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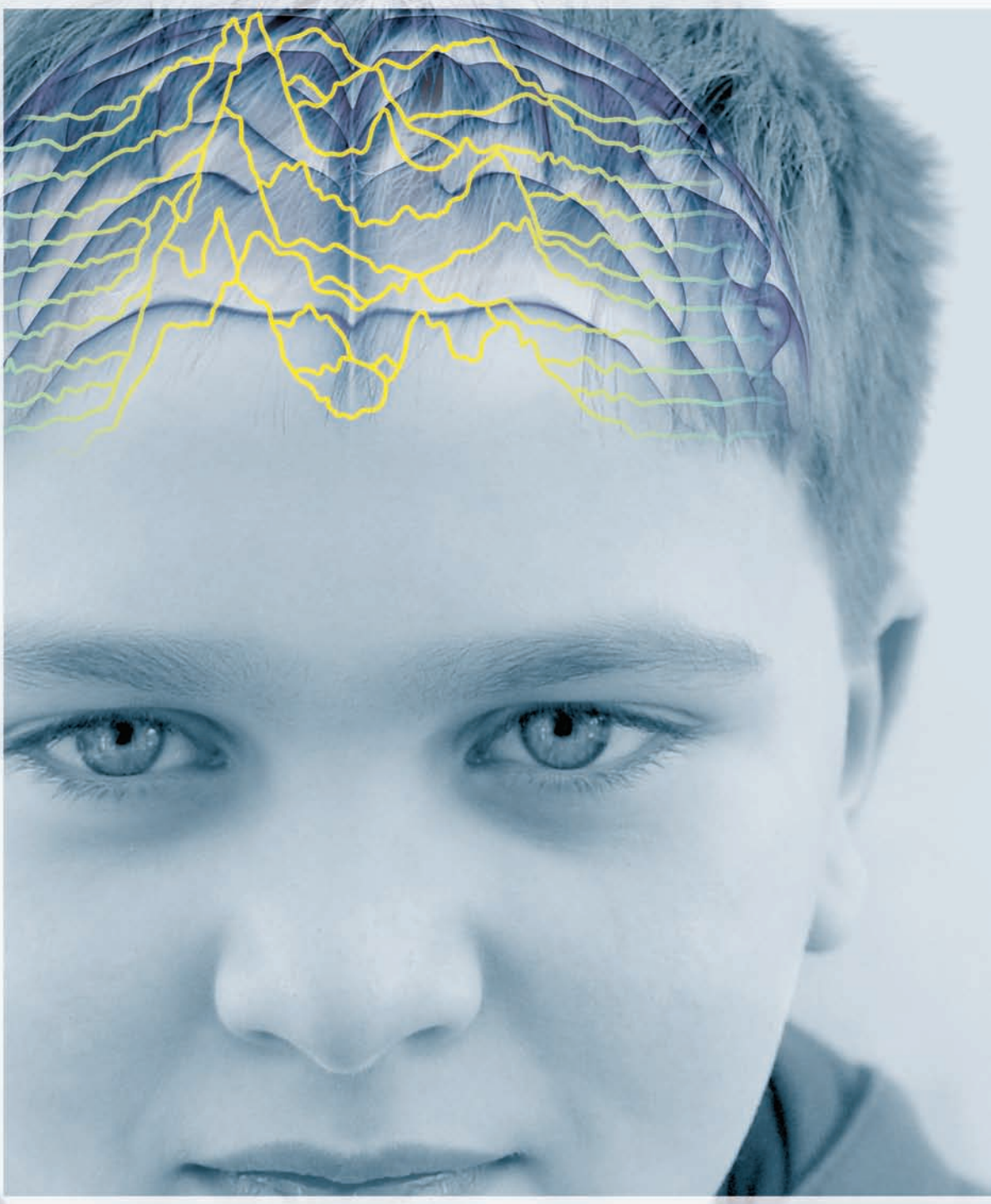

Toxic Tic-Tac-Toe

Is Organic Food Worth the Extra Cost?

What's in a Picture?

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STUDENT EDITION

If the brain were so simple we could understand it, we would be so simple we couldn't.
Lyall Watson, philosopher

NEUROLOGY

Marine Toxin Hinders Cognitive Development

Domoic acid, a naturally occurring marine toxin, causes acute symptoms of diarrhea, vomiting, seizures, and memory loss in people sickened by eating contaminated shellfish. Now a recent study reveals that exposure to even tiny amounts of domoic acid *in utero* may produce subtle, long-term cognitive impairment in rats. The new findings raise the possibility that pregnant women who inadvertently eat shellfish tainted with low levels of domoic acid may put their unborn children at risk for lifelong behavioral consequences, says Edward D. Levin, a professor of psychiatry at Duke University Medical Center in Durham, North Carolina. Levin coauthored the study, which is described in the September/October 2005 issue of *Neurotoxicology and Teratology*.

Harmful algal blooms that produce domoic acid are increasing, possibly due to warming ocean waters and human impacts such as farm and sewage runoff. Shellfish take in the toxin when they filter water and incorporate it into their tissues. Along the Florida coast, pigments in the "red tides" caused by *Karenia brevis* and other dinoflagellates signal toxic blooms. However, no distinctive color characterizes toxic blooms elsewhere, such as those along the Oregon coast caused by *Pseudonitzschia*, a phytoplankton that generates domoic acid. "The first warning sign we get for these toxic blooms is when domoic acid shows up in routine testing of shellfish," says Peter Strutton, a biological oceanographer at Oregon State University in Corvallis. Thus, it's possible that shellfish with potentially dangerous levels of domoic acid are being harvested and consumed (Strutton notes that, in Oregon at least, most of the shellfish of concern are those that people catch recreationally on their own).

In the Levin study, the researchers injected pregnant rats with single doses of 0.3, 0.6, or 1.2 milligrams of domoic acid per kilogram body weight at the end of the second trimester. The highest dose was in the low end of the range known to cause acute illness in rats.

During adolescence and adulthood, the offspring underwent a battery of behavioral tests. In a radial-arm maze, which looks like a wagon wheel without its rim, the rats searched for sugary cereal at the ends of arms extending from a central hub. Once eaten, the cereal is not replaced, and the rats must remember which arms they've already explored. This test therefore measures working memory. The females performed the same regardless of dose, while the males performed progressively worse as the dose increased (male rats normally perform better than females in this maze).

Next Levin gave the rats low doses of scopolamine, a drug that causes amnesia and memory impairment. Slightly stressing the brain with a low dose of scopolamine helped to uncover subtle neurological defects caused by domoic acid. Compared to controls, rats exposed to domoic acid had greater memory loss following administration of scopolamine, with the highest-dose group performing the worst. "Animals can normally deal with a low dose of scopolamine," says Levin, "unless there's prior neurotoxic damage that adversely affects the brain."

Now researchers wonder whether domoic acid may negatively affect unborn children even at levels that do not cause symptoms in expectant mothers. The U.S. Food and Drug Administration based its current limits for domoic acid in shellfish on levels that are assumed to be safe for adults. "We may need to re-evaluate the monitoring of waters and seafood to make sure that the most sensitive members of the population are protected from toxic exposure to domoic acid," says Levin. However, he adds, it's important to ensure that fisherman are not unnecessarily cut off from their livelihood and that people are not deprived of the nutritional benefits of uncontaminated seafood.

Strutton and Michelle Wood at the University of Oregon in Eugene are developing a new tool to improve early surveillance of toxic blooms. They are combining satellite data on physical attributes of the ocean such as water color and surface temperature to identify early markers for toxic blooms. In collaboration with the National Oceanic and Atmospheric Administration's CoastWatch program, they plan to develop products for coastal managers such as charts of conditions that raise the risk of domoic acid poisoning of shellfish. CoastWatch also plans to post satellite maps of regions where blooms exist or are developing on its website. "It will warn [managers] to ramp up their shoreline sampling of shellfish beds," Strutton says. **–Carol Potera**

A meal for mothers to skip? New studies in rats show that the marine toxin domoic acid may impair fetal cognitive development when mothers consume contaminated shellfish.

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POLICY

Cloud Banks: Airlines Save Halon

The airlines of the developing world are being advised by the United Nations Environment Programme (UNEP) to bank their stocks of halons—chemicals vital for extinguishing aircraft fires—as the 2010 deadline to cease production approaches. Most developed nations already have plans for halon recycling and banking systems—registries of who has excess halon to sell. For developing countries, however, the challenges of starting up such systems may leave some airlines grounded.

Halons have been used for years in many kinds of fire-extinguishing systems. However, when they escape into the atmosphere, UV light causes them to release highly reactive bromine radicals that deplete the ozone layer. Indeed, halons are thought to be three to ten times more ozone-unfriendly than chlorofluorocarbons. For this reason the Montréal Protocol obliged developed nations to cease halon production in 1994, and set a 2010 target date for the developing world.

Fear of firing. Halon, used to put out fires on aircraft, is being phased out with no suitable replacement in sight.

The trouble is that, while replacements for halons now exist for nearly all other applications, these chemicals remain essential for aircraft safety. Jim Curlin, information manager of the UNEP Division of Technology Industry and Economics, OzonAction Branch, explains, "Aircraft fire-extinguishing systems must have good dispersion and fire-suppression functions, must work at low temperatures, be of low toxicity to humans for the time that they are trapped in an affected plane and have an excellent weight-to-volume ratio." Currently, he says, there is no drop-in replacement for halons that has all these characteristics, making halon availability critical to airlines.

Even developed countries are not without halon banking problems. Developed nations have enough halon 1301—which is used in cargo bay and engine fire-fighting equipment—to last some 25 years, by which time a replacement should be available, explains John O'Sullivan, a member of the UNEP Halons Technical Options Committee and fire representative for the International Air Transport Association in Montréal. But there isn't enough halon 1211, which is used by aircrew in handheld extinguishers. "[Halon 1211] can still be made in developing countries, so at least in this respect [developing nations] should have fewer problems," says O'Sullivan. "But European regulations, for example, make it difficult to import. This is a problem we still have to address."

Starting up halon banking systems is certainly in the best interest of developing world airlines. With passenger safety a top priority, aircraft that do not maintain their halon-based systems would eventually fail airworthiness inspections and be banned from flying to many destinations. But how easy will it be for developing countries to start such systems, and where does halon banking figure on their priority list?

"Most focus first on economic problems and then on the environment," says Wilman Rajiman, the Indonesia Halon Bank Project manager at Soekarno-Hatta International Airport in Jakarta. "In Indonesia we started to discuss a national halon bank in 1995, but due to an economic crisis in 1998 it was not launched until March 2000. The major problems we faced were capital investment, knowledge, training, and local regulations."

For many countries, cash flow will be the major obstacle. Rajiman explains that Indonesia received a grant from the World Bank, but must spend its own money and then ask for reimbursement. Poorer nations may find that stipulation difficult, yet airline-servicing companies worldwide must comply strictly with the halon specifications laid down by aircraft manufacturers and foreign aviation authorities. Maintaining proper halon stocks is therefore vital to their business.

Flyers may be comforted to know that the Montréal Protocol contains a clause that allows developing nations to temporarily restart halon production for critical systems if supplies fail—always supposing the necessary infrastructure exists. "That's a situation we all want to avoid," says Curlin, "and one of the reasons we are encouraging companies and countries to develop halon banks."

–Adrian Burton

Top to bottom: Chris Reuther/EHP; Furlong Photography/Alamy; Shutterstock

Back-Door Cigarette Marketing?

At a time when marketing restrictions make it harder for tobacco manufacturers to reach the youth market, a number of new candy- and liqueur-flavored tobacco products are hitting the market. A review of internal tobacco industry documents published in the November/December 2005 issue of *Health Affairs* showed that the industry has long sought to target youth through new flavors, with one document stating that young people's interest in unusual flavors "may indicate new opportunities for enhanced-flavor tobacco products that could leverage [brand's] current strength among younger adult smokers." The authors write that flavored cigarettes can promote youth smoking initiation and help young occasional smokers become daily smokers by reducing or masking the unpleasant taste of tobacco smoke. The authors add there is little information on the potential health effects of the flavorings themselves.

