing, and include soy protein isolate and soy flour. These products become primary ingredients in items such as meatless burgers, dietary protein supplements, and infant formula, and are also used as nonnutritive additives to improve the characteristics of processed foods.

Health Effects of Soy

Soybeans and soy foods contain a variety of bioactive components, including saponins, protease inhibitors, phytic acid, and isoflavones. Isoflavones belong to a class of compounds generally known as phytoestrogens, plant compounds that have estrogen-like structures.

The dominant isoflavone in soy is genistein, with daidzein and glycitein composing the remainder. Within soy, isoflavones are almost entirely bound to sugars, producing the respective compounds genistin, daidzin, and glycitin. Soy isoflavones have been linked with numerous health effects, but the strength of the relationships and whether the effects are beneficial are strongly debated.

Soy isoflavones are frequently referred to as weak estrogens, and depending upon the specific circumstance, they can act as agonists, partial agonists, or antagonists to endogenous estrogens (such as estradiol) and xenoestrogens (including phytoestrogens) at estrogen receptors. They are not especially potent, however, and activity varies by tissue concentration, cell type, hormone receptor type, and stage of differentiation. In addition to their estrogen receptor activity, isoflavones may also interfere with steroid metabolism by inhibiting aromatase, hydroxysteroid dehydrogenase, and steroid α-reductase, and by altering the ratio of estradiol metabolites. Soy isoflavones may also act as antioxidants; inhibitors of proteases, tyrosine kinases, and topoisomerases; inducers of Phase I and/or Phase II enzymes such as cytochrome P450s, glutathione *S*-transferase, and quinone reductase; and inhibitors of angiogenesis.

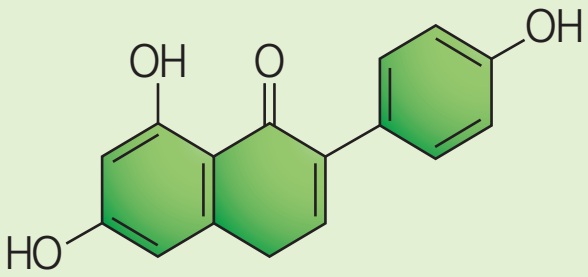
Such activities have potential benefits—if they occur in the body. Caution is necessary when predicting *in vivo* potency from *in vitro* systems. *In vitro* systems are valuable for investigating the structure–activity relationships and the mechanisms of isoflavone actions, but *in vitro* tests have used genistein concentrations that may be five times higher than the peak concentrations seen in human serum, 95% of which occurs as glucuronide conjugate. Animal studies also require careful extrapolations due to how exposure occurs, interspecies differences in metabolism, and comparability of the stage of development at which exposure occurs.

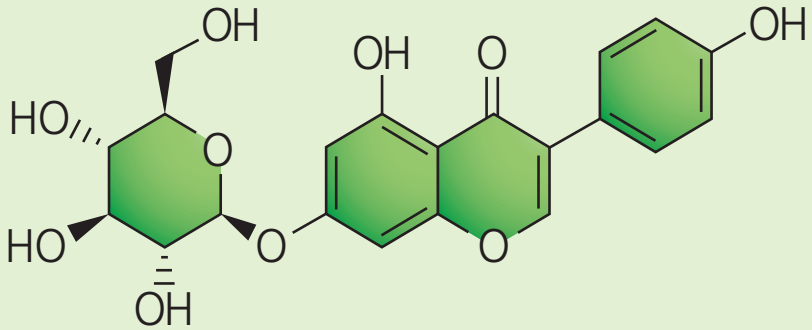
Retha R. Newbold, a supervisory research biologist at the NIEHS, is well aware of these factors. Concerns about genistein's effects on reproduction and development are due in part to her extensive research in mice. Newbold believes caution is warranted, because her studies, as well as others, have shown that genistein has such effects as inducing uterine adenocarcinoma in mice and premature puberty in rats. A recent study led by biologist Wendy Jefferson in Newbold's laboratory and published in the October 2005 issue of *Biology of Reproduction* linked genistein with effects such as abnormal estrous cycle, altered ovarian function, and infertility in mice.

The original interest in soy was fueled by geographic epidemiology—the observation that populations that consume a lot of soy, particularly those in eastern Asia, have less breast cancer, prostate cancer, and cardiovascular disease, and fewer bone fractures. Additionally, women in these populations report fewer menopausal symptoms, such as hot flashes, and both men and women have a lower incidence of aging-related brain diseases. Since lifestyle can affect chronic disease development, and diet is a major lifestyle factor, traditional Asian diets drew considerable attention.

Although initial research overestimated the amount of soy consumed by Asians, the cumulative evidence of numerous biomarker studies has confirmed that their diets are significantly higher in both isoflavones and lignans (another phytoestrogen) compared to the typical Western diet. Studies have further shown that when Asians emigrate to Western nations such as the United States and adopt the prevailing diet, their disease rates change.

What's interesting, says Jay Kaplan, head of comparative medicine at Wake Forest University School of Medicine, is that people who switch to an American-





G-force. The dominant isoflavone in soy is genistein (above), which within soy is almost always bound to a sugar molecule, producing genistin (below). Once genistin enters the digestive tract, it releases its sugar. Most of the "free" genistein is subsequently reconjugated into glucuronides or sulfates.

Matt Ray/EHP

The culture question. Early research looked to the differences in soy consumption between Asian and Western diets to explain differences in disease rates, but results are far from conclusive.

style diet from a traditional diet high in plant protein take on the disease characteristics of the host population, not those of their ancestral population. "It does seem to be something that's in the environment, and it looks like this reliance on plant proteins is one of these things that goes away after [immigrants have] been here a while," Kaplan says. "What also goes away is any protection from chronic disease that we ascribe to those populations."

By the late 1990s, the epidemiologic and experimental data seemed strong enough to support recommendations to incorporate soy in the diet. Still, not everyone was convinced.

Isoflavone Variables and Risks

Soy research is complicated because there's considerable variation in isoflavone exposure among people classified as soy consumers. Agronomic factors (such as the soybean cultivar and the environmental conditions under which the crop grew) affect a food's isoflavone profile, as does the way a soy food is processed. For example, soy protein concentrate produced by alcohol extraction may have only 12.5 milligrams (mg) total isoflavones per 100 g, in contrast to the nearly 199.0 mg total isoflavones per 100 g of full-fat roasted soy flour. Additionally, the fact that most of the isoflavones in food occur bound to sugar affects how they are digested.

Once genistin enters the digestive tract, it releases its sugar and becomes "free" genistein. Some of this free genistein is absorbed. However, most is reconjugated into glucuronides or sulfates, the primary circulating forms of genistein, which are thought to have either low or no biological activity. Only a very small amount of free genistein escapes conjugation by the liver and circulates in that form.

"People need to know that as it occurs in soy and other plant products, genistin is the compound that's there. The amount of actual genistein is very low, one percent or less probably," says Michael Shelby, director of the National Toxicology Program's Center for the Evaluation of Risks to Human Reproduction (CERHR). Key exceptions are fermented products, such as miso and tempeh, which may contain up to 40% free genistein.

Several researchers say that figuring out the pharmacokinetics of genistin and genistein is a vital piece of missing information. "It's a matter of finding out how much genistin is converted to genistein in the digestive system, and that information is not known," says Jefferson. "I don't think a lot of this was understood years ago when some of the animal experiments started, and at that time we didn't have a clear understanding of the metabolism and fate of these chemicals. We did the best we could, as a community, to try to use the compound we thought would be the one we should look at. I think it's given us some excellent starting types of data, where we know that these compounds are capable of causing reproductive and developmental effects."

According to Thomas Badger, director and senior investigator at the Arkansas Children's Nutrition Center in Little Rock, however, these effects are seen only under certain experimental conditions that are not likely to occur in humans—and therein lies the crux of the debate. Criticisms of many studies of genistein's effects on reproduction and development have centered on exposure occurring by injection and consequently bypassing the usual metabolic pathways. There is also disagreement about the use of neonatal mice—commonly used in studies of reproduction and development—as a suitable model for predicting effects in human infants.

Despite these criticisms, Newbold stands by her data. "There was some confusion on the fact that in all of our work we have injected genistein," she says. "We went back and did some of the pharmacokinetics with that to show that the total circulating amounts of genistein are very similar to what's been reported in feeding rats and also in infants. Metabolism doesn't have to be the same, but you have to know that the active compounds are getting to the target tissue. Ultimately, a mouse and a rat are not the human, though. You just have to accept it and be as careful with your extrapolations as possible."