In My Skin

Rates of melanoma, the deadliest skin cancer, continue to climb, more than tripling in Caucasians between 1980 and 2002, according to the American Cancer Society. Now skin cancer experts at the University of Newcastle upon Tyne have developed a novel test that uses a small skin sample and responses to a ten-page questionnaire to produce highly personalized assessments of the risks individuals face from their sun exposure to date. Patients also receive personalized skin protection advice and can re-take the test to see how changes they've made have affected their skin cancer risk. The "skinphysical" test was launched at British clinics in the autumn of 2005.

The Healing Quiet

A new study from The Johns Hopkins University shows that a noisy hospital environment may make patients sicker and lead to higher stress levels and burnout among staff. The study, presented at the 2005 annual meeting of the Acoustical Society of America, found that hospital noise levels worldwide have grown steadily over the past five decades and now on average exceed WHO hospital noise guidelines. This disturbs those within the hospital's confines, raises the risk of medical errors, and can even slow the pace of healing and contribute to lapses in short-term memory. Two possible solutions are to equip hospital personnel with hands-free personal communicators (eliminating the need for loudspeakers) and to wrap fiberglass insulation with an antibacterial fabric to form a sound-absorbent tile for ceilings and walls.

PARKINSON DISEASE

PD Gene and Oxidative Stress

A gene linked to familial Parkinson disease (PD) may protect neurons from oxidative damage, according to two independent studies in the fruit fly *Drosophila*. Flies lacking the *DJ-1* gene showed selective sensitivity to widely used agricultural toxicants that kill neurons mainly through oxidative stress. The studies, published 6 September 2005 in *Current Biology*, suggest that normally "*DJ-1* has a neuroprotective role against different oxidative stimuli," says Darren Moore, an instructor in neurology at the Johns Hopkins University School of Medicine who has studied the gene. However, if *DJ-1* stops working—because of either an inherited mutation or toxicant exposure—oxidative stress may wreak havoc on the brain, killing dopamine-producing neurons.

Experiments in cultured cells and in knockout mice have hinted that *DJ-1* mutations may sensitize cells to the harmful effects of oxidative stress. This type of oxidative damage happens when unstable oxygen molecules react with certain components inside cells in a manner similar to the process that converts iron to rust. Many environmental insults—including exposure to certain agricultural chemicals—can generate these unstable oxygen molecules.

To pin down how *DJ-1* interacts with such oxidative stress agents, Nancy Bonini, a professor of biology at the University of Pennsylvania, Philadelphia, and her colleagues first identified *DJ-1* in *Drosophila*, which they found exists in two forms: *DJ-1*α (expressed primarily in the testes) and *DJ-1*β (expressed everywhere). They then created a line of *Drosophila* mutants completely lacking both forms.

The flies with no *DJ-1* had normal life spans and showed no neuronal degeneration. However, when Bonini and her colleagues exposed flies to the herbicide paraquat, *DJ-1* mutants died much sooner than normal flies. The mutants also showed marked sensitivity to the insecticide rotenone and to hydrogen peroxide—both agents that promote oxidative stress. These results suggest that *DJ-1* normally protects against oxidative stress and that its inactivation may leave neurons susceptible to oxidative damage. The team also found that exposure to paraquat led to biochemical modification of the DJ-1β protein, a change Bonini says may somehow influence the ability of *DJ-1* to protect neurons from oxidative damage.

In the other paper, Kyung-Tai Min, an investigator at the National Institute of Neurological Disorders and Stroke, and his colleagues examined a different type of *Drosophila DJ-1* mutant. They disrupted the function of *DJ-1*β by inserting a mutation into the middle of the gene. Surprisingly, Min says, they found that dopaminergic neurons in these mutants survived longer into old age than did neurons of normal flies. They also found that their *DJ-1*β mutants were much more resistant to paraquat insult than were normal flies.

Further examination revealed that these flies had elevated *DJ-1*α expression—the loss of *DJ-1*β somehow encouraged a compensatory upregulation of *DJ-1*α, which the authors believe protected the fly from paraquat-induced oxidative damage. When they treated the same flies with hydrogen peroxide, however, they found that the *DJ-1*β mutants were extremely susceptible to early death. Min says this suggests that *DJ-1*α and *DJ-1*β may normally protect cells against different types of agents that promote oxidative stress.

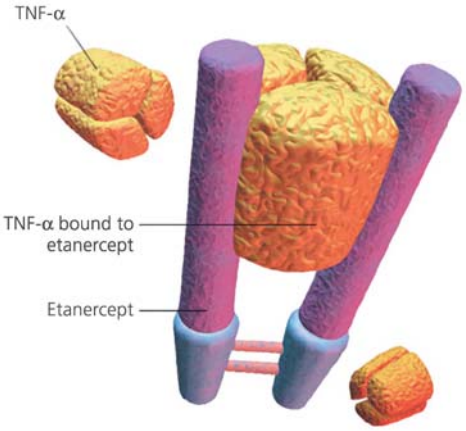
Neither of the mutant *DJ-1* fly strains will probably make an ideal model for PD, according to Moore, because the flies don't suffer from the neurodegeneration seen in humans with *DJ-1* mutations. Nevertheless, says Bonini, studies of *DJ-1* in *Drosophila* will provide greater understanding of fundamental activities of the gene, helping to elucidate how its function may be critical in PD. **–Melissa Lee Phillips**

ASTHMA

Expanding the Medicine Chest

About 10% of the asthmatic population has a severe form of the disease that can require progressively higher doses of corticosteroid drugs to manage the symptoms. Now a new therapeutic approach, described in the December 2005 issue of *Thorax*, may help people whose severe asthma symptoms no longer respond to steroid treatments.

Early research suggested that asthma was a so-called Th2 cytokine disorder involving certain white blood cells known as eosinophils. However, Stephen T. Holgate, a professor in the Infection, Inflammation, and Repair Division of Southampton General Hospital in the United Kingdom, and other researchers noticed that severe asthma was actually associated with neutrophils, another type of white blood cell that is associated with Th1 diseases such as rheumatoid arthritis and psoriasis. These diseases respond well to treatments that block the action of an immune system molecule called tumor necrosis factor–alpha (TNF-α). If severe asthma was truly a Th1 disease, Holgate hypothesized, it stood to reason that it too would respond to a TNF-α blocker.



Asthma attacker? The drug etanercept binds to TNF-α to block its action on the immune system. The drug may be an effective treatment for severe asthma aggravated by factors that affect immunity, such as viruses and air pollution.

To test this idea, Holgate and his colleagues administered the drug etanercept (Enbrel®) to 17 subjects with severe asthma in a 12-week study. Etanercept is a soluble receptor that binds to TNF-α. Treatment was associated with significant improvements in asthma symptoms and lung function, and reduction of bronchial hyperresponsiveness (abnormal sensitivity to agents that narrow the airways) in the 15 patients who completed the regimen.

Some research now suggests that asthma is not a single disease. "Mild and moderate forms of asthma may be disorders primarily characterized by a Th2-type immune response associated with allergen-specific IgE antibodies," Holgate says. "In contrast, severe asthma, which is aggravated by viruses and air pollution, may be a separate Th1-type immune disorder that involves the excess production of TNF-α." This could explain why the use of etanercept in previous studies of mild asthma produced no improvement in symptoms.

Although the improvement in asthma symptoms and airway hyperresponsiveness are impressive, placebo-controlled studies are needed to assess the efficacy of anti-TNF-α therapy. According to Holgate, such studies are now under way in his and other laboratories, and the preliminary results look quite promising. "In twenty-seven years of asthma research, this is the biggest breakthrough that [our research group has] had," he says. **–Michael Szpir**

Wyeth Pharmaceuticals

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National Institute of Neurological Disorders and Stroke

More than 600 disorders affect the nervous system, and neurological disorders strike an estimated 50 million Americans annually. The National Institute of Neurological Disorders and Stroke (NINDS) is the NIH institute charged with overseeing research on these conditions. The NINDS website at http://www.ninds.nih.gov/ provides the latest news concerning the institute, its programs, and neurological science in general, as well as a resource on the entire spectrum of neurological diseases.

At the top of the homepage is a Disorder Index of the many neurological conditions that the NINDS addresses. Selecting one of the hundreds of disorders takes visitors to in-depth information about the disorder's symptoms, methods of diagnosis, treatment options, research being done on the disorder, organizations devoted to the disorder, related NINDS publications (including information in Spanish) and additional resources from MedlinePlus.

The site's homepage features the latest news about neurological diseases, with an archive of older items. The homepage also includes a section listing studies that are seeking subjects. Here, visitors can learn more about what clinical trials really are; those who wish to participate in a clinical trial can choose from an extensive list of neurological conditions, from ADHD to Zellweger syndrome, to see what research is in progress or coming up at the NINDS and elsewhere across the country.

Two sections of the homepage allow researchers to find out about funding opportunities. Under the Funding Opportunities header, visitors can retrieve lists of NINDS opportunities either for the last 60 days or all current opportunities. This section also has information on electronic submission of grant applications and answers questions potential grantees may have about new government requirements for submitting grant applications online. Under the Funding Newsletters header, visitors can sign up for the free *NINDS Notes* newsletter, published three times a year, which contains information on grant applications, requests for applications, and studies that need volunteers. The most current newsletter, plus links to previous editions, are available on the site.

The Neuroscience at NIH section of the homepage contains three sections. The Research at NINDS (Intramural) section contains links to information about NINDS faculty, research facilities, events, and training programs such as summer programs and fellowships. The NIH Blueprint section contains an overview of and link to an NIH framework "to enhance cooperative activities among fifteen NIH Institutes and Centers that support research on the nervous system." This section also has links to requests for information and requests for applications related to the blueprint. Finally, the Neuroscience@NIH section contains information about NIH neuroscience faculty, areas of research interest, seminars, interest groups, and postdoctoral openings. **–Erin E. Dooley**

New Guidelines for Pediatric Asthma

More than 6 million U.S. children have asthma, the leading cause of school absenteeism attributable to chronic conditions and the third leading cause of hospitalization among children under age 15. In November 2005, the National Environmental Education & Training Foundation released *Environmental Management of Pediatric Asthma: Guidelines for Healthcare Providers*. Funded by the NIEHS, the peer-reviewed guidance was built on current best practices and includes competencies for managing environmental asthma triggers in pediatric care, an environmental history form for clinicians to use, and intervention guidelines and fliers for specific triggers such as dust mites, cockroaches, and mold spores. Incorporating these guidelines into medical and nursing curricula could give future generations of primary care providers the tools to better manage pediatric asthma.

Own Private Kyoto

Despite producing 24% of all greenhouse gas emissions worldwide, the United States has not signed on to the Kyoto Protocol to reduce greenhouse gases. An analysis in the 17 November 2005 *Nature* shows, however, that as much as one-third of the U.S. population lives in areas that have adopted their own climate change abatement policies. Together, these regions contribute almost half of the U.S. GDP, a slightly larger share of the global GDP than Japan, the world's second largest economy. The authors warn that compliance could be challenging, though, especially since there are currently no mechanisms for enforcement.

Software for Sorting Satellite Images

NASA satellites generate enough data daily to fill 1,500 copies of the *Encyclopedia Britannica*, but satellite data maps often have blank spots where a satellite wasn't able to record data on a particular day. Now statisticians at The Ohio State University have developed new software that can help researchers rapidly process incoming data to produce complete, detailed maps. In an example given by lead statistician Noel Cressie, it could take one person 500 years to fill in the gaps in a map depicting the thickness of the ozone layer, while the same job would take three minutes with the new software. The software also calculates a measure of map precision.

Top to bottom: Banana Stock; NINDS; NASA

Making Progress on Breast Cancer

For the last two years, scientists across the country have been working together in a highly collaborative effort to uncover the links between exposure to environmental pollutants, puberty, and development of breast cancer. On 10–11 November 2005 the Breast Cancer and the Environment Research Centers (BCERCs), a joint effort of the NIEHS and the National Cancer Institute, came together in Michigan to share what they have accomplished to date. Each center reported on advances made over the past year in their two research components: 1) research on the basic biology of mammary gland development, and 2) epidemiologic studies of how environmental factors affect puberty in girls.

The impetus for creation of the centers came from appeals from advocates, who urged the NIH to support a more comprehensive approach to research on the environmental causes of breast cancer. "We wanted a research approach that focused on environmental causes, and we wanted more involvement from the advocates' perspective," said Dale Eastman, vice president of the Alamo Breast Cancer Foundation.

"The centers represent a great opportunity to conduct transdisciplinary science to explore the problem of breast cancer from many different perspectives, including the valuable perspective of [breast cancer survivor] advocates," said Robert Hiatt, principal investigator for the BCERC at the University of California, San Francisco, which collaborates with Lawrence Berkeley National Laboratory and serves as the coordinating center for the BCERC network. The other three centers are housed at the Fox Chase Cancer Center in Philadelphia (which collaborates with Mount Sinai School of Medicine in New York and the University of Alabama at Birmingham), the University of Cincinnati in Ohio (which collaborates with Cincinnati Children's Hospital Medical Center), and Michigan State University in East Lansing.

Three important features characterize the BCERC program. First, the four centers work together as a network in which experimental methods are coordinated in order to maximize the pooling and comparison of the data generated. Second, to allow adequate time to track the subjects and collect comprehensive information on the onset and progress of puberty, the BCERCs will be funded for seven years, an unusually long time, given that most NIH grants are funded for a maximum of five years. Third, representatives of breast cancer survivor advocacy organizations are integral members of the centers. The advocate members participate in many aspects of the decision-making processes, collaborate with the Community Outreach and Translation Cores of the centers, and added much to the discussion at the annual meeting.

Basic Biology Advances

Many of the significant advances of the past year were made in basic biology studies that use laboratory rodents and cell cultures as models. "This is not surprising, as the human studies are prospective and will yield their most valuable information over time," said Les Reinlib, a program official at the NIEHS who directs the BCERC program.

At the November meeting, Deborah J. Clegg, an assistant professor of psychiatry at the University of Cincinnati, described the insights of her team into understanding the links between obesity, body fat distribution, and postmenopausal breast cancer. Based on her observations of how fat distributes in various body regions, Clegg hypothesized that women who accumulate fat in the upper body—the pattern typically seen in men—would have a greater breast cancer risk than women who accumulate fat in the lower body and thighs. Her team is the first to directly test this idea in humans. Clegg's hypothesis is supported by reports linking insulin resistance, increased recurrence of tumors, and lower survival in obese breast cancer patients with the male pattern of fat distribution. Clegg suggested that body fat distribution may be a better measure than body mass index in determining breast cancer risk.

The team lead by Jose Russo, director of the Fox Chase Cancer Center BCERC and a senior member of the Fox Chase Medical Science Division, is studying the impact of endocrine disruptors on mammary gland development in rats. In this study, animals are exposed at different times in development to environmental pollutants of concern, including bisphenol A and butyl benzyl phthalate. Bisphenol A is an estrogenic substance that is used in the production of some plastics and in food container coatings, while butyl benzyl phthalate is used to plasticize polyvinyl chloride and other polymers. These investigators are finding that, depending on the time of exposure and the age of the young rats upon examination, different genes are up- or down-regulated by the exposures.

Their studies demonstrate that these compounds modify the genomic profile of the rat mammary gland and that these changes are age-specific, indicating windows of vulnerability in the development of the gland. "Interestingly, we are finding that some of these genes, such as glutamic decarboxylase 1, that are affected by exposure to estrogenic plasticizers have been implicated in other diseases such as autism, bipolar disorders, schizophrenia, diabetes, and cancer," said Russo. "So there is an opportunity here to investigate not only how these compounds affect the genomic profile of the mammary gland but also how other organs are affected, explaining other diseases as well."

At Michigan State University, center director and physiology professor Sandra Haslam is conducting studies in both rats and mice. Her work focuses on the study of the normal mammary gland in order to understand how progesterone, a hormone secreted in the second half of the menstrual cycle, is involved in breast development and perhaps in carcinogenesis. She said, "For prevention methods to be produced, we must understand the normal developmental process." To do this, her team is defining the architecture and timing of two forms of progesterone receptors (PR-A and PR-B) as they are expressed on mammary gland cells from rats and mice. From the patterns of expression of these receptors and their colocalization with proliferation markers, Haslam infers the roles of the receptors on cell growth and maturation.

Haslam showed that the rat model is more similar than the mouse model to the human breast in terms of the patterns of receptors expressed. However, the mouse model exhibits novel differences that may

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allow a better understanding of the differences in the functional roles of PR-A and PR-B. Each animal model may provide information about certain aspects of the human condition but not others, given the many differences that exist among the species. Said Haslam, "Ideally, we would have access to human breast tissues from different times in development, but it is extremely difficult [to get breast tissue from women who do not have cancer]."

Human cells have been used in the laboratory to try to study some aspects of carcinogenesis that may be uniquely human and thus are difficult to study in rodents. Paul Yaswen, a staff scientist at the Lawrence Berkeley National Laboratory, is using human breast epithelial cells to define molecular events and cellular characteristics that allow tumor progression. When mammary cells become exposed to a carcinogen, their genomic tumor suppression pathways fail, and the cells become immortalized, or able to reproduce without proper controls. At the meeting, Yaswen presented work on two main tumor suppression pathways involved in blocking indefinite proliferation of breast cells derived from normal human tissue. These tumor suppression pathways represent a gauntlet that carcinogen-exposed cells must overcome in order to acquire cancerous properties.

Listening and Learning

In the closing session, Gwen Collman, chief of the NIEHS Susceptibility and Population Health Branch, presented a framework upon which the results from the different BCERCs will fill in the gaps of scientific understanding about mammary gland development and breast cancer in the context of likely environmental carcinogens. "It was always our intention that [the BCERC program] would spark dialogue between laboratories, scientists of different disciplines, and advocates," said Collman.

Although the data presented at the scientific sessions reflect advances in uncovering the link between environmental exposures and breast cancer, there's still a long way to go before a cure for breast cancer is found. "We have been with the scientists from the beginning, and we have seen the progress, but what do we tell our communities of women who are dying of breast cancer right now?" asked Virginia Regnante, president of the West Islip Breast Cancer Coalition. She suggested that scientists should concentrate on testing the many chemicals that are likely to cause cancer in communities with high rates of breast cancer. "It is an inspiration to have the advocates not be afraid to tell us what they need from us scientists," answered Irma Russo, an investigator in the Fox Chase BCERC. **–Luz Claudio**

Rubberball

Headliners | Breast Cancer

NIEHS-Supported Research

Decreased Melatonin Production Linked to Light Exposure

Blask DE, Brainard GC, Dauchy RT, Hanifin JP, Davidson LK, Krause JA, et al. 2005. Cancer Res 65(23):11174–11184.

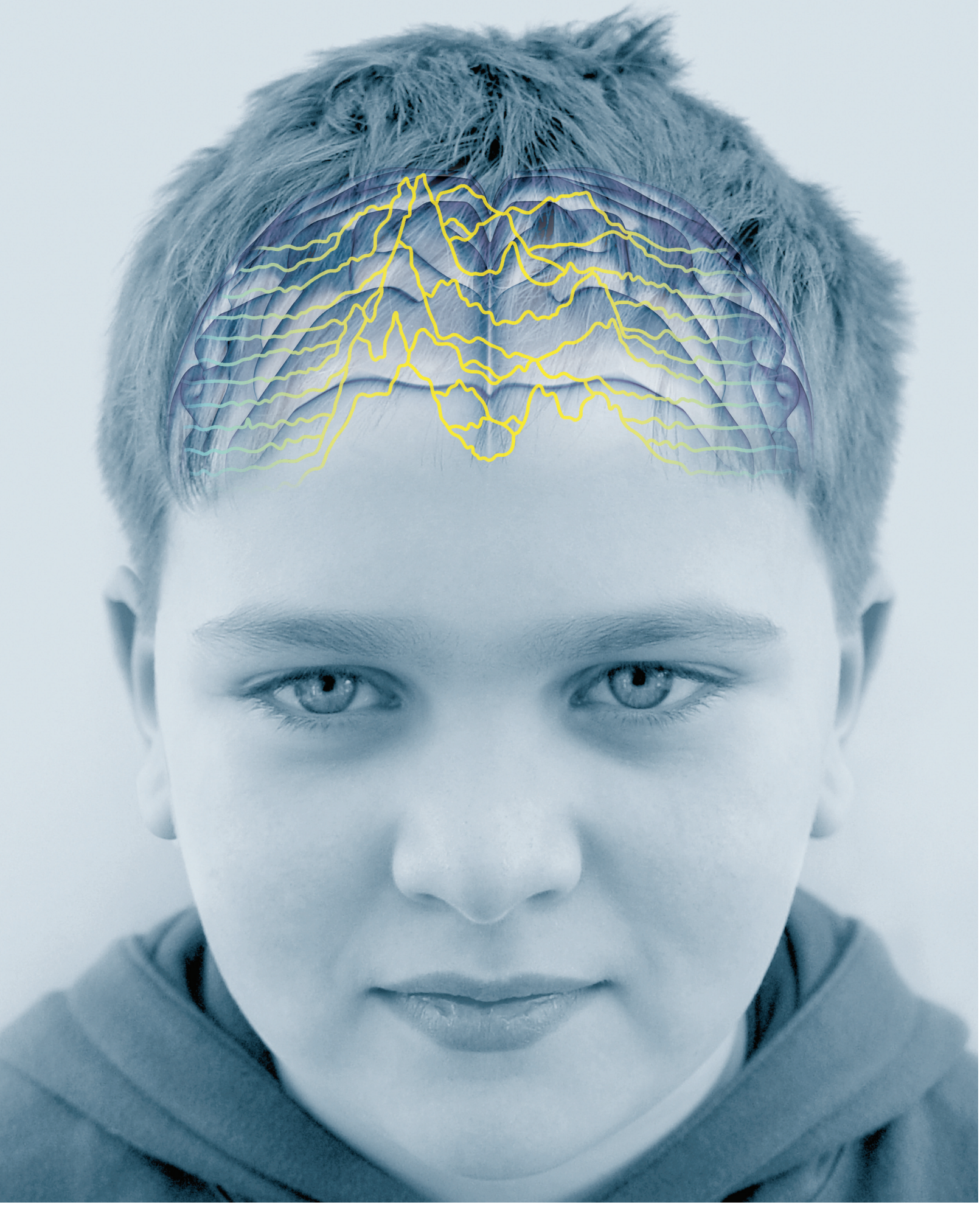
The incidence of breast cancer is up to fives times higher in women living in industrialized nations compared to those living in developing countries, and female night shift workers have particularly high rates of the disease. Given that Western nations have become "24-hour societies," with more people awake around the clock, one hypothesis holds that nighttime exposure to artificial light suppresses the nocturnal production of melatonin. This hormone, produced by the pineal gland, helps regulate the body's circadian rhythm and immune function, and also suppresses tumor growth. Now NIEHS grantees David E. Blask and George C. Brainard and their colleagues have confirmed that ocular exposure to bright artificial light at night inhibits the production of melatonin, which in turn may lead to an increased risk of developing breast cancer.