Further controversy surrounds the fact that most of the epidemiologic studies of Asian populations involved whole soy foods, but animal and human intervention studies have generally used soy concentrates or isolated isoflavones; some animal

From left: Buretsu/Stockphoto; lisaaadphotography/ShutterStock

Boon or bane? The touted benefits of consuming soy as part of a healthy diet or ingesting soy supplements as a remedy for menopause symptoms are many, but some data suggests cause for concern.

studies used pure genistein. This difference may have obscured what the health effects of soy actually are.

"I'm reasonably sure that any time you take one of those isoflavones and give it separately, you don't see the same effects as when all three of the isoflavones of soy are given," says Kaplan. "Based on everything that we know, the best health effects probably come from the whole isolated soy protein given together. There's something about the intact product that seems to be bioactive that is not able to be replicated when you begin chopping it up."

In *Expert Panel Report on the Reproductive and Developmental Toxicity of Genistein,* a March 2006 review of the literature on this compound, an expert panel convened by the CERHR scrutinized what has been learned about human exposure to genistein and the associated reproductive and developmental consequences. The most highly exposed adult population was Japanese, with a daily average intake of 0.43 mg per kilogram body weight, which was approximately 10-fold less than the no-effect levels found in rodent studies. Based on the conclusions presented at a meeting held on 15–17 March 2006, the panel found little cause for concern about human exposure to genistein. However, no consideration was made for the amount of genistin found in the diet or how much of it is hydrolyzed in the digestive system to genistein. Further, the panel's conclusions were not unanimous.

Considerably less attention has attached to daidzein, though there are currently indicators that it may play a larger role than genistein in soy's apparent beneficial health effects. Like genistein, daidzein in soy exists primarily in linkage with a sugar molecule. This complex, daidzin, is hydrolyzed and the sugar molecule removed in the gut. Daidzein can also be conjugated to glucuronic acid or sulfate in the gut and liver. It may also be converted to equol (suspected of having a higher estrogenic potency than the original daidzein) by gastrointestinal bacteria. There is considerable variability in individuals' ability to produce equol, and the metabolic pathways for both genistein and daidzein may vary due to factors such as a person's particular microflora, intestinal transit time, and current or recent use of antibiotics and other drugs.

Thomas Clarkson, a professor of comparative medicine at the Wake Forest University School of Medicine, points out that although soy protein has a very large beneficial effect on cardiovascular health in monkeys, the effects are much less clear in women. Daidzein metabolism may be the key.

"Our best clue is that all monkeys are equol producers, but only about twenty-five or thirty percent of women are equol producers," Clarkson explains. "There's some suggestion now that those women who are equol producers do derive some cardiovascular benefits. The fact that [the effects are] so profound in monkeys may have to do with the fact that they're all equol producers, and [those effects] may only be translatable to the women who are equol producers."

There have been only a few studies that have looked exclusively at glycitein, the third soy isoflavone, but those have not been on health effects. There are indicators from a couple of recent *in vitro* studies that glycitein may be protective of bone. Most glycitein research has focused on determining how to detect the compound, and its estrogenicity and metabolism.

Soy-Based Infant Formula

Approximately 20–25% of U.S. infants receive at least some soy-based formula in their first year (there are no numbers on

Top to bottom: jaymast/Shutterstock; MR/ShutterStock

how many are exclusively fed soy formula). Unlike soy milk, which is sometimes mistakenly—and tragically—used in its place, soy formula contains soy protein isolate supplemented with additional amino acids, minerals, vitamins, and fats necessary to support infant growth and development. Parents may choose soy formula for babies who are allergic to cow's milk–based formula or if they themselves do not consume dairy products.

Steroid hormones affect myriad processes during development, including the formation of hormone-responsive tissues and organizing and activating effects in the central nervous system. Some researchers are therefore concerned that isoflavones from soy-based infant formula might perturb that system, with long-term consequences.

In a study led by Kenneth Setchell at the Children's Hospital Medical Center in Cincinnati and published 5 July 1997 in *The Lancet*, infants fed soy formula were found to receive 28–47 mg of soy isoflavones per day. Isoflavones were detected in blood, showing that infants absorbed the compounds from the intestine. This was not a given, since the infant gut is significantly different from the adult gut and continues to develop through the first year.

Biological effects are plausible but not necessarily detrimental. For example, in a study comparing the short-term, long-term, and multigenerational effects of soy protein isolate, casein, and whey on the health and development of rats, a group led by Badger found that soy protein isolate was protective against chemically induced breast cancer. This study appeared in the 1 May 2001 issue of the *International Journal of Toxicology*.

In advising caution in feeding infants soy formula, several groups cite a study led by Richard Sharpe at the Centre for Reproductive Biology in Edinburgh, Scotland. The study, published in *Human Reproduction* in July 2002, compared infant marmosets fed cow's milk–based formula with others that were fed soy-based formula. The soy-fed marmosets had comparatively lower testosterone levels and higher numbers of Leydig cells per testis. However, a follow-up study published in April 2006, also in *Human Reproduction*, indicated no obvious effects on reproduction.

One of the few human studies was led by Brian Strom of the University of Pennsylvania in Philadelphia, in conjunction with the University of Iowa, and published in the 15 August 2001 issue of *JAMA*. In this study, adults who had been in a controlled feeding study during infancy completed a telephone interview about their health, development, and reproductive history. The only significant differences reported were that women who received soy formula as infants had slightly longer menstrual bleeding and more discomfort than women who had received cow's milk–based formula. A follow-up letter to the *JAMA* editor pointed out that both the Strom study and a retrospective epidemiological study published in the April 1990 issue of the *Journal of the American College of Nutrition* suggested that consumption of soy formula could adversely affect immune function in children.

More human data are clearly needed, as described in the recent CERHR *Expert Panel Report on the Reproductive and Developmental Toxicity of Soy Formula*. "The findings for soy formula were that there's just not enough information to make the call. I'm not surprised by that at all," says Newbold. "Hopefully, the next step that will come from this is that there certainly will be more research with soy formula and more epidemiology studies. That's definitely what we're missing."

For their part, both Jefferson and Newbold caution against using their results to determine the safety of soy formula, although they believe their findings of adverse effects in rodents provide strong evidence that concern is warranted. Their findings are not definitive proof that soy formula is harmful, however. "The studies in our laboratory are to determine if these compounds can cause an effect at any dose level," says Jefferson. "The studies we do in our laboratory were designed to study mechanism, and not specifically intended for risk assessment."

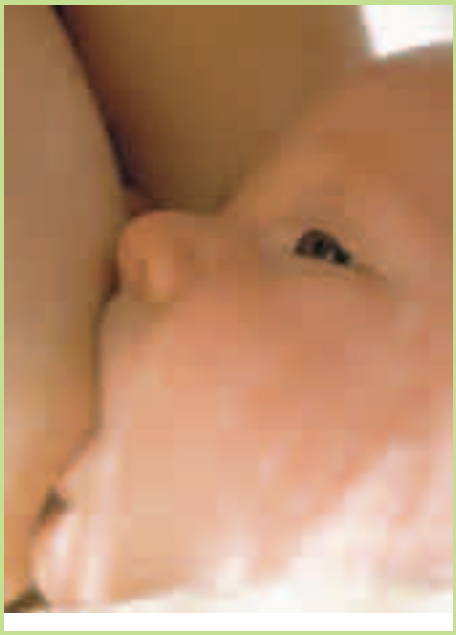
Given the limited evidence for the health effects of soy isoflavones in infants, pediatric and health organizations in several countries suggest caution in feeding soy to infants and young children. If an infant is not receiving breast milk (either its mother's or a donor's), cow's milk–based infant formula is the first recommendation. If there seems to be a problem with that option, parents shouldn't automatically switch to soy formula, assuming dairy allergy or lactose intolerance. Soybeans are a major allergen, and a significant percentage of children who are sensitive to dairy are also sensitive to soy.

However, other experts indicate that soy formula is an adequate source of nutrition for infants with more than 40 years of apparently safe use. "It's always true that there could be something subtle that we didn't look for or didn't know to look for, but so far we haven't seen any major health problems," says Susan Baker, a pediatric gastroenterologist at the Children's Hospital of Buffalo and former chair of the American Academy of Pediatrics Committee on Nutrition. Marisa Salcines, director of communications for the Atlanta-based International Formula Council, which represents infant formula manufacturers, adds that there's no conclusive evidence for alarm in terms of genistein in soy formula. "There haven't been any studies that have shown

Scott Dunlap/iStockphoto

Bottled 'beans. About one-quarter of U.S. children receive some soy-based formula. Parents sometimes choose soy formula in the belief that it is less allergenic than cow's milk–based formula, even though soybeans themselves are a major allergen.





Baby steps toward better understanding. A prospective longitudinal study now in its fourth year seeks to clarify whether concerns about soy-based infant formula are justified. The study compares the growth and development of children fed cow's milk–based formula, soy-based formula, or breast milk, from birth through puberty.

any negative effects in adults who consumed soy-based infant formulas as babies," she says.

To clarify whether or not concerns are justified, Badger is leading the world's largest longitudinal, prospective study of children comparing soy-based formula, cow's milk–based formula, and breast milk. During the study, now in its fourth year, children receive multiple in-depth checkups, including assessments of bone development and health, imaging of reproductive tissues, and assessments of brain development and function, metabolism, growth, development, and body composition. The research team aims to enroll 600 pregnant women, whose children will be followed from birth through puberty.

Additionally, Walter Rogan, a senior investigator in the NIEHS Epidemiology Branch, is heading the Study of Estrogen Activity and Development. Through pilot studies conducted in late 2004 at the Children's Hospital of Boston and the Children's Hospital of Philadelphia, researchers gathered physical, sonographic, and biochemical data from infants fed soy formula, cow's milk formula, or breast milk. Data analysis is currently under way.

Finessing Investigations

On balance it does not seem that soy and its constituent isoflavones have met original expectations. Clinical results with regard to soy's ability to reduce the risk of cardiovascular disease have been inconsistent; a review in the 21 February 2006 issue of *Circulation* indicated there was little to no effect. The only apparent impact of soy and soy isoflavones on cardiovascular disease risks seems to be a slight reduction in low-density lipoproteins in individuals who had very high levels of cholesterol. An August 2005 report from the DHHS Agency for Healthcare Research and Quality, *Effects of Soy on Health Outcomes*, also concluded that there was little evidence to support a beneficial role of soy and soy isoflavones in bone health, cancer, reproductive health, neurocognitive function, and other health parameters.

Nevertheless, there remain tantalizing clues that soy may benefit human health. For example, *in vitro* studies with human breast cancer cells suggest that genistein may induce detoxification enzymes and inhibit growth of both estrogen receptor–positive and estrogen receptor–negative cancers. Additionally, *in vitro* studies demonstrate that genistein inhibits prostate cancer cell growth, and epidemiologic studies continue to find an inverse relationship between consumption of isoflavone-rich foods and prostate cancer. Rodent models and *in vitro* systems have suggested beneficial effects on bone density; similar results have not been observed in humans, although clinical trials have shown a promising effect on biomarkers of bone turnover.

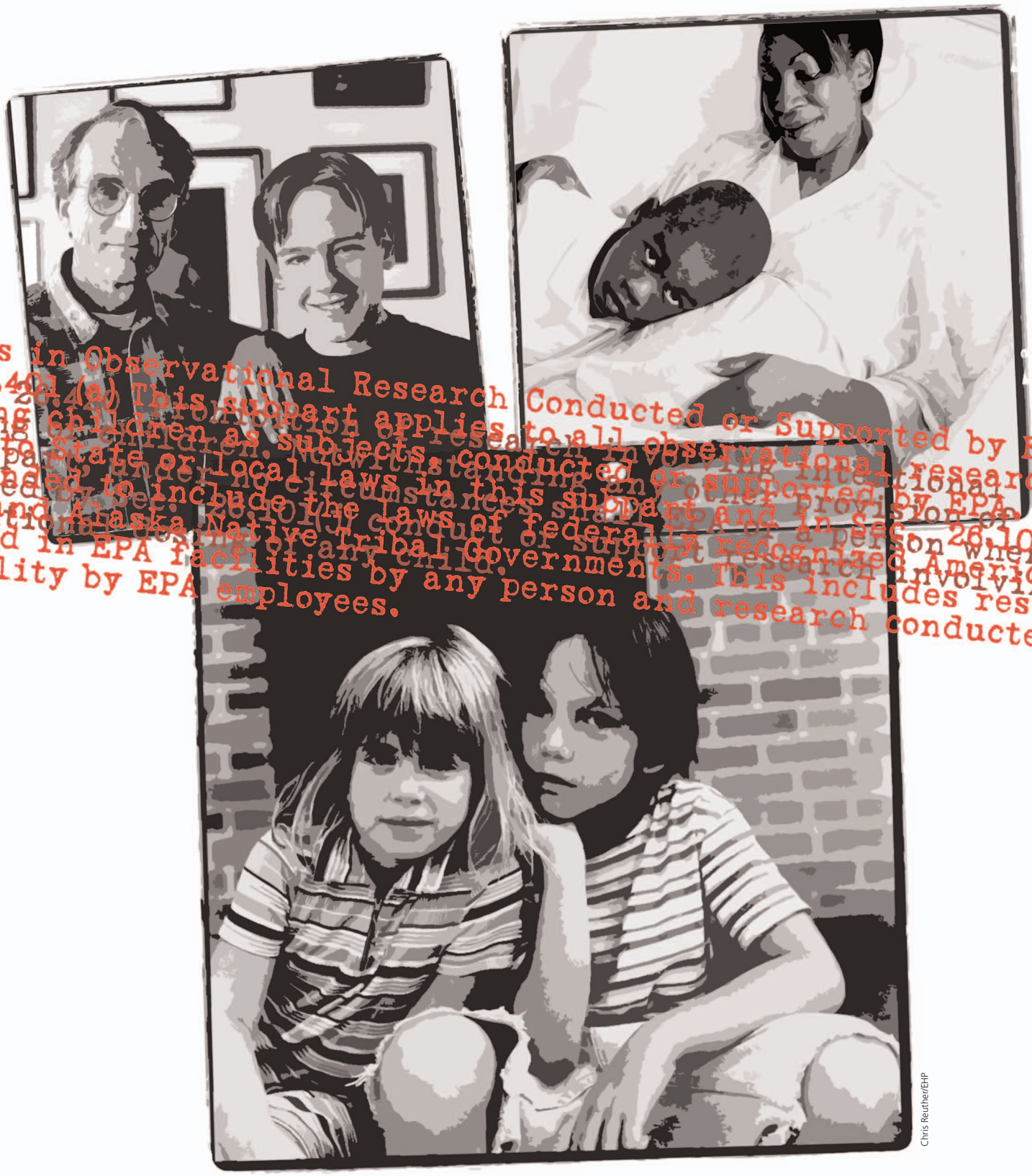
Although there has been comparatively little research on the effects of soy and isoflavones on cognition and other brain activity, Clarkson says this area may also hold some promise. "Our group has done some work [in monkeys] showing that [soy] modifies serotonin metabolism in a direction that should be useful in the prevention of depression," he says.

Michael R. Adams, a professor of pathology at Wake Forest University School of Medicine, has expanded the scope of the research beyond isoflavones. He is currently looking at one of soy's protein fractions, 7S, which may have a role in inhibiting the development of atherosclerosis by acting directly on the artery wall rather than on plasma lipids or low-density lipoprotein cholesterol receptors.

What most researchers do agree on is that we are only just beginning to truly understand the nature of soy, and that much more research is needed before it is possible to make firm health recommendations. "If you look at nutritional research in general," Kaplan says, "there are kinds of proteins that are described as being 'bioactive.' Most people had assumed that if soy is bioactive, it's because of the isoflavones. We're no longer certain of that at all."

Julia R. Barrett

From left: George Green/ShutterStock; P.T. Lee/ShutterStock; EyeWire



Chris Reuther/EHP

Human Experimentation

A Rule Gone Awry?

The U.S. EPA's new Protections for Subjects in Human Research rule, which came into force on 7 April 2006, was born of a need to tighten the ethical guidelines controlling nonmedical human experimentation. The rule was ostensibly designed to offer people greater protection in pesticide toxicity experiments. But just two weeks after its coming into force, a coalition of labor and environmental interest groups filed suit against the EPA, challenging the rule's legality and ethics. Against a backdrop of claims of industry influence, financial interests, and bipartisan rhetoric, the Second Circuit Court of Appeals in New York City must now determine whether this rule safeguards Americans against unethical experimentation or sells them out to big business.