The researchers implanted human breast cancer cells into female laboratory mice, then transferred the malignant tumors that formed to female rats for continued development. They then collected blood from several healthy premenopausal volunteers under three different conditions: during the day, during the night following two hours of complete darkness, and during the night following 90 minutes of exposure to bright fluorescent light.

Next, they infused the collected blood directly through the developing tumors. Melatonin-rich blood collected following complete darkness slowed the growth of cancer tumors, while melatonin-depleted blood collected from volunteers exposed to both daylight and bright fluorescent light stimulated tumor growth.

The team also exposed tumor-bearing rats to varying intensities of light during the darkness phase of an alternating 12-hour light/12-hour dark cycle. They found that the extent to which melatonin production was suppressed depended on the magnitude of the light intensity that the rats were exposed to during the dark phase.

The authors say these results establish a role for the natural, nocturnal production of melatonin as a preventive agent in human disease. They also emphasize the risks of extensive exposure to bright artificial light at night, and point to the possibility that preserving the integrity of the circadian melatonin signal could help prevent breast cancer. **–Tanya Tillett**



New Thinking on Neurodevelopment

The notion that some substances in the environment can damage the nervous system has an ancient history. The neurotoxicity of lead was recognized more than 2,000 years ago by the Greek physician Dioscerides, who wrote, "Lead makes the mind give way." In the intervening millennia many other substances have been added to the list of known or suspected neurotoxicants. Despite this accumulation of knowledge, there is still much that isn't understood about how neurotoxicants affect the developing brain, especially the effects of low-dose exposures. Today researchers are taking a hard look at low-dose exposures *in utero* and during childhood to unravel some of the mysteries of impaired neurodevelopment.

About 17% of school-age children in the United States suffer from a disability that affects their behavior, memory, or ability to learn, according to a study published in the March 1994 issue of *Pediatrics* by a team from the Centers for Disease Control and Prevention (CDC). The list of maladies includes attention deficit/hyperactivity disorder (ADHD), autistic spectrum disorders, epilepsy, Tourette syndrome, and less specific conditions such as mental retardation and cerebral palsy. All are believed to be the outcome of some abnormal process that unfolded as the brain was developing *in utero* or in the young child.

These disorders have an enormous impact on families and society. According to the 1996 book *Learning Disabilities: Lifelong Issues*, children with these disorders have higher rates of mental illness and suicide, and are more likely to engage in substance abuse and to commit crimes as adults. The overall economic cost of neurodevelopmental disorders in the United States is estimated to be $81.5–167 billion per year, according to a report published in the December 2001 issue of *EHP Supplements*.

Potentially even more disturbing is that a number of epidemiologic studies

Duncan Walker/Stockphoto; geopaul/Stockphoto; Matt Ray/EHP

suggest that the incidence of certain disorders is on the rise. In the United States, the diagnosis of autistic spectrum disorders increased from 4–5 per 10,000 children in the 1980s to 30–60 per 10,000 children in the 1990s, according to a report in the August 2003 *Journal of Autism and Developmental Disorders*. Similarly, notes a report in the February 2002 issue of *CNS Drugs*, the diagnosis of ADHD grew 250% between 1990 and 1998. The number of children in special education programs classified with learning disabilities increased 191% between 1977 and 1994, according to an article in *Advances in Learning and Behavioral Disabilities, Volume 12*, published in 1998.

17

Percentage of school-age children in the United States who suffer from a disability that affects their behavior, memory, or ability to learn. Pediatrics, March 1994

So what is going on? The short answer is that no one really knows. There's not even consensus on what the soaring rates actually mean. Heightened public awareness could account for the surge in the numbers, or it may be that physicians are getting better at diagnosing the conditions. Some autism researchers believe the rise in that condition's prevalence simply reflects changes in diagnostic criteria over the last 25 years. On the other hand, some scientists believe that the rates of neurodevelopmental disease are truly increasing, and that the growing burden of chemicals in the environment may play a role.

With that in mind, investigators are considering the effects of gene–environment interactions. A child with a mild genetic tendency toward a neurodevelopmental disorder might develop without clinically measurable abnormalities in the absence of environmental "hits." However, children in industrialized nations develop and grow up in a veritable sea of xenobiotic chemicals, says Isaac Pessah, director of the University of California, Davis, Center for Children's Environmental Health and Disease Prevention. "Fortunately," he says, "most of us have a host of defense mechanisms that protect us from adverse outcomes. However, genetic polymorphisms, complex epistasis, and cytogenetic abnormalities could weaken these defenses and amplify chemical damage, initiating a freefall into a clinical syndrome."

Pessah cites the example of autism. He says susceptibility for autism is likely conferred by several defective genes, no one of which can account for all the core symptoms of social disinterest, repetitive and overly focused behaviors, and problems in communication. Could multiple genetic liabilities and exposure to a chemically complex environment act in concert to increase the incidence and severity of the condition?

Despite the uncertainties, many scientists believe it would be wise to err on the side of caution when it comes to a research agenda. As Martha Herbert, a pediatric neurologist at Harvard Medical School, puts it, "Even though we may have neither consensus nor certainty about an autism epidemic, there are enough studies coming in with higher numbers that we should take it seriously. Environmental hypotheses ought to be central to research now. The physiological systems that have been harmed by environmental factors may also point to treatment targets, and this might be a great way to help the children."

81.5 to 167

Annual cost in billions of dollars for neurodevelopmental disorders in the United States. EHP Supplements, December 2001

The Parade of Neurotoxicants

Among the most intensely studied neurotoxicants are metals (lead, mercury, and manganese), pesticides, polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs). A number of these compounds were identified as neurotoxicants when individuals were exposed to high doses during occupational accidents or childhood poisonings. Scientists are now exploring the potential consequences of low-dose exposures, especially to children and fetuses. Epidemiologic studies play a central role, and these are often complemented by experimental work on animals and cell cultures. These days, researchers are looking not only at associations between toxicants and disease, but also at the underlying cellular and molecular mechanisms.

Lead. Studies dating to the 1970s show that children exposed to lead have deficits in IQ, attention, and language. In response, the CDC revised its limits for acceptable blood levels of the metal in several steps, from 60 micrograms per deciliter (µg/dL) in the 1960s to the current level of 10 µg/dL, set in 1991. But many scientists think that limit is still too high. A study reported in the September 2005 issue of *EHP* found that there were significant effects on a child's IQ even when blood lead concentrations were below 10 µg/dL. Upon the July 2005 release of the *Third National Report on Human Exposure to Environmental Chemicals* by the CDC, Jim Pirkle, deputy director for science at the CDC's Environmental Health Laboratory, stated, "There is no safe blood [lead] level in children."

Several groups have also found evidence that lead exposure may shape a child's social behavior. An article in the May 2000 issue of *Environmental Research* reports a strong correlation, dating back to 1900, between violent crime and the use of lead-based paint and leaded gasoline. The research complements studies by Herbert Needleman, a professor of psychiatry and pediatrics at the University of Pittsburgh School of Medicine, who found that bone lead levels in young males were correlated with aggression and criminality. "Lead is significantly associated with a risk for delinquency," says Needleman. His research appeared

Top to bottom: Ahmed Hussam/iStockphoto; Photodisc

in the November–December 2002 issue of *Neurotoxicology and Teratology* and the 7 February 1996 issue of *JAMA*.

Another new area of research links early lead exposure to changes in the aging brain. Nasser Zawia, an associate professor of pharmacology and toxicology at the University of Rhode Island, Kingston, and his colleagues found increased expression of amyloid precursor protein (APP) and its product, β-amyloid (which is a hallmark of Alzheimer disease), in aging rats that were exposed to lead shortly after birth. In contrast, old rats that were exposed to lead did not show an increased expression of APP and β-amyloid. The work, published in the 26 January 2005 issue of *The Journal of Neuroscience*, suggests that early exposure to lead can "reprogram" gene expression and regulation later in life. According to Zawia, preliminary research also shows that "monkeys exposed to lead as infants exhibit similar molecular changes as well as exaggerated Alzheimer's pathology."

4–5 to 30–60

Increase from the 1980s to the 1990s in the number of U.S. children per 10,000 diagnosed with autistic spectrum disorders. Journal of Autism and Developmental Disorders, August 2003

250

Percentage of increase in the number of U.S. children diagnosed with ADHD between 1990 and 1998. CNS Drugs, February 2002

Mercury. The current Environmental Protection Agency (EPA) reference dose for methylmercury (an organic, toxic form of mercury) is 0.1 micrograms per kilogram per day (µg/kg/day). Humans are exposed to methylmercury primarily through consumption of contaminated fish; a good 70% of this contamination comes from anthropogenic sources such as emissions from coal-fired power plants. High-level exposure to methylmercury in the womb is linked to a number of impairments, including mental retardation, cerebral palsy, seizures, deafness, blindness, and speech difficulties. An article in the May 2005 issue of *EHP* puts the economic cost to the United States of methylmercury-induced toxicity (in terms of lost productivity) at $8.7 billion annually.

The effects of low-dose exposures are not so apparent. Two large epidemiologic studies of fishing populations in the Faroe Islands and the Seychelles have produced conflicting results regarding low-dose effects. Both studies sought to examine the association between methylmercury exposure and neurodevelopment in children whose mothers ate contaminated seafood during pregnancy.

The leader of the Faroe Islands study, Philippe Grandjean, an adjunct professor of environmental health at the Harvard School of Public Health, and his colleagues reported in the November 1997 issue of *Neurotoxicology and Teratology* that 7-year-old Faroese children had significant cognitive deficits and neurological changes after prenatal exposure to methylmercury. Grandjean's team followed up on the children at age 14. According to a report in the February 2004 issue of *The Journal of Pediatrics,* the children continued to have problems, including neurological changes and decreased nervous control of the heart.

In contrast, the authors of the Seychelles study found little evidence of lasting harm on a cohort of 66-month-old children, according to their report in the 26 August 1998 issue of *JAMA*. A follow-up study, published in the 17 May 2003 issue of *The Lancet,* similarly found no lasting effects on language, memory, motor skills, or behavioral function when the children were 9 years old.

The different outcomes of the two studies are puzzling because the children of both populations appeared to be exposed to similar amounts of methylmercury. Several explanations have been proposed, including the possibility that genetic differences between the populations may alter their relative predispositions to harm from mercury exposure. The source of methylmercury is also different in the two populations. The Faroese are exposed primarily through the consumption of pilot whale meat, whereas the Seychelles population relies heavily on ocean fish. According to Gary Myers, a professor of neurology and pediatrics at the University of Rochester Medical Center and one of the principal investigators of the Seychelles study, whale meat contains many other contaminants (including PCBs) besides methylmercury. "There is also evidence," he says, "that the effects of concomitant PCB and mercury exposure are synergistic."

Researchers continue to look at whether there is a danger from methylmercury at the levels of exposure achieved by fish consumption. Another layer of uncertainty was added with findings published in the October 2005 issue of *EHP* showing that fish consumption during pregnancy appeared to boost infant cognition—but only as long as mercury intake, as measured in maternal hair, wasn't too high.

The question of whether low levels of mercury are harmful has also manifested itself in a controversy over the use of vaccines containing thimerosal, a preservative. Although thimerosal was removed from many of these vaccines in 2001, children that were immunized before that date could have received a cumulative dose of more than 200 µg/kg of mercury with the routine complement of childhood vaccinations, according to a study in the May 2001 issue of *Pediatrics*. Thimerosal is nearly half ethylmercury by weight. Because ethylmercury is an organic form of mercury, there is some suspicion that it acts like methylmercury in the brain, although research published in

Top to bottom: Photodisc, RMAX/iStockphoto

the August 2005 issue of *EHP* suggests that the two forms differ greatly in how they are distributed through and eliminated from the brain. Developing countries continue to use pediatric vaccines that contain thimerosal. In the United States, thimerosal is still present in influenza vaccines, which the CDC recommends be given to pregnant women and children aged 6–23 months.