The plaintiffs—the Natural Resources Defense Council (NRDC), Pesticide Action Network North America, San Francisco Bay Area Physicians for Social Responsibility, and Northwest Treeplanters and Farmworkers United, Oregon's union of farm, nursery, and reforestation workers—filed their suit on 23 February 2006. They claim the new rule does not meet the demands of Congress to afford the fullest protection to human subjects—especially pregnant women and children—in pesticide experiments, and charge that the rule is undercut by numerous loopholes that ultimately encourage, rather than deter, human testing.

"EPA is giving its official blessing for pesticide companies to use pregnant women, infants, and children as lab rats in flagrant violation of [the EPA Appropriations Act of August 2005] cracking down on this repugnant practice," said Erik Olson, senior attorney for the NRDC, in a 23 January 2006 press release from that organization. "There is simply no legal or moral justification for the agency to allow human testing of these dangerous chemicals. None."

The Need for a New Rule

The new rule expands on the existing Federal Policy for the Protection of Human Subjects (or "Common Rule"), which covers the ethics of medical trials and governs human research sponsored or regulated by federal agencies. The Common Rule largely reflects the aims of the Nuremberg Code, a document drawn up after World War II providing the basis for modern human experimentation ethics. But according to *Human Pesticide Experiments*, a June 2005 report drawn up for Senator Barbara Boxer (D–CA) and Representative Henry Waxman (D–CA), the Common Rule offers insufficient protection against pesticide companies that pay people to be intentionally exposed to their products.

Why would a pesticide company even want to conduct experiments on humans in the first place? One key reason can be found in the provisions of the 1996 Food Quality Protection Act, which was passed to provide greater protection for vulnerable populations (such as children) against pesticide exposures via food. The act applied a 10-fold safety factor to permissible levels of pesticide residues in food to account for children's greater vulnerability. Under the act, pesticides could be granted a lower safety factor "only if, on the basis of reliable data . . . [the lower factor] will be safe for infants and children." According to a 2004 National Academy of Sciences report titled *Intentional Human Dosing Studies for EPA Regulatory Purposes: Scientific and Ethical Issues*, several pesticide manufacturers conducted human dosing studies in pursuit of lower safety factors.

But human testing raises important ethical questions that need to be answered. For example, although the subjects in these pesticide dosing studies were supposed to have given their consent to participate, was it ethical to have asked them in the first place? Would the benefits of these studies outweigh the risks to the subjects (as recommended by the 1964 Declaration of Helsinki on human medical experimentation), or just help the bottom line of the companies involved?

Given the dilemma surrounding human experimentation, in 1998 the Clinton administration placed a moratorium on the EPA reviewing human experiments for setting permitted exposure levels. When the moratorium was lifted by the Bush administration after a court found procedural errors in its establishment, Congress reacted by way of the 2005 EPA Appropriations Act, demanding that the agency draw up new ethical guidelines governing itself and all third parties wishing to submit results to the agency for regulatory purposes. Championing this cause was Representative Hilda Solis (D–CA), along with Boxer and Waxman, whose 2005 report claimed that 22 human pesticide experiments submitted to the EPA for possible use in regulatory decision making were in violation of ethical and scientific standards, for reasons such as failure to obtain fully informed consent, dismissal of adverse outcomes, and the use of unethical liability waivers.

In obedience to Congress, the EPA drew up a proposed rule, which evolved into the final rule published on 2 February

2006 after a 90-day public comment period. During this time the EPA received thousands of criticisms that it took into account for preparing the final draft—a document that ended up little to the liking of the litigating coalition or indeed of the politicians who had demanded it.

The coalition members allege that the rule's wording—with what they perceive to be inherent loopholes—now actually encourages rather than prohibits human experimentation, and suggest that pesticide companies could take advantage of this to further their interests. "EPA's rule allows pesticide companies to use intentional tests on humans to justify weaker restrictions on pesticides," said Margaret Reeves, a senior scientist and program coordinator with Pesticide Action Network North America, in a press release from that group announcing the filing of the lawsuit. In the same press release, Robert Gould, president of San Francisco Bay Area Physicians for Social Responsibility, was quoted as saying, "Pesticide companies should not be allowed to take advantage of vulnerable populations by enticing people to serve as human laboratory rats."

In a press release issued by his office the same day, Waxman commented: "Unethical human pesticide experiments must be stopped. It is morally wrong to encourage chemical companies to dose humans with pesticides in order to argue for weaker public health standards."

Question of Intent

One of the major intentions for the new rule—and now a major bone of contention—was that it ban the experimental use of pregnant women and children. Indeed, the EPA insists the rule does just that. "This rule provides far-reaching protections for all Americans and absolute protections for children and expectant mothers," explains senior policy advisor William Jordan of the EPA Office of Pesticide Programs. "It categorically inhibits EPA or any researcher from using [such subjects] in intentional dosing studies. The rule further extends those protections by banning any researcher for pesticides from using pregnant women or children as participants in any intentional dosing study intended for submission to EPA."

Jordan says the rule also prohibits the EPA from relying on any intentional pesticide dosing study involving pregnant women or children regardless of the intent of those conducting the study or the country where the study was conducted. Finally, he says, the rule directs the EPA to waive that prohibition "only if the agency were to become aware of information that would indicate the need for stricter regulatory controls for a pesticide."

The coalition, however, points to what it considers to be several exceptions to the rule. They note that while Americans may be offered some protection from intentional dosing studies, the rule does nothing to prevent U.S. pesticide companies from performing experiments on nationals in other countries.

The term "intended for submission to the EPA" worries them too. "The wording of the rule means you are not allowed to do a study on pregnant women or children that you admit was intended from the beginning for submission to the EPA," explains Olson. "If that was not your original idea—or if you say it wasn't—then you apparently could do those experiments. A second scenario could be where a company performs a study on infants or pregnant women and submits it to a state or, say, a European country, saying that it does not intend to submit it to the EPA. We have plenty of experience to show that decisions made by other bodies are very influential on the EPA. [So], you can avoid the EPA rule and still get the result you want." In addition, pesticide studies on pregnant women and children submitted under clean water, drinking water, clean air, hazardous waste, or other laws are not covered by the new rule's restrictions, Olson explains.

Jordan rejoins that the rule does not, in fact, permit a registrant to claim at the outset of a study that they have no intent to submit a human pesticide study and then later submit that study. "If the agency were ever to receive such a study done in this deceptive fashion," he insists, "the rule prevents EPA from using it."

Children's Consent

Another major area of contention is consent. In the proposed rule, section 26.408 of the text clearly stated that "if the [institutional review board] determines a research protocol is designed for conditions or for a subject population for which parental or guardian permission is not a reasonable requirement to protect the subjects (for example, neglected or abused children), it may waive the consent requirements in subpart A of this part and paragraph (b) of this section." Subpart A refers to the requirement that a child must assent to be included in an experiment (although this was apparently not necessary "if the capability of some or all of the children is so limited that they cannot reasonably be consulted"), while paragraph (b) refers to soliciting permission from parents or guardians. Many critics believed this waiver suggested that abused or mentally impaired children could be freely used in commercial pesticide experiments.

In comments published with the final rule, the EPA says such a sinister reading is incorrect: "Many commenters misinterpreted EPA's proposed language. Contrary to public comments, none of the alleged 'loopholes' ever existed, because the prohibition in proposed Sec. 26.420 stated 'Notwithstanding any other provision of this part, under no circumstances shall EPA or a person when covered by Sec. 26.101(j) conduct or support research involving intentional dosing of any child.'"

According to the EPA, the words "Notwithstanding any other provision of this part" meant that the provisions in proposed Section 26.420 overrode all other provisions of the entire regulation, including those in 26.408. So even though the latter section would have appeared to give the EPA the authority to waive certain requirements, it did not, the agency claims, authorize any departure from the ban declared in Section 26.420. In Jordan's words, no child is going to be used in intentional dosing studies—period.

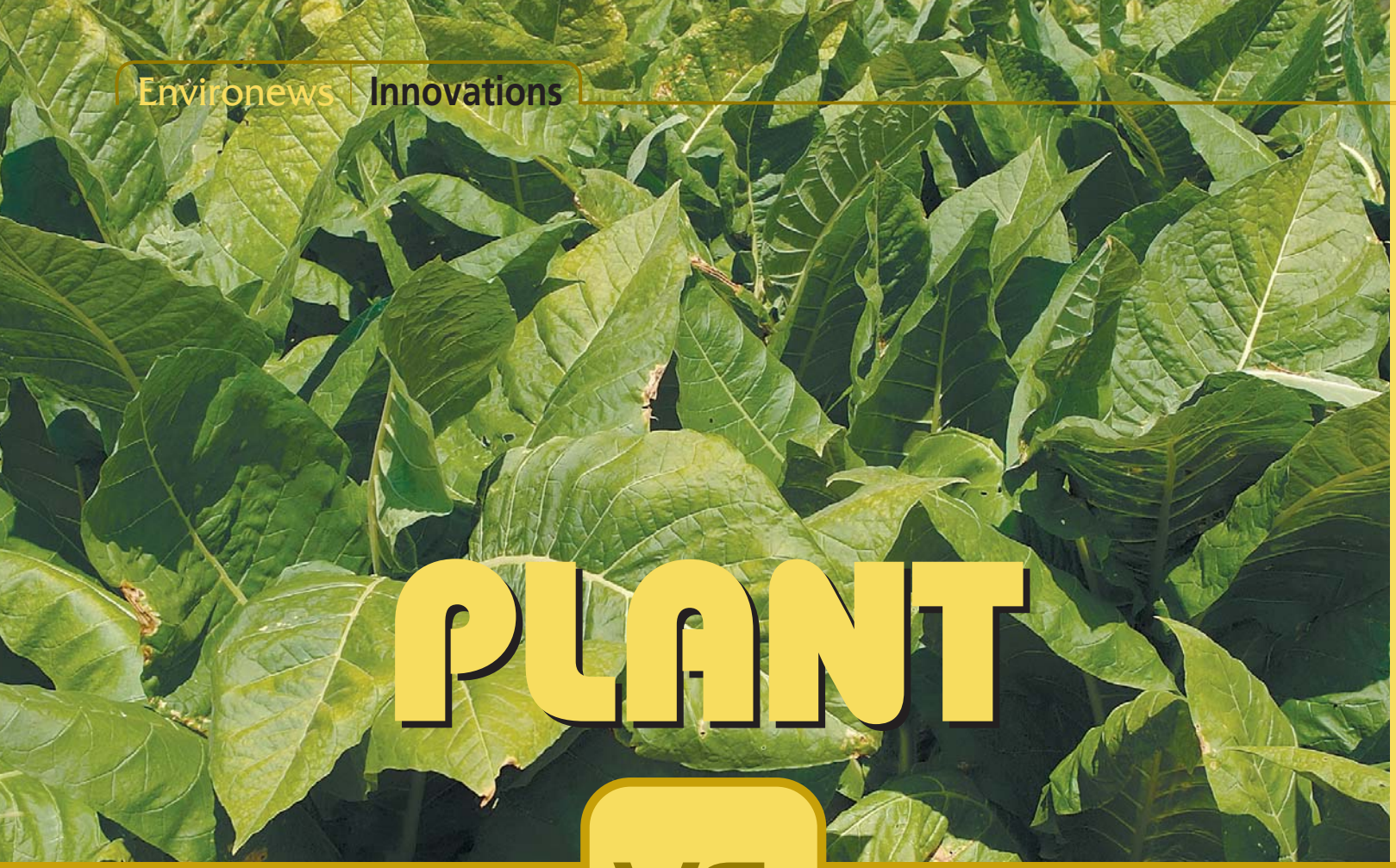
"But if this is what it means, why doesn't it simply say that?" asks Olson. "It sure appears that if there is no 'intent to submit' to EPA then you could use such children."

The wording in the final rule is scarcely different from that in the original version. The proposed Section 26.408, now named Section 26.406, remains virtually intact, while the promise in the proposed Section 26.420 has been consolidated into a blanket statement in the final Section 26.203 that "under no circumstances shall EPA conduct or support research involving intentional exposure of any human subject who is a pregnant woman (and therefore her fetus) or child."

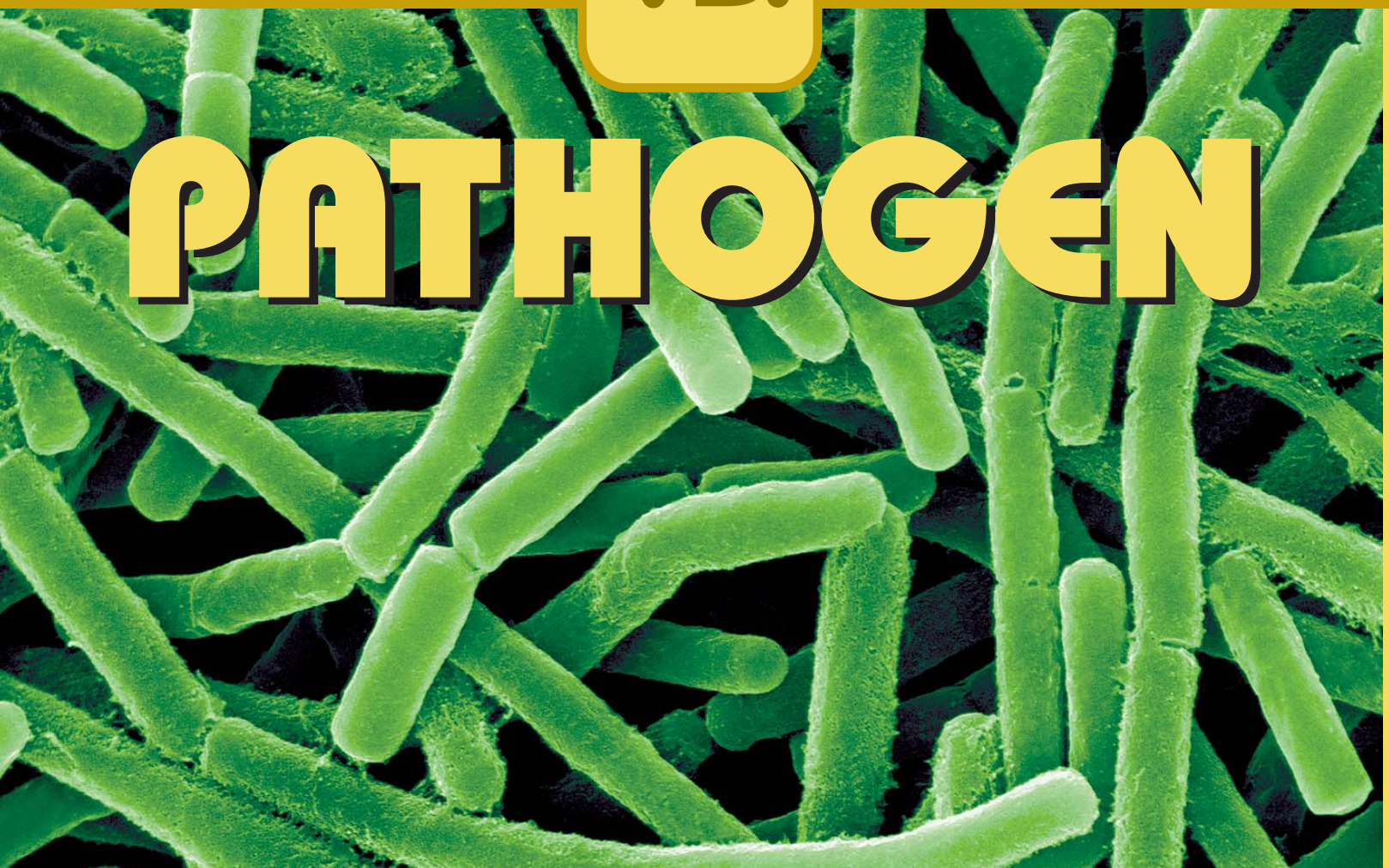
Other concerns voiced by the coalition include claims that the ethics review board established by the new rule is powerless to prevent experiments it deems unethical (its role is merely advisory), that nowhere is any sanctioning power mentioned, and that a clause in the text requires that any studies presented need only "substantially" comply with the rule—a quantity of compliance that is never defined.

Given the EPA's funding of the now-cancelled CHEERS study (which would have paid parents to let EPA and industry scientists observe the effects on their children of spraying their homes with pesticide, but which was abandoned by the agency in the face of overwhelming criticism; the EPA declined to comment to *EHP* on the study), the fundamental question arises as to whether the agency should be allowed to write its own rules. It is now the job of the courts to decide whether the EPA has done a good job. Briefings will begin 5 June 2006, but a ruling could take a year or more to come through. In the meantime, the new rule is in force.