Advocacy groups, such as SafeMinds, have suggested that the decades-long rise in the diagnosis of autism is related to the presence of thimerosal in vaccines. In May 2004, however, the Institute of Medicine (IOM) issued a report, *Immunization Safety Review: Vaccines and Autism*, stating that several epidemiological studies published since 2001 "consistently provided evidence of no association" between thimerosal-containing vaccines and autism. However, the IOM's report has been severely criticized by a number of advocacy groups, including the National Autism Association, for relying too heavily on a specific set of epidemiologic data while dismissing clinical evidence and other epidemiologic studies that showed evidence of a link.

Despite the assurances of the IOM, some scientists continue to explore the mechanisms underlying the potential neurotoxic effects of thimerosal. In the January 2005 issue of *NeuroToxicology*, S. Jill James, a professor of pediatrics at the University of Arkansas for Medical Sciences, and her colleagues report that the neuronal and glial cell toxicity of methylmercury and ethylmercury (as dosed via thimerosal) are both mediated by the depletion of the antioxidant peptide glutathione. Of the two cell types, neurons were found to be particularly susceptible to ethylmercury-induced glutathione depletion and cell death, according to James, and pretreatment of the cells with glutathione reduced these effects. Other studies by James and her colleagues, reported in the December 2004 issue of the *American Journal of Clinical Nutrition*, showed that autistic children had lower levels of glutathione compared to normal controls, and may therefore have had a significant reduction in the ability to detoxify reactive oxygen species.

83

Percentage of decrease in the CDC acceptable level for lead in blood from 1960 to the current level, set in 1991. EHP, September 2005

James says the abnormal profile "suggests that these children may have an increased vulnerability to pro-oxidant environmental exposures and a lower threshold for oxidative neurotoxicity and immunotoxicity." Speaking at the XXII International Neurotoxicology Conference in September 2005, she presented evidence that multiple genetic polymorphisms affecting glutathione pathways may interact to produce a chronic metabolic imbalance that could contribute to the development and clinical symptoms of autism. Her paper in the *American Journal of Clinical Nutrition* reported that low glutathione levels in many autistic children were reversible with targeted nutritional intervention, but the ramifications of this finding are still unclear.

191

Percentage of increase in the number of U.S. children in special education programs classified with learning disabilities between 1977 and 1994. Advances in Learning and Behavioral Disabilities, Volume 12, 1998

Manganese. As an essential nutrient, manganese is required for normal development; the reference dose for manganese is 0.14 mg/kg/day. Chronic occupational exposure to high levels of this metal is associated with manganism, a condition reminiscent of Parkinson disease that is characterized by tremors, rigidity, and psychosis. The illness is seem primarily among miners.

Animal studies published in the August 2005 issue of *Neurotoxicology* by David Dorman, director of the division of biological sciences at the CIIT Centers for Health Research in Research Triangle Park, North Carolina, suggest that the fetus is protected to a certain extent from maternally inhaled manganese. According to Dorman, children are exposed to manganese primarily by ingesting it, but he knows of no link between childhood exposure to manganese and later Parkinson disease.

Nevertheless, because manganese affects the adult brain, people suspect that the developing brain may be even more susceptible to harm from this metal, and recent research has unveiled a new cause for concern: In the January 2006 issue of *EHP*, child psychiatry professor Gail Wasserman and colleagues from Columbia University reported that Bangladeshi children who drank well water with high concentrations of naturally occurring manganese had diminished intellectual function. The researchers noted that the bioavailability of manganese in water is higher than that of manganese in food. They also pointed out that about 6% of U.S. wells have a high enough manganese content to potentially put some children at risk for diminished intellectual function.

The cellular and molecular mechanisms of manganese neurotoxicity are not well understood. The dopaminergic system in the basal ganglia, which is affected in Parkinson disease, may be involved, but this hypothesis is controversial. Tomás Guilarte, a professor of molecular neurotoxicology at the Johns Hopkins Bloomberg School of Public Health, described research on these systems in nonhuman primates at the XXII International Neurotoxicology Conference. According to Guilarte, unpublished positron-emission

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tomography studies of the basal ganglia show that "manganese does appear to have an effect on dopaminergic neurons." Guilarte found that the more manganese the animals received, the less dopamine was released through the actions of amphetamine (which is used to induce the release of the neurotransmitter). "This does not mean that manganese causes Parkinson's disease, merely that it has an effect on those neurons," he says. This is the first report of an *in vivo* effect on dopamine release by manganese.

PCBs, PBDEs, and pesticides. Many chemicals raise concerns because of their persistence in the environment and their tendency to bioaccumulate in animal tissues. They are typically synthetic molecules that were designed for use in everyday products, such as electrical equipment, computers, furniture, and pesticides.

PCBs appear to be present in all parts of the food chain, and humans are exposed to these molecules primarily through the ingestion of animal fat. The toxicity of these chemicals was first recognized after mass poisonings in Japan in 1968 and Taiwan in 1979. Children born to women who had ingested contaminated cooking oil in Taiwan had a number of developmental abnormalities, including psychomotor delay and lower scores on cognitive tests, according to a report in the 15 July 1988 issue of *Science*.

Since those earlier observations, several studies have described a connection between prenatal exposure to PCBs and delayed cognitive development and lower IQ. For example, a study in the 10 November 2001 *Lancet* reports those infants and young children exposed to PCBs through breast milk scored lower on tests of psychomotor and mental development. The mothers were exposed to normal background levels of PCBs in Europe. In response to such studies, the U.S. Food and Drug Administration set tolerance levels for PCBs in a number of consumer products, such as milk and manufactured dairy products (1.5 parts per million), poultry (3.0 parts per million), and baby food (0.2 part per million).

PBDEs are widely used as flame retardants in consumer products. The effects of PBDEs on humans is not clear, but animal toxicity studies described in volume 183 (2004) of *Reviews of Environmental Contaminants and Toxicology* show that PBDEs can cause permanent learning and memory impairments, hearing deficits, and behavioral changes. There is a growing concern about PBDEs because they appear to be accumulating in human tissues. Andreas Sjödin, a toxicologist at the CDC, and colleagues found a trend toward increasing concentrations of PBDEs in human serum taken from sample populations in the southeastern United States from 1985 through 2002, and in Seattle, Washington, from 1999 through 2002. This report appears in the May 2004 *EHP*. Several studies have also discovered PBDEs in human breast milk. The current EPA reference dose for PBDEs is 2 mg/kg/day.

As for pesticides, it's been suggested by zoologist Theo Colborn of the University of Florida that every child conceived today in the Northern Hemisphere is exposed to these chemicals from conception through gestation and beyond. Some pesticides appear to be more harmful than others, and so the reference dose varies somewhat from one compound to another.

The effects of pesticides on the developing brain have been investigated in human epidemiologic studies and in laboratory experiments with animals. Vincent Garry, a professor of environmental medicine at the University of Minnesota, and his colleagues found that children born to applicators of the fumigant phosphine were more likely to display adverse neurological and neurobehavioral developmental effects. The herbicide glyphosate was also linked to neurobehavioral effects, according to the same report, which appeared in the June 2002 issue of *EHP Supplements*. Another epidemiologic study, reported in the March 2005 issue of *NeuroToxicology*, showed that women who were exposed to organophosphate pesticides in an agricultural community in California had children who displayed adverse neurodevelopmental effects, and that higher levels of pesticide metabolites in maternal urine were associated with abnormal reflexes in the women's newborn children.

Many PCBs, PBDEs, and pesticides are the subject of the 2001 Stockholm Convention on Persistent Organic Pollutants, which became international law in May 2004. The goal of the treaty is to "rid the world of PCBs, dioxins and furans, and nine highly dangerous pesticides," according to the United Nations Environment Programme. Implementation of the treaty has significant practical challenges, however, including the difficulty of eliminating one persistent pollutant without creating another (for example, when burning PCBs yields by-products such as dioxins and furans).

Not Immune to Harm

Exposure to a neurotoxicant may not be the only way to disrupt the natural growth of the brain. Scientists are now looking at the subtle physiological effects of immunotoxicants and infectious agents on biological events during development.

It turns out that mothers who experience an infection during pregnancy are at a greater risk of having a child with a neurodevelopmental disorder such as autism or schizophrenia. For example, prenatal exposure to the rubella virus is associated with neuromotor and behavioral abnormalities in childhood and an increased risk of schizophrenia spectrum disorders in adulthood, according to an article in the March 2001 issue of *Biological Psychiatry*. Rubella has also been linked to autism: some 8–13% of children born during the 1964 rubella pandemic developed the disorder, according to a report in the March 1967 *Journal of Pediatrics*. The same study also noted a connection between the rubella virus and mental retardation.

Some epidemiologic studies have found an increased risk of schizophrenia among the children of women who were exposed to the influenza virus during the second trimester of pregnancy, according to a report in the February 2002 *Current Opinion in Neurobiology*. In the August 2004 *Archives of General Psychiatry*, Ezra Susser, head of epidemiology at Columbia University's Mailman School of Public Health, and his colleagues reported that the risk of the mental disorder was increased sevenfold if the schizophrenic patient's mother had influenza

8.7

Annual cost in billions of dollars of methylmercury-induced toxicity (in terms of lost productivity). EHP, May 2005

Corbis

during her first trimester of pregnancy. A prospective birth cohort study in the April 2001 *Schizophrenia Bulletin* found that second trimester exposure to the diphtheria bacterium also significantly increased the risk of schizophrenia.

6

Percentage of U.S. wells with a high enough manganese content to potentially put some children at risk for diminished intellectual function. EHP, January 2006

How might infectious agents cause these disorders? According to John Gilmore, a professor of psychiatry at the University of North Carolina at Chapel Hill, maternal infections during pregnancy can alter the development of fetal neurons in the cerebral cortex of rats. The mechanism is far from clear, but signaling molecules in the mother's immune system, called cytokines, have been implicated. Speaking at the XXII International Neurotoxicology Conference, Gilmore described *in vitro* experiments showing that elevated levels of certain cytokines—interleukin-1β, interleukin-6 and tumor necrosis factor–alpha (TNF-α)—reduce the survival of cortical neurons and decrease the complexity of neuronal dendrites in the cerebral cortex. "I believe that the weight of the data to date indicates [that the maternal immune response] can have harmful effects," says Gilmore.

Inflammatory responses in the mother may not be the only route to modifying the fetal brain. The University of California, Davis, Center for Children's Environmental Health and Disease Prevention is conducting a large study of autistic children in California called CHARGE (Childhood Autism Risks from Genetics and the Environment), which suggests that the child's immune system may also be involved. According to Pessah, the study principal investigator, children with autism appear to have a unique immune system. "Autistic children have a significant reduction in plasma immunoglobulins and a skewed profile of plasma cytokines compared to other children," he says. "We think that an immune system dysfunction may be one of the etiological cores of autism."

He continues, "We know that many of the things that kids are exposed to these days are immunotoxicants. . . . We have evidence that ethylmercury and thimerosal alter the signaling properties of antigen-presenting cells, known as dendritic cells, at nanomolar levels." Since each dendritic cell can activate 250 T cells, any dysregulation will be magnified, he says. "Add to that a genetic abnormality in processing immune information, and there could be a problem."

Such problems might extend to the central nervous system. The brains of individuals who have a neurodevelopmental disorder also show evidence of inflammation. In the January 2005 issue of the *Annals of Neurology*, Carlos Pardo, an assistant professor of neurology and pathology at the Johns Hopkins University School of Medicine, and his colleagues report finding high levels of inflammatory cytokines (interleukin-6, interleukin-8, and interferon-γ) in the cerebrospinal fluid of autistic patients. Glial cells, which serve as the brain's innate immune system, are the primary sources of cytokines in the central nervous system. So it may not be surprising that Pardo's team also discovered that glia are activated—showing both morphological and physiological changes—in postmortem brains of autistic patients.