Adrian Burton



PLANT VS. PATHOGEN



Enlisting Tobacco in the Fight against Anthrax

Just weeks after the September 11 terrorist attacks in New York and Washington, letters containing anthrax spores were mailed to newspapers and television stations in New York and to two U.S. senators on Capitol Hill. Although only a few letters were sent, 22 people were infected and 5 died. More importantly, the bioterror attacks fueled fears that future attacks might be more extensive. Now researchers at the University of Central Florida are helping to prepare for the possibility of anthrax attacks by developing a new technique that can quickly produce hundreds of millions of doses of a potentially safer anthrax vaccine.

Since the 1960s American microbiologists have produced a vaccine for anthrax from the very microbe itself, *Bacillus anthracis*. The microbe's toxin is made up of three key parts: edema factor (EF), lethal factor (LF), and protective antigen (PA). EF causes fluid to build up in the area of infection, while LF kills cells or prevents them from working. However, both of these factors require PA to create a passageway into the cells—the PA bonds to protein receptors, creating a new complex to which the other two factors attach.

According to Stephen Leppla, a senior researcher at the National Institute of Allergy and Infectious Diseases (NIAID), anthrax bacteria that don't have PA cannot cause an infection. "In essence," he says, "they are inactivated and become much less virulent." The current anthrax vaccine works on this very

Facing page, top to bottom: Anne Kitzman/Stockphoto; Dennis Kunkel Microscopy

principle by introducing nonvirulent PA into the body so antibodies are created. PA introduced in the event of a future anthrax exposure would be inactivated by these antibodies, stopping the infection in its tracks.

In Pursuit of PA

But obtaining large quantities of PA has been a problem. Only one company—BioPort of Lansing, Michigan—is licensed by the FDA to produce the vaccine in the United States, and it can produce only 8 million doses each year through a fermentation process, according to BioPort spokeswoman Kimberly Brenne Root. That's enough to fill the company's contracts with the Department of Defense (DOD) and the Department of Health and Human Services, which stockpile the vaccine and administer it to military personnel, but not enough to vaccinate a large civilian population in the event of a widespread attack.

In 2004, in an attempt to procure more doses of vaccine, the U.S. government awarded an $877.5-million contract to VaxGen of Brisbane, California, to produce 75 million doses by the end of 2006. Setbacks have resulted in major delays, however; on 10 May 2006, company officials confirmed that the first shipments of the vaccine won't be delivered before late 2007 at the very soonest.

As well, there have been concerns that the vaccine produced by BioPort was not safe. Several Gulf War service members reported health problems after being vaccinated. Anecdotal reports suggest the vaccine may contribute to heart problems, cardiovascular illness, seizures, Gulf War syndrome, even death. Documented side effects include pain and swelling at the injection site, inflammation, flu-like symptoms, malaise, rash, joint pain, and headache. The BioPort vaccine can be contaminated with small amounts of LF and EF, which may contribute to the adverse effects associated with it.

To overcome these problems, Henry Daniell, a professor of molecular biology and microbiology at the University of Central Florida, has been on the hunt for a way to produce large quantities of "clean" PA, free of EF and LF. Now he thinks he has finally found it.

Turning Over a New Leaf

Daniell and his team began by isolating the gene for PA from *B. anthracis*. Then they inserted the gene into tobacco plants. "There are a lot of advantages to tobacco plants," says Daniell. "They produce a lot of biomass. . . . Also, we didn't want to produce a vaccine in a food crop in case there was cross-contamination or some package got mixed up on some truck somewhere." (Although tobacco shipments also could get mixed up, burning the tobacco in the course of smoking would destroy the PA it contained.) Furthermore, Daniell says, "[Tobaccco plants] are very easy to genetically manipulate."

Daniell's team chose to insert the gene into the chloroplast rather than the cell nucleus since they could get far more copies of the PA protein that way. After harvesting the tobacco plants, Daniell's team found that each plant produced about 150 milligrams of anthrax PA. That adds up to 360 million doses' worth of PA from one acre of tobacco plants. And because only PA is produced, the resulting vaccine will be cleaner than one produced through fermentation.

When the PA was introduced into mice, the rodents responded by producing very high levels of anti-PA antibodies. The immunized mice were sent to the NIAID, where they underwent anthrax toxin challenge. There, Leppla injected the mice with 150 micrograms of anthrax toxin, 1.5 times the amount needed to kill a mouse. Yet, the mice survived, proving that the new technique could produce an effective vaccine. These findings were published in the December 2005 issue of *Infection and Immunity*.

Practical Considerations

Rakesh Bhatnagar, chairman of the Centre for Biotechnology at Jawaharlal Nehru University in New Delhi, India, has researched plant-based anthrax vaccines, and has signed a commercial agreement to produce larger quantities of anthrax PA than BioPort while still using a fermentation system. He believes Daniell's research is significant because it shows that PA produced in plants can protect animals from anthrax. Yet he also believes plant-based vaccines still belong to the future.

"At this point in the road [plant-based vaccine researchers] have only expressed the protein in a few plants and only tested on small animals," says Bhatnagar. "Plant vaccines are a long way off, because industry wants higher levels of productivity to be successful. Plus, everything requires approval from government regulators, so it all takes time. But, if I had to estimate, it might be ten years down the road."

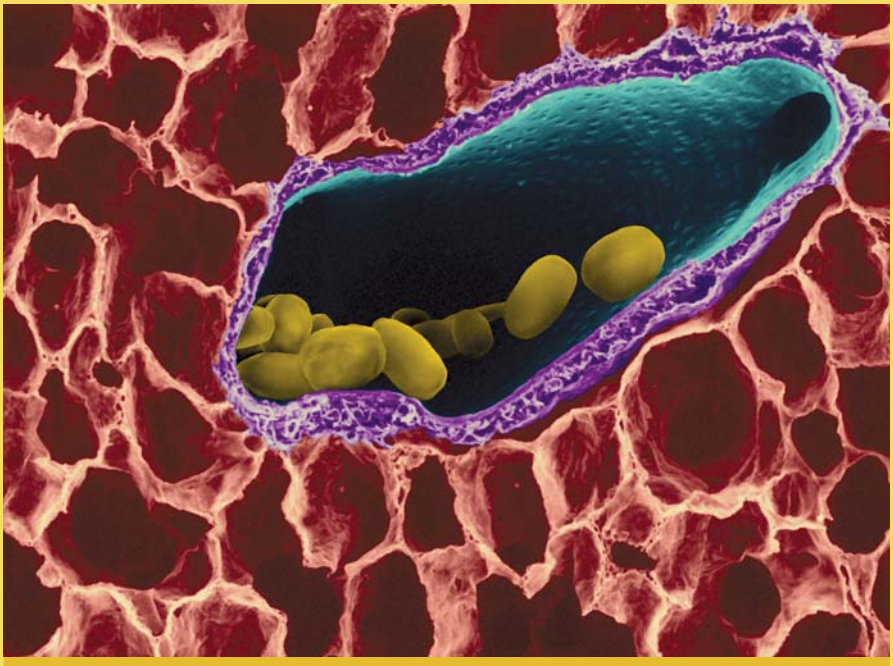
Daniell disagrees with this assessment, however. He says that vaccines against agents of bioterrorism are now on fast-

DOD

Fighters first, but then what? The government has stockpiled enough anthrax vaccine to supply military personnel but not nearly enough for public citizens. (above) Monica Carpenter, a medical services journeyman in the U.S. Air Force, administers anthrax vaccine to Technical Sergeant Ricky Anderson in Iraq.

track approval, and approval should come much sooner than 10 years.

A DOD spokesperson, who asked to remain anonymous, says that a plant-based anthrax vaccine would be of interest but that such a vaccine would have to be approved by the FDA. Also, says the spokesperson, "At present, the DOD has sufficient FDA-licensed anthrax vaccine to fulfill its policy. If the supply of anthrax vaccine was suddenly expanded, it might be that civilian purchasers of the vaccine would be less constrained than at present."



Sleeping giant? Although no one can predict if, when, or where an anthrax outbreak might occur, the magnitude of the threat makes the development of adequate vaccine resources a priority. (above) *Bacillus anthracis* spores in lung tissue.

Dennis Kunkel Microscopy

A Growth Industry?

Meanwhile, Daniell and his team aren't content with producing 360 million doses of anthrax vaccine. Rather, they consider this a preliminary step towards an even greater goal: vaccines that are actually grown in and consumed along with a piece of fruit.

The idea of putting vaccines in plants or fruits was pioneered in 1992 by Charles Arntzen, currently codirector of the Center for Infectious Disease and Vaccinology at Arizona State University, after he observed a mother feeding her child a banana during a research trip to Thailand. Arntzen's idea was simple: what if we could cut through the obstacles to vaccination by simply growing vaccines in fruit?

Many vaccines are hard to produce because of expensive fermenters, hard to ship because they often need to be kept refrigerated, and hard to distribute widely because it can take a trained health professional to administer the vaccine. All these factors make it particularly difficult to vaccinate populations in developing countries. Arntzen and his colleagues have continued exploring this line of thinking, and in the 1 March 2005 issue of *Proceedings of the National Academy of Sciences*, they conclude that a plant-based oral vaccine against hepatitis B, as delivered via potato, "should be considered as a viable component of a global immunization program."

However, before we can eat bananas or potatoes for our booster shots, researchers need to figure out a few key problems.

First, there needs to be a way to standardize the vaccine's dose. "Other vaccines are very exact on the dosages," says Bhatnagar, "but with plant-based vaccines, what are you going to say? In the plant, levels might vary widely."

The other major problem is that it takes months for a crop to grow, even a quick-growing one like tobacco, whereas the bacteria used in a fermentation system take only days or even hours. On the other hand, a crop system could be cheaper and produce more vaccine, compared to a fermentation system.

Despite the remaining hurdles, Daniell believes that his developments in tobacco plants will lead to an anthrax vaccine someday in the future. His team is also working on growing vaccine antigens against other diseases such as cholera, amebiasis, plague, and hepatitis C in tobacco plants.

"If a vaccine was produced in a plant cell, dried cells could be put in a capsule and delivered because the plant cell wall protects the protein already," says Daniell. "Different delivery methods still need approval, but the cost of vaccines could drop from [up to] a hundred dollars to a few cents since basically all you are doing is powdering the plant and putting it in a capsule. For that reason, it is worth every regulatory hurdle, because it will pay off big time."

Graeme Stemp-Morlock

Suggested Reading

Joellenbeck LM, Zwanziger LL, Durch JS, Strom BL, eds. 2002. The Anthrax Vaccine: Is It Safe? Does It Work? Committee to Assess the Safety and Efficacy of the Anthrax Vaccine, Medical Follow-Up Agency. Washington, DC: National Academies Press. Available: http://darwin.nap.edu/books/0309083095/html/.

Koya V, Moayeri M, Leppla SH, Daniell H. 2005. Plant-based vaccine: mice immunized with chloroplast-derived anthrax protective antigen survive anthrax lethal toxin challenge. Infect Immun 73:8266–8274.

Leppla SH, Robbins JB, Schneerson R, Shiloach J. 2002. Development of an improved vaccine for anthrax. J Clin Investig 110:141–144.

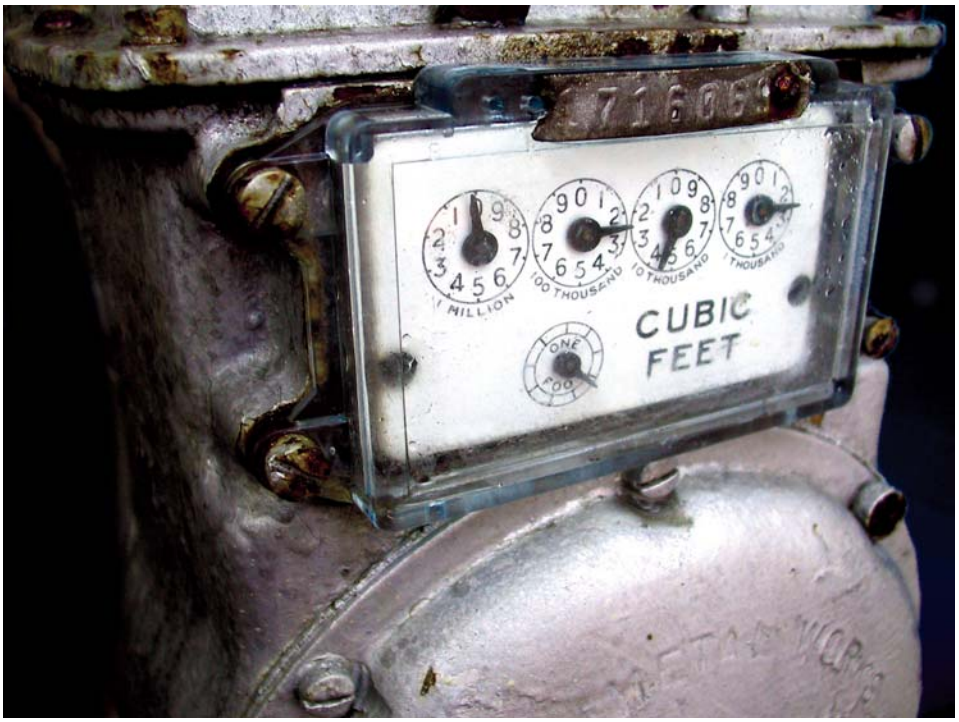
National Institute of Allergy and Infectious Diseases. December 2005. Anthrax [fact sheet]. Available: http://www.niaid.nih.gov/factsheets/anthrax.htm.

Watson J, Koya V, Leppla SH, Daniell H. 2004. Expression of *Bacillus anthracis* protective antigen in transgenic chloroplasts of tobacco, a non-food/feed crop. Vaccine 22:4374–4384.

A Most Irregular Threat

Old Gas Regulators Can Present Mercury Exposure

Residential gas regulators reduce the pressure of gas from main feeder lines to usable levels for household pipes. Gas regulators built before 1961 were commonly located within dwellings and incorporated a component that contained, on average, 136 grams (about 2 teaspoons) of elemental mercury. Newer units do not contain mercury, but as a group of Chicago-area researchers report, careless replacement of the older units can result in potentially hazardous mercury contamination [***EHP* 114:848–852; Hryhorczuk et al.**].



Old equipment poses new problem. Old gas regulators that contain mercury can present a potential health hazard to residents if care is not taken when they are replaced.

The authors describe how this newly identified source of residential mercury exposure came to light in 2000, when a suburban Chicago family discovered a pool of the silvery element on the floor of their basement workroom six weeks after a gas company contractor removed and replaced their gas regulator and meter. An investigation showed that the air in the house contained elevated levels of mercury vapor, and the father and the 9-year-old son, who spent more time in the basement area than the mother, had blood and urine mercury levels above recommended background limits. Short-term exposure to high levels of metallic mercury vapors can cause lung damage, nausea, vomiting, diarrhea, elevated blood pressure or heart rate, skin rash, and eye irritation.

Fortunately, ventilation and remediation of the home brought mercury concentrations in the home's air down to safe levels, and several weeks after exposure ceased, the father's and son's blood and urine mercury levels returned to normal background ranges. Neither ever manifested overt clinical signs of mercury poisoning.

This case and a cluster of similar cases quickly caught the attention of area authorities. Ultimately, two area gas companies were required to conduct inspections in 361,000 homes where their employees or subcontractors had replaced old gas regulators. Free urine mercury screenings were offered to concerned residents.

Of the 301,000 homes inspected by one of the companies, 1,308 (0.43%) were found to be contaminated, and 1,033 were remediated. Of 60,000 homes screened by the other company, 55 (0.09%) were found to be contaminated and were remediated. The risk was considerably higher in homes where the equipment had been replaced by one particular subcontractor—of 120 homes screened, 20 (16.7%) were found to have been contaminated by mercury.

Of the 625 residents who elected to undergo urine mercury screenings, 9 (1.4%) had positive bioassays, defined as a 24-hour urine mercury concentration equal to or higher than 10 micrograms per liter. Although none of the subjects showed overt symptoms of mercury poisoning, as the authors point out, the screenings were not designed to detect subclinical effects of mercury exposure.

Interestingly, positive urine mercury in residents was more strongly associated with elevated air mercury concentrations on the first floor of the homes than with elevated basement air levels, even though the air concentrations were considerably higher in basements, where the spills typically occurred. The authors attribute this result to the simple fact that people generally spend less time in their basements than in the living quarters aboveground.