The recognition that the immune system is involved in neurodevelopmental disorders is changing people's perceptions of these conditions. "Historically, scientists have focused on the role of neurons in all kinds of neurological diseases," Pardo says, "but they have generally been ignoring the [glia]." He adds, "In autism, it could be that the [glia] are responding to some external insult, such as an infection, an intrauterine injury, or a neurotoxicant."

According to Pardo, it's still not clear whether the neuroimmune responses associated with autism contribute to the dysfunction of the brain or whether they are secondary reactions to some neural abnormality. "John Gilmore's work [showing that cytokines can be harmful to brain cells] is quite interesting and important," he says. "However, *in vitro* studies may produce results that don't reflect what occurs under *in vivo* conditions. Cytokines like TNF-α may be beneficial for some neurobiological functions at low concentrations, but may be extremely neurotoxic at high concentrations."

Lending Brain Power to Exposure Assessment

The medical and scientific communities recognize the colossal challenges involved in identifying the ultimate causes of neurodevelopmental disorders. This is complicated by the sheer numbers of potential exposures involved. More than 67% of the nearly 3,000 chemical compounds produced or imported in amounts exceeding 1 million pounds per year have not been examined with even basic tests for neurotoxicity, according to *Toxic Ignorance*, a 1997 analysis by Environmental Defense.

In the past few years, several large projects have been proposed, and funding

7-fold

The increased risk of schizophrenia in offspring if the mother had influenza during her first trimester of pregnancy. Archives of General Psychiatry, August 2004

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by the NIH has been increased. For example, the NIH boosted its support for autism research from $22 million in 1997 to $100 million in 2004. In 2001, the NIEHS and the EPA jointly announced the creation of four new children's environmental health research centers (including the one at the University of California, Davis), which focus primarily on neurodevelopmental disorders. More recently, the proposed multibillion-dollar National Children's Study, which is cosponsored by the Department of Health and Human Services and the EPA, has been designed to follow nearly 100,000 children over the course of 21 years. The investigators plan to study the effects of environmental factors on children's growth and development, including impacts on learning, behavior, and mental health. Study investigators hope to enroll the first participants in early 2007.

Scientists also see the need for designing better studies. In neurodevelopmental studies, as in any other field, the quality of a study is only as good as all of its parts. Jean Harry, head of the NIEHS Neurotoxicology Group, says, "You can have a valid assessment of behavior, but in the absence of good exposure data, a causative association with environmental factors will be compromised."

In a bid to address the difficulties faced by epidemiologic studies that look for neurodevelopmental effects from *in utero* chemical exposure, a working group of 20 experts gathered in September 2005 under the auspices of the Penn State Hershey Medical Center, coincident with the XXII International Neurotoxicology Conference. The goal of their day-long session was to develop a scheme of best practices for the design, conduct, and interpretation of future investigations, as well as the practical inclusion of new technologies, such as imaging.

At one point in the dialogue, the group recognized that perhaps the greatest challenge in these studies was determining how to evaluate *in utero* exposures to environmental chemicals. "Quite often the very nature of epidemiological studies limits the ability to perform accurate exposure assessments," says Harry, who was part of the expert group. "Such exposures may have occurred in the distant past, they may have been unknown, or they may have been in conjunction with many other compounds."

The group therefore recommended that actual measurements, even if indirect, are better than methods based on subject recall. It also recommended that a well-defined hypothesis should form the foundation of *in utero* studies for assessing neurodevelopmental outcomes. "[These and other] conclusions will move the science forward by describing methods that should improve interstudy comparisons, and they offer ways in which research results should be reported to the scientific and medical communities," says Judy LaKind, an adjunct associate professor of pediatrics at the Hershey Medical Center and a member of the workshop steering committee. The complete workshop report will be published in an upcoming issue of *NeuroToxicology*.

67

Percentage of high-production-volume chemicals produced in or imported into the United States that have not been examined for neurotoxicity.
Toxic Ignorance, 1997

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Imagining the Big Picture

The challenges of addressing neurodevelopmental disorders are more than scientific. The difficulties come together at a crossroads where the communication of knowledge, the treatment of patients, and the regulation of potentially toxic chemicals meet. Says Herbert, "Evidence-based medicine has not yet developed standards for assessing, or practices for treating, the impacts of chronic, multiple low-dose exposures." Rather than waiting, she says, patients and parents of patients are turning to alternative medicine to address their concerns.

That's not always a good thing, especially when patients and parents may be misinformed. Kathy Lawson, director of the Healthy Children Project at the Learning Disabilities Association of America, says there is a disconnect between scientific knowledge and the public's awareness of ways to reduce the incidence of some disorders. "In my visits to various organizations, I've discovered that people are completely unaware that there is a connection between environmental toxicants and their health," she says. "Even pediatricians often don't know about these things," she adds.

Educating the public is only part of the solution. Elise Miller, executive director of the nonprofit Institute for Children's Environmental Health, thinks that federal regulatory agencies do not adequately protect children's health. "The Toxic Substances Control Act, which was passed thirty years ago, needs a major overhaul to ensure neurotoxicants and other chemicals are prioritized, screened, and tested properly," she says. "Currently, there are too many chemicals on the market and in the products we use every day for which there is no toxicity data."

Some politicians agree with these sentiments. In July 2005, Senator Frank R. Lautenberg (D–NJ) introduced the Child, Worker, and Consumer Safe Chemicals Act, which initially calls for chemical manufacturers to provide health and safety information on the chemicals used in certain consumer products, among them baby bottles, water bottles, and food packaging. If passed into law, the bill, coauthored by Senator James Jeffords (I–VT), would require all commercially distributed chemicals to meet the new safety measures by 2020.

The human brain is often touted as the most complex structure in the known universe. The developmental process that produces this remarkable entity may also be among the most delicate in nature. As one scientist put it, "The brain doesn't like to be jerked around." That kind of fragility makes it difficult for scientists to untangle genetic influences from what often may be subtle environmental assaults. Even so, the catalogue of harmful environmental agents will undoubtedly continue to grow as scientists learn more about the interactions between the developing brain and its environment. The hope is that enough good minds will use that catalogue to create a future with healthier brains and more peace of mind for parents and society alike.

Michael Szpir

Image Zoo

Flu Vaccine Production Gets a Shot in the Arm

The world is holding its collective breath. Governments and health organizations are feverishly preparing, stockpiling drugs and vaccines, and formulating contingency plans. Headlines are coughing out dire predictions of up to 100 million deaths worldwide and devastating economic consequences. Experts are chillingly warning that it's not a matter of if, but when—when a pandemic of avian influenza will strike the human population.

Although the H5N1 avian flu is already a serious problem in Asia, it will not become a major threat to human health worldwide until and unless the virus mutates into a strain that is both highly virulent and highly communicable from human to human. At present that is not the case, but influenza viruses are notorious for their ability to mutate via a process called antigenic drift.

Mutation into a strain with the potential for pandemic may never happen, but if it does, mortality could be extremely high. Rapid global travel could spread the disease quickly, and, unlike with the seasonal flu strains that come around every winter, our bodies are not immunologically familiar with this type of avian influenza—no one will have native defenses to ward off or minimize infection.

Massive efforts are under way both in the United States and internationally to respond should an avian flu pandemic occur. One of the most important elements in controlling a pandemic will be the development and production of an effective vaccine. Now Yoshihiro Kawaoka and his colleagues at the University of Wisconsin–Madison School of Veterinary Medicine and the University of Tokyo have perfected an advanced method of producing the inactivated "seed" virus used to produce influenza vaccine, a technique known as

reverse genetics. This breakthrough may represent a critical step forward in accelerating the production of enough vaccine in a short enough time to prevent massive loss of life.

The Chicken and the Egg

To be effective, the nonvirulent virus used to make an influenza vaccine must match the genetic makeup of the viral strain that is circulating in the human population. Stimulation of the immune system by exposure to the nonpathogenic form of the viral strain produces antibodies that will confer future resistance to the pathogenic strain. The key is to first identify and then recreate the subtypes of two of the virus's surface proteins—hemagglutinin (HA) and neuraminidase (NA). These are the "active ingredients" of the virus, determining the strain's virulence and communicability, and are the targets of vaccine intervention. There are 16 HA subtypes and 9 NA subtypes—the combination of these surface protein subtypes describes the viral strain (for example, H5N1).

Reassortment, the traditional method of seed virus production, has been around for more than 50 years and remains in almost universal use, particularly in the production of annual seasonal flu vaccines. In reassortment, scientists inject fertilized chicken eggs with both a standard nonpathogenic influenza strain known to grow well in eggs and the circulating strain that carries the genes expressing the desired HA and NA protein subtypes. The two viruses multiply, and their genes mix with each other in up to 256 possible combinations of eight genes each. The resultant viruses are then screened, with the desired virus being the one with the six genes that allow the standard strain to grow so well in eggs and the HA and NA genes from the circulating strain. This seed virus is then injected into millions of eggs for mass production of that year's vaccine.

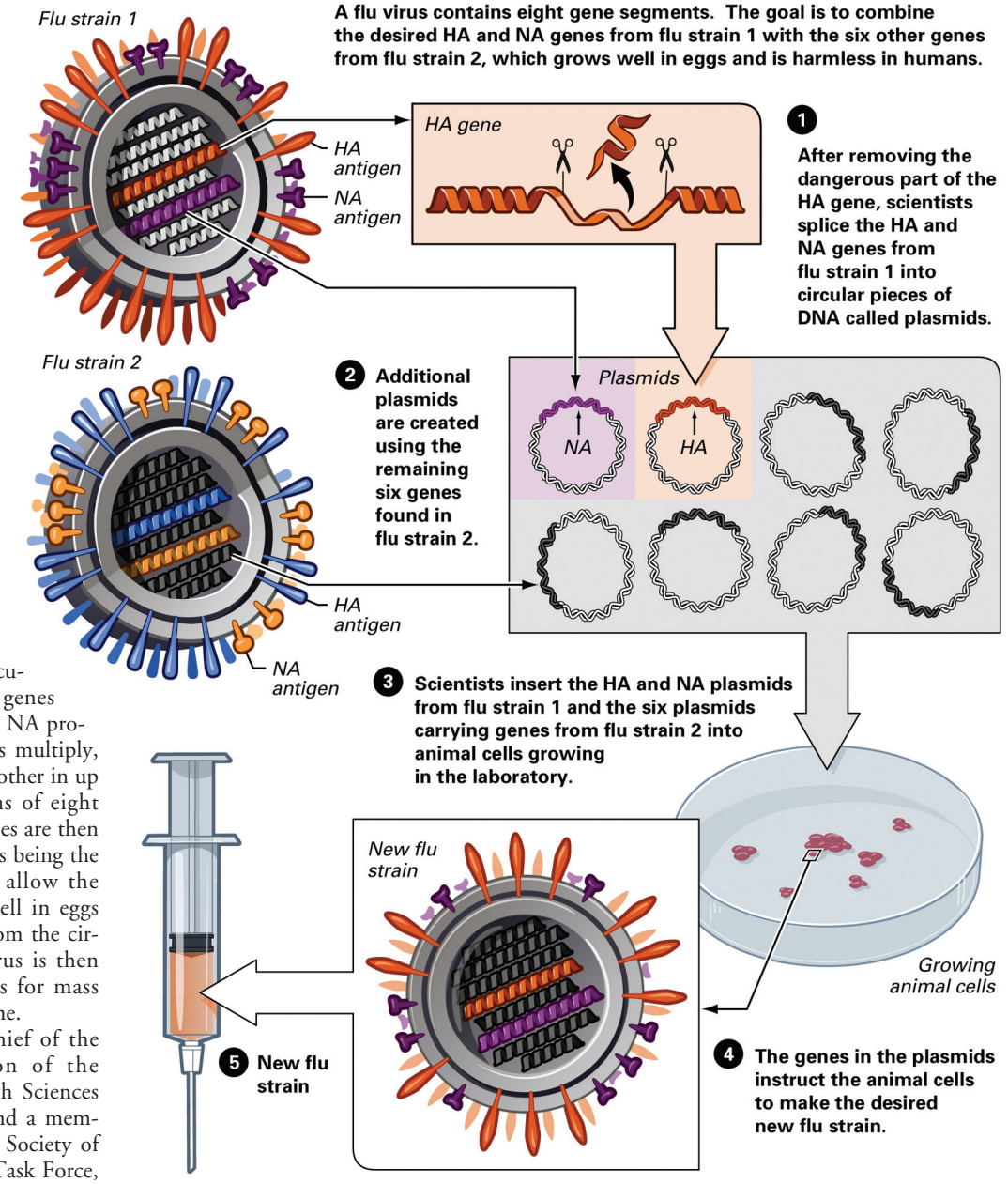
Edward Janoff, who is chief of the infectious diseases division of the University of Colorado Health Sciences Center School of Medicine and a member of the Infectious Diseases Society of America Pandemic Influenza Task Force, describes the reassortment process as "very tedious." According to Andrew Pekosz, an assistant professor of molecular microbiology at the Washington University in St. Louis School of Medicine, the whole process to generate seed stocks "could take two weeks optimistically, but more realistically one to two months." As Kawaoka bluntly puts it, "Classical reassortment? I don't know why people are still using that method."