The Chicago episode not only revealed a previously unidentified environmental exposure hazard but also provided valuable lessons on how to respond quickly and efficiently. As the authors note, gas companies and their subcontractors, clinicians, public health and environmental officials, and residents all need to be aware of the potential for contamination in older homes or other buildings where mercury-containing gas regulators have been replaced in the past, or where they may still exist. **–Ernie Hood**

PCBs Are Endocrine Disruptors

Mixture Affects Reproductive Development in Female Mice

Polychlorinated biphenyls (PCBs) are a broad group of chemicals that includes 209 aromatic chlorinated hydrocarbons used for products ranging from fluorescent light fixtures to coolant fluids

Lisa C. McDonald/ShutterStock

inside parts of consumer electronics. Short-term exposure to large amounts of PCBs can cause liver damage; the effects of smaller concentrations can be more subtle, affecting the reproductive development of children of exposed mothers. But are these compounds actually endocrine disruptors? New work by researchers at the Mount Sinai School of Medicine confirms that they can be, and for the first time connects a molecular mechanism to the life-long phenotypic changes seen after exposure to environmental estrogens [***EHP* 114:898–904; Ma and Sassoon**].

Sold predominantly as mixtures under the trade names Aroclor and Pyranol, PCBs have been banned in the United States since 1977 and are out of use or highly restricted throughout much of the world. These compounds, which are fat-soluble and structurally similar to DDT, are highly stable: PCBs made and used for nearly 50 years before the ban remain in the environment and are found throughout the food chain, including in human tissues and breast milk.

The researchers tested the hypothesis that PCBs are endocrine disruptors by comparing their effects on expression of *Wnt7a* in mice. Down-regulation of *Wnt7a* is a known factor in the reproductive deficits found in mice exposed to diethylstilbestrol (DES), a synthetic estrogen once used to supplement estrogen levels in pregnant women. After exposure to DES, development of the reproductive system is impaired when production of the regulatory protein coded by *Wnt7a* is temporarily squelched. A passing loss of appropriate regulation in very young animals leads to reproductive changes—for example, in the number of uterine glands or the thickness of the myometrium, the uterus's muscular outer layer—that give way to more pronounced changes through life. This suggests that exposed organisms are sent down an abnormal developmental path from which they cannot return.

Using *in situ* hybridization, immunohistochemistry, and quantitative reverse-transcriptase polymerase chain reaction, the authors showed that the effects of environmentally relevant levels of Aroclor 1254, a commercial mix of PCBs, were similar but not identical to those of low levels of DES. Both led to decreases in expression of the *Wnt7a* regulatory gene and showed qualitative changes in uterine development like those described above. The authors also demonstrated that measurement of *Wnt7a* provides a molecular tool that lays to rest any doubt that PCBs are endocrine disruptors.

The authors further showed that genetic makeup matters in terms of vulnerability to endocrine disruption. Mice with only one good copy of the *Wnt7a* gene were more sensitive to DES or PCBs than mice with both copies intact, providing a concrete example of how gene variation can leave some individuals more susceptible than others to the effects of PCB exposure. **–Victoria McGovern**

Putting a Load on Your Bones

Low-Level Cadmium Exposure and Osteoporosis

High-level exposure to cadmium is known to cause bone damage, including osteoporosis, but the effects of low-level exposure have been less clear. A group of Swedish researchers now shows for the first time that low-level exposure to cadmium also can be associated with negative effects on bone in humans [***EHP* 114:830–834; Åkesson et al.**]. Although the impact may be slight, even a limited role for cadmium in the etiology of osteoporosis could be important at the population level, given the prevalence of osteoporosis and our ubiquitous, life-long low-level exposure to the substance through diet.

Razvan Radu/Shutterstock

From cadmium to canes? New data show that even low-level exposure to cadmium may contribute to osteoporosis.

Unlike lead (another contributor to osteoporosis), which is retained in bone tissue, cadmium is retained mainly in the kidneys. Exposure mostly comes from cereals, vegetables, shellfish, and tobacco, all of which absorb cadmium. Some cadmium occurs naturally, and more is released in industrial emissions and vehicle exhaust.

To investigate associations between cadmium retention and bone effects, the scientists assessed a cohort of women ranging in age from 53 to 64 years. This segment of the population is the most susceptible to both cadmium retention (which appears to decrease slightly past this point) and osteoporosis. The 820 subjects were recruited from a large (n = 10,766) population-based survey of upper-middle-aged women in the community of Lund, Sweden. The lack of known history of excessive cadmium contamination in this area implied that exposures were fairly constant over time.

The team measured cadmium in blood and urine; lead in blood; several biochemical markers of bone metabolism; and forearm bone mineral density (BMD), a test used to assess osteoporosis status. Statistical analysis of the results incorporated a comprehensive array of potential confounders and effect modifiers, including weight, menopausal status, use of hormone replacement therapy, age at menarche, alcohol consumption, smoking history, and physical activity level.

Increasing urinary cadmium concentrations were associated with decreasing BMD. Furthermore, urinary cadmium was negatively associated with parathyroid hormone (a bone metabolism hormone) and positively associated with urinary deoxypyridinoline (a bone resorption marker). Those associations were present even in the subgroup with the lowest cadmium exposure—those who had never smoked. The study also showed that the negative bone effects appeared to intensify after menopause.

The authors calculated that women in the 99th percentile of urinary cadmium concentration had an average of 5–6% lower BMD than those in the 1st percentile. This difference was similar to what could be expected from a 6-year-greater age or 11-kilogram-lower body weight. Although the researchers acknowledge that this contribution of low-level cadmium exposure to the development of osteoporosis is small, they emphasize that the observed effects should be considered "early signals of potentially more adverse health effects." The findings thus lend increased urgency to efforts to reduce cadmium pollution of the environment. **–Ernie Hood**

The Public Health Payoff of "No Smoking Allowed"

Quantifying Decreases in SHS Exposure

Secondhand smoke (SHS), the cigarette smoke involuntarily inhaled by a nonsmoker, was recognized as a serious health problem as early as 1972. Studies have shown that SHS causes lung cancer and leads to other adverse effects, including lower respiratory tract infections, bronchitis, pneumonia, fluid in the middle ear, and sudden infant death syndrome. Fortunately, a new report by researchers at the CDC shows that over the last 14 years, SHS exposure has decreased substantially—by an average of 70% in people over the age of 4, regardless of sex, age, or race [***EHP* 114:853–858; Pirkle et al.**].

The researchers analyzed data from the National Health and Nutrition Examination Survey (NHANES), conducted by the CDC's National Center for Health Statistics, which collected data on the U.S. population during four distinct time periods from 1988 through 2002. Over the 14-year span, 29,849 nonsmoking participants completed a home interview followed by a physical exam.

SHS exposure was measured by testing blood samples from each participant for serum levels of cotinine, the primary byproduct formed when the body metabolizes nicotine. Serum cotinine levels indicate SHS exposure that occurred in the past few days. Nonsmokers exposed to typical levels of SHS usually have serum cotinine concentrations of less than 1 nanogram per milliliter (ng/mL), while active smokers generally have concentrations greater than 15 ng/mL. For this study, participants with serum cotinine concentrations of less than 10 ng/mL were considered nonsmokers (the relatively high cutoff accommodates heavy exposure to SHS).

During the first time period studied, 1988 through 1991, 65% of nonsmokers had serum cotinine concentrations greater than 0.1 ng/mL. On that basis, Healthy People 2010 (a DHHS initiative) had earlier established an objective of reducing that percentage to 45% by the year 2010. The current study results suggest that this goal had already been met by 2000. The authors say the decrease is most likely due to restrictions on smoking at work and in other public places, since adult smoking itself did not decrease dramatically in the 1990s.

Although public health efforts have been successful overall, the results suggest that further efforts should focus on two groups that showed relatively higher levels of risk of SHS exposure—children and blacks. In the most recent time period studied, 2001 through 2002, the median exposure level for children aged 4 to 11 was almost twice that of adults: 0.067 ng/mL, compared to 0.035 ng/mL. The median exposure level for blacks was even higher, at 0.135 ng/mL, compared to 0.034 ng/mL for whites.

Previous studies have shown that blacks have consistently higher serum cotinine concentrations per cigarette smoked than do whites. But other studies have found higher SHS levels among blacks even after accounting for differences in metabolism. The consistently higher serum cotinine concentrations for black nonsmokers in the current study appear to reflect higher SHS exposure, the authors state, though metabolism differences may have influenced the numbers somewhat.

To focus on these at-risk groups, further public health efforts are needed to discourage smoking where children are present, inside homes, and inside cars, the authors conclude. **–Angela Spivey**

Signs of progress. A new study shows that antismoking campaigns have greatly reduced exposures to SHS, but additional measures are still needed to protect at-risk groups such as children and blacks.

philpell/iStockphoto