Monkeying Around with Plasmids

Kawaoka and his colleagues were among the groups who originally developed reverse genetics in the 1990s. With the reverse genetics method, scientists can splice the desired genes—six from the harmless strain and the HA and NA genes from the circulating strain (which have already been adjusted to be nonvirulent)—into small circular pieces of DNA called "plasmids." The plasmids are then transfected into animal cells, and the vaccine seed virus grows. The seed stock can then be grown in mass quantity for vaccine production either in the traditional chicken egg or in cell culture.

Although the laboratory techniques used in reverse genetics are fairly routine at

Using Reverse Genetics to Produce Vaccine



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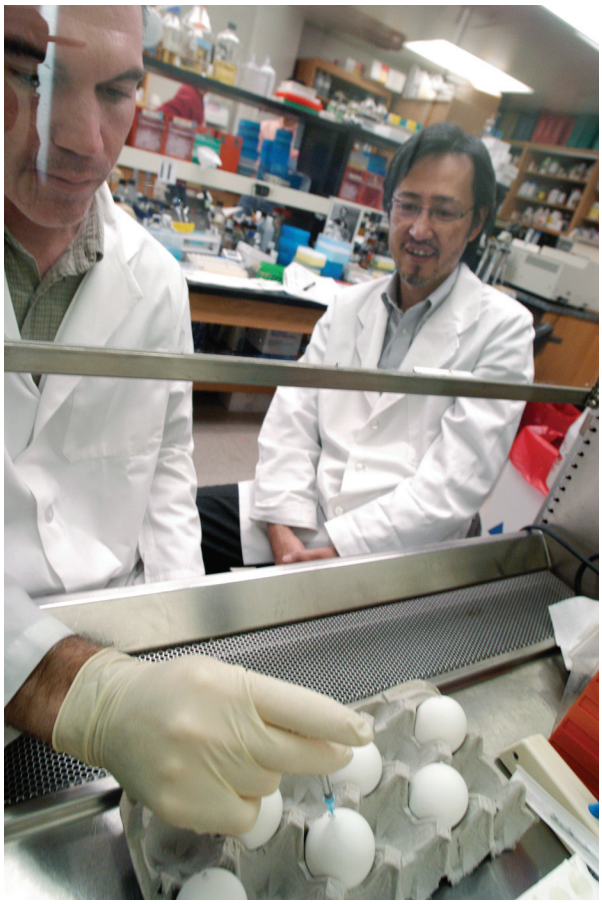

Egg-citing approach to producing vaccines. Yoshihiro Kawaoka (right) and lab technician Barry McClernon (left) oversee an experiment in Kawaoka's laboratory at the UW–Madison School of Veterinary Medicine, where they are working to refine vaccine manufacturing technologies to ensure a faster response to flu outbreaks.

Michael Forster Rothbart/University of Wisconsin–Madison

this point, safety and efficiency issues have presented obstacles to it completely supplanting the reassortment method. The first challenge was the safety of the animal cell line itself. Researchers were concerned that the cells could cause cancer or carry other dangerous viruses. But now a line of African green monkey kidney cells known as Vero cells has been cleared for use in reverse genetics. "These Vero cells have been vetted fairly carefully to be safe," says Janoff, "and the cell line has now been approved for production of human viruses."

The second obstacle was the difficulty of transfecting the cell line with plasmids and growing enough virus to be of use as seed stock. "Many of these cell lines that we'd like to use in a cell culture–based vaccine are very hard to transfect with plasmids," says Karen Lacourciere, an influenza program officer at the National Institute of Allergy and Infectious Diseases. Until now, it was thought to be necessary to transfect eight to twelve plasmids carrying the various viral elements into the Vero cells, and results have been less than ideal in terms of the efficiency of viral rescue—that is, the generation of sufficient numbers of viruses for vaccine use. It can and has been done; the H5N1 vaccine currently in clinical trials (based upon the existing H5N1 strain) was the first one developed via reverse genetics. But clearly, reverse genetics has not been quite ready for prime time.

The refinement to reverse genetics that Kawaoka and colleagues describe in the 15 November 2005 edition of the *Proceedings of the National Academy of Sciences* overcomes this second hurdle. The advance is quite simple. Kawaoka's group has shown that by combining the viral elements in certain ways, the number of plasmids needed to generate large amounts of virus in Vero cells can be reduced. In short, the team tried several different combinations of genes and numbers of plasmids, until they narrowed down which one seemed to work the best in terms of virus production.

Four plasmids appears to be the ideal number: "If we don't worry about just generation of virus, we can make a virus with one plasmid," says Kawaoka. "But in a practical sense, we would use four plasmids, and we would be changing only one plasmid, which encodes HA and NA genes. . . . Our method simply allows one to make vaccine candidate strains easily, so any laboratory can now easily make any H5N1-containing strain."

A Small Step with Big Implications

Kawaoka is modest about the achievement, but observers see it as a crucial step forward. "This new reverse genetics system will allow a cell culture–based vaccine strategy to be developed and become more efficient," says Lacourciere. This is particularly good news given certain problems associated with the egg production system—the need for huge quantities of eggs, and the fact that a significant number of people are allergic to eggs (although no prevalence studies have been done on the general population, 1.5% of children under age 3 are known to have this allergy, according to The Food Allergy & Anaphylaxis Network).

Should a pandemic avian flu strain emerge, time will be of the essence. "What this [method] allows you to do," says Pekosz, "is generate the seed stock for a pandemic virus twenty-four hours after the pandemic is detected—it could speed up the process that quickly."

Janoff, who has his finger on the pulse of preparations for a pandemic, agrees. "One of the concerns about a pandemic is that it would spread more quickly than a regular flu," he says. This means vaccine producers would have a shorter window of time from selecting the virus to having enough vaccine on hand for people both at the source of the epidemic and across the globe as the disease spreads. "So if you can reduce the time from identification and selection to actual vaccine," he says, "that would really potentially save millions of lives."

If and when the H5N1 virus mutates into a strain that retains its lethal effects and becomes highly transmissible from human to human, the clock will start ticking, and the race against time to control the pandemic will begin. Thanks to Kawaoka and his colleagues, at least now the human race will have a bit of a head start.

Ernie Hood

Suggested Reading

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OP Pesticides in Children's Bodies

The Effects of a Conventional versus Organic Diet

Conventional agriculture includes the use of pesticides to control insects in vegetable, fruit, wheat, and other crops, so it's no surprise that foods derived from these crops can therefore contain pesticide residues. What's in question, though, is what these exposures amount to in terms of body burden. Risk-defining data are lacking, and scant data exist on diet-derived pesticides levels in children's bodies. Now researchers from Seattle and Atlanta characterize the relationship between eating a diet of conventionally grown food products and the amount of organophosphorus (OP) pesticide residues that make it into children's bodies [***EHP* 114:260–263**].

According to a 1993 National Research Council report titled *Pesticides in the Diets of Infants and Children*, diet delivers the bulk of children's exposure to pesticides. This exposure poses a greater health risk to children as compared to adults, because not only do children consume more food on a per-weight basis than adults and consequently have higher exposure, they also may be more vulnerable to the effects of toxicants because they are still developing.

The researchers employed a longitudinal design in which 23 children aged 3 to 11 years accustomed to eating a conventional diet switched to organic foods and back again during a 15-day study period. For the first three days, the children consumed their regular conventional diets. During the next five days, they substituted organic equivalents of their usual plant-derived food items (including fresh produce, juice, processed fruits and vegetables, and grain-based products). For the last seven days, they resumed their conventional diets. Each day, for the entire 15-day period, parents collected a urine sample in the morning when the children woke and again at bedtime.

The urine samples were analyzed for metabolites of several OP pesticides. The most commonly detected metabolites were MDA (a metabolite of malathion) and TCPY (a metabolite of chlorpyrifos). During both conventional phases, 60% of samples contained MDA, and 78% of samples contained TCPY. When children switched to organic foods, the percentage of samples containing MDA dropped to 22% and the proportion with TCPY fell to 50%.

Average concentrations of MDA and TCPY also were significantly lower during the organic phase compared to the conventional phases. During the two conventional phases, mean urinary MDA concentrations were 2.9 and 4.4 micrograms per liter (μg/L) compared with 0.3 μg/L in the organic phase. The mean TCPY level decreased from 7.2 to 1.7 μg/L between the first and second phases, and rose to 5.8 μg/L when the children resumed their conventional diets.

Metabolite levels varied widely among the samples, however. Recent research suggests that fractions of MDA and TCPY form as the parent compounds degrade in foods and the environment. Therefore, some proportion of the children's exposure may have been to the metabolites themselves in the foods.

The current study provides insight into how residual OP pesticides in food correspond with the absorbed dose, and the researchers conclude that a diet of organic foods protects children from exposure. They caution against applying results to the general population, however. Given that people from different backgrounds and living in different areas may have different and more significant OP exposures, it would be a mistake to assume that switching to an organic diet would eliminate all exposure to these pesticides. The study does support the National Research Council's conclusion that dietary intake is a major source of OP pesticide exposure, but some children may receive even more exposure from the use of pesticides in the home, and further research is needed. **–Julia R. Barrett**

No beef here. A study of organophosphate metabolites in children eating an alternating conventional/organic/conventional diet shows that eating organic plant-derived foods really can reduce pesticide exposure.

PhotoAlto

Endocrine Disruption and Flame-Retardant Chemicals

PBDE-99 Effects on Rat Sexual Development

Chemical flame retardants containing polybrominated diphenyl ethers (PBDEs) have attracted growing attention in recent years from toxicologists. Unlike their chemical cousins, dioxins and polychlorinated biphenyls (PCBs), environmental levels of PBDE compounds are increasing, as are concentrations found in human breast milk. This is of concern because of the potential that PBDEs may cause developmental toxicity due to endocrine-modulating effects, as has previously been documented with dioxins and PCBs. Results of a new German study examining developmental exposure in rats to a commonly used and environmentally abundant PBDE compound, PBDE-99,

lend support to suspicions that PBDEs can have detrimental effects on sexual development in the offspring of exposed mothers [***EHP* 114:194–201**].

The researchers examined sexual development end points—circulating sexual steroid concentrations, reproductive organ development, and sexually dimorphic behavior—that have been shown in similar rodent experiments to change following exposure to a mixture of PCBs reconstituted according to the congener pattern found in human breast milk. In the current study, weight-matched groups of dams received daily subcutaneous injections of PBDE-99 from gestational days 10 through 18 at doses of 1 or 10 milligrams per kilogram body weight (mg/kg). For comparison, another group was dosed with 30 mg/kg of the PCB mixture Aroclor 1254 (A1254).

In PBDE-exposed male offspring, pronounced decreases in circulating sex steroids (estradiol, testosterone) were seen both at weaning and in adulthood. Anogenital distance, an androgen-dependent marker of sexual development, was also reduced in the male offspring. The males further displayed a dose-dependent increased preference for sweets, which is a sexually dimorphic behavior in rodents—this finding indicates feminization in the males. A slight acceleration in onset of puberty was noted in the low-dose group.

The PBDE-exposed females had less severe effects. Onset of puberty was mildly delayed in the high-dose group. Also, the number of primordial/primary (rudimentary) ovarian follicles was reduced in the low-dose group, with a more pronounced decline in secondary (more developed) follicles within the high-dose group. These results could indicate an impaired reproductive life span, in that ovarian follicles are a key indicator of ovarian health. The females also had an insignificant increase in sweet preference.

The researchers found no effect on sweet preference in male or female offspring exposed to the PCB mixture, but in the females they did see a significant increase in the number of tertiary (still larger, more developed) follicles. The exposure also adversely affected steroid concentrations in males and sexual development in both sexes. Thus, it appears that the pattern of endocrine-disruptive effects differs for PBDE-99 and A1254, which may be due to the higher content of dioxin-like compounds in the PCB mixture.

The scientists also examined organ weights as an indicator of overall development in the rodents. They found that pituitary gland weights were slightly decreased in males in the PBDE-99 high-dose group at weaning, while they were increased in females in the low-dose group. The authors speculate that these effects may indicate a biphasic response to PBDE-99, and suggest that the phenomenon should be studied further in future research. Results also included a marked dose-dependent decrease in thyroid gland weight in adult rats of both sexes exposed to either PBDE-99 or A1254.

This study adds to a growing body of literature indicating that chemical flame retardants are endocrine-disrupting compounds with the potential to profoundly affect sexual development and sexually dimorphic behaviors. The fact that several effects were seen in adulthood, long after termination of the exposure, indicates that the rats' gestational exposure caused permanent physiologic damage. With increases in human body burden and environmental levels, and with the compounds' apparent persistence in both venues, it is safe to assume that PBDEs will be the focus of more urgent research activity. **–Ernie Hood**

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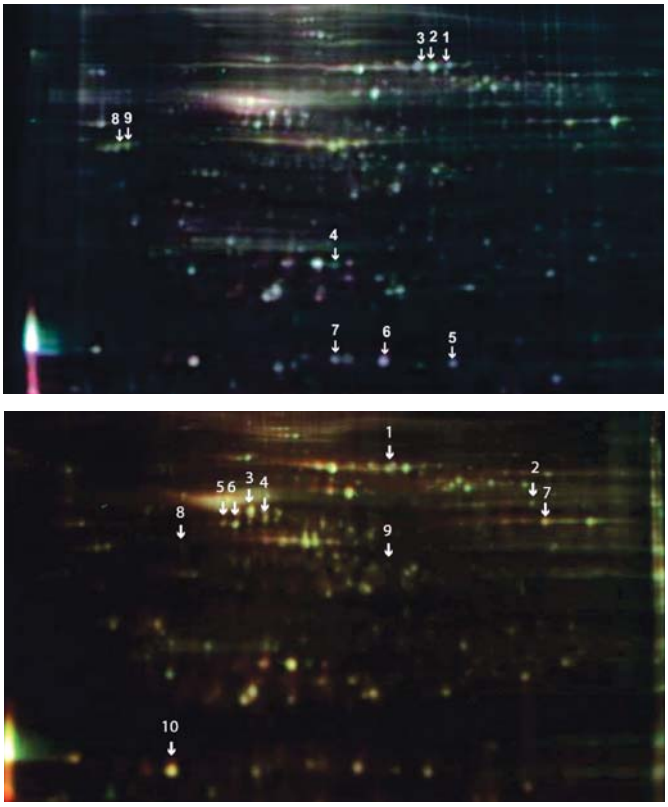


Lights in the darkness. 2D-DIGE reveals nine striatal proteins (top) and ten hippocampal proteins (bottom) that may serve as biomarkers of early-stage PBDE-99 toxicity.

Proteomic Insights into Brain Development

Neurotoxic Effects of PBDE-99 in Mice

The amounts of polybrominated diphenyl ethers (PBDEs) found in human tissues, particularly breast milk, are rising steadily. PBDEs are flame retardants that reduce fire risks when added to electronic equipment and household products. Epidemiologic studies have yet to show conclusive evidence of PBDE toxicity in humans. However, studies in animals have shown these chemicals to produce effects such as liver damage, altered thyroid hormone levels, developmental changes, and neurotoxicity. In the present study, a Swedish team uses proteomic methods to investigate the early-stage neurotoxic effects of PBDE-99 on two parts of the developing mouse brain: the striatum and the hippocampus [***EHP* 114:254–259**]. The findings help deconstruct the mysterious mechanisms that underlie PBDE-99 neurotoxicity.

The striatum and the hippocampus are both part of the cholinergic and monaminergic systems, which play roles in neurotransmitter functioning and other aspects of cognition. The authors sought specifically to uncover PBDE-induced cellular events leading from the neonatal brain growth spurt to permanent neurological problems in adult animals.

PBDE-99 is among the most common PBDE congeners found in environmental and human tissue samples. This penta-brominated

compound impairs spontaneous behavior and habituation among adult mice exposed neonatally to moderate doses. These effects are progressive, worsening with age.

During the study, 10-day-old mice of both sexes were given a single dose of 12 milligrams PBDE-99 per kilogram body weight. After 24 hours, the mice were sacrificed, their brains dissected, and the striatum and hippocampus isolated for study. The team used 2D-DIGE to compare protein expression patterns between treatment and control groups. The analysis illuminated 40 proteins in the striatum and 56 proteins in the hippocampus whose expression was altered by PBDE-99 exposure.

From this initial grouping, nine striatal proteins and ten hippocampal proteins were selected for identification using MALDI-ToF-MS. Among the striatal proteins affected by PBDE-99 were several (including Gap-43/neuromodulin and stathmin) that participate in neurodegeneration and neuroplasticity. The affected hippocampal proteins (including α-enolase, γ-enolase, Atp5b, and α-synuclein) tend to participate in metabolism and energy production. Many of these proteins are linked to protein kinase C, a signaling molecule whose role in development and function, as well as in learning and memory, has been intensively studied.

Based on these findings, the authors conclude they have identified potential protein biomarkers that reflect the immediate consequences of early-stage PBDE-99 toxicity, in addition to the processes that drive its neurological effects in older animals. The researchers suggest that protein kinase C signaling is a target of PBDE-99 toxicity in the developing mouse brain. Moreover, the authors propose that neonatal cell stress induced by PBDE-99 exposure, in addition to related neurodegenerative processes and aberrant neuroplasticity, may contribute to the latter-stage behavioral effects observed in adult mice. The responses within the striatum and hippocampus differ, however, reflecting the underlying heterogeneity between different brain parts and cell populations. **–Charles W. Schmidt**

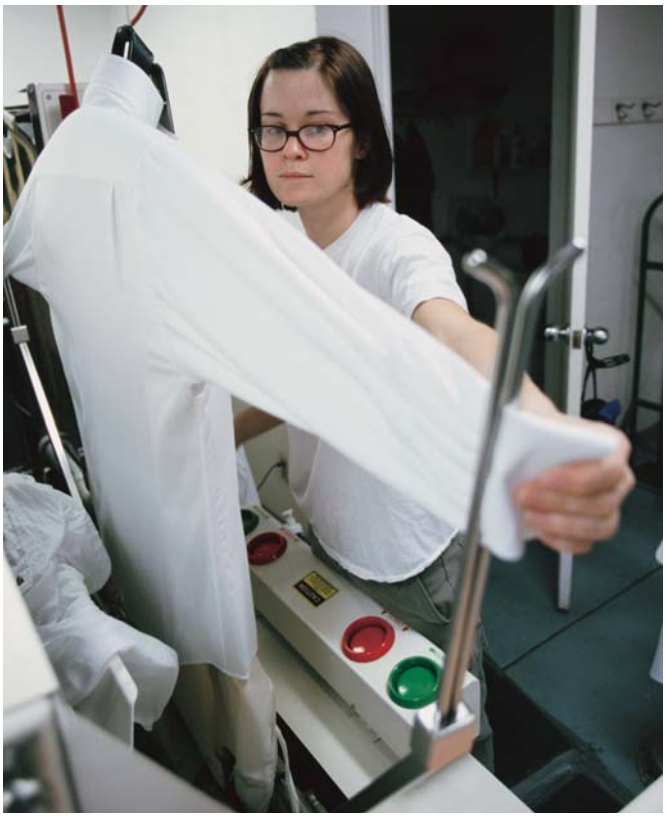
Pressed for Hard Facts

Multiple Tetrachloroethylene–Cancer Links Go Unconfirmed

Recent U.S. studies have linked dry cleaners' exposure to tetrachloroethylene, a solvent used in the industry, to an increased risk for a number of cancers, including esophageal, lung, and cervical cancer. Results of other studies on bladder and pancreatic cancer are equivocal. Still other studies have shown an increased risk of non-Hodgkin lymphoma. Now a study by a team of Nordic researchers of cancer risk among dry-cleaning workers in Denmark, Finland, Norway, and Sweden finds that, with the exception of bladder cancer, exposure to the solvent showed no link to the disease [***EHP* 114:213–219**].

The researchers identified 46,768 dry-cleaning and laundry workers from the 1970 censuses in the four countries. The investigation was a series of case–control studies nested within this cohort of workers. Controls were matched by country, sex, and five-year group for age and year of cancer diagnosis. The team studied the period from 1964 to 1979, when tetrachloroethylene was the primary solvent used for dry cleaning in these countries.

The team considered four categories of exposure: exposed workers in dry-cleaning shops with fewer than ten workers (reflecting a probability of sharing tasks and working in more cramped quarters), other workers in dry-cleaning shops (such as seamstresses and office workers), unexposed laundry workers and others not working in a dry-cleaning shop, and those who could not be classified.



Clean sweep for dry cleaners? Tetrachloroethylene, a solvent widely used in the dry-cleaning industry, has been implicated in many cancers, but a new study of Nordic dry cleaners fails to corroborate most of those links.

Blinded telephone interviews were done with cases, controls, and, eventually, next of kin in Norway and Sweden. If the subject's occupation was dry cleaning, the interview covered length of employment in the dry-cleaning shop, number of workers in the shop, solvents used, and the subject's smoking and drinking habits. For Denmark and Finland, pension records and other data sources were used to gather comparable information.

Although exposure to tetrachloroethylene varied greatly among shops, the average annual level of exposure was fairly stable between 1964 and 1979. The team found no increase in risk of cancers of the esophagus, gastric cardia, liver, pancreas, or kidney. There was also no link to non-Hodgkin lymphoma. The study did, however, find a 44% excess risk in bladder cancer concentrated in Norway and Denmark, the two countries with the best data.

The authors point to several strengths of the study, particularly its completeness. It covered all persons working in dry cleaning in 1970 in the Nordic countries. It also compared two cohorts, dry-cleaning workers and laundry workers, who had similar jobs except for the exposure to tetrachloroethylene.

The authors also acknowledge the study had some weaknesses. For a high proportion of cases and controls from Sweden and Finland the authors could not determine whether the subjects worked in either a laundry or dry-cleaning business. Consequently, estimates of cancer risk were reported for all four countries together and for Denmark and Norway together. Furthermore, subjects could not be classified by exposure level to tetrachloroethylene. The researchers note, though, that because the data indicated a fairly stable level of exposure during employment, they consider length of time on the job an adequate surrogate measure of a cumulative dose. **–Harvey Black**

Rubberball