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Tracing the Origins of Autism: A Spectrum of New Studies

Mutations, Proteins, and Autism: Modeling a Pathway

Beauty or the Beast?

NIEHS National Institute of Environmental Health Sciences

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STUDENT EDITION

Discovery consists of seeing what everybody has seen and thinking what nobody has thought.
Albert Szent-Györgyi (1893–1986)

LEGISLATION

California Enacts Safe Cosmetics Act

Californians frustrated with what they consider the FDA's loose control over cosmetic safety have taken matters into their own hands with the country's first state cosmetics regulatory act, which takes effect in January 2007. The California Safe Cosmetics Act of 2005 will require manufacturers to report the use of potentially hazardous ingredients to the state Department of Health Services (DHS), which in turn will alert consumers. The DHS has the authority to investigate whether the product could be toxic under normal use and to require that manufacturers submit health effects data. Manufacturers that continue marketing products deemed unsafe in California could face legal action.

A safer smooch. California recently enacted legislation that will require manufacturers to report potentially hazardous ingredients used in cosmetic and personal care products.

"The legislation's sponsors believe that the basis of the law is the public's right to know," says Kevin Reilly, DHS deputy director of prevention services. The new law uses the list of toxicants drawn up under California's Proposition 65, which mandates that the governor publish a list, updated at least yearly, of chemicals that are known to the state of California to cause cancer, birth defects, or other reproductive harm.

Although the new act applies only in California, its effects are likely to reverberate nationwide. Consumer advocates predict that manufacturers seeking to avoid negative publicity will remove, rather than report, suspect ingredients. Those formulas would then be marketed coast to coast.

Impetus for the law stems from consumers' concerns over long-term exposure to certain cosmetic ingredients. Cosmetic use has not been linked to chronic illnesses, but some products do contain carcinogens (such as formaldehyde, used in nail treatments), teratogens (such as lead acetate, used in two hair dyes), and other reproductive toxicants (such as di-*n*-butyl phthalate, used in nail treatments and dandruff shampoos).

Studies in recent years have shown that humans absorb and inhale sometimes surprisingly high levels of toiletry ingredients. In the November 2005 issue of *EHP*, a team led by Susan M. Duty of the Harvard School of Public Health demonstrated that urine concentrations of phthalate metabolites increased by 33% with each personal care product—hair gel or spray, lotion, deodorant, cologne, aftershave—that subjects used.

Historically, cosmetics safety has been in the hands of manufacturers; the FDA requires no premarket testing. Each year, an expert panel convened by the industry-funded Cosmetic Ingredient Review (CIR) identifies priority ingredients for which it conducts literature reviews and analyses to determine safety. The panel—made up of independent academic researchers and representatives from industry, consumer interests, and the FDA—has declared 9 of the 1,286 ingredients reviewed since 1976 unsafe for normal cosmetic use. But manufacturers are not obligated to eliminate any ingredients—at least one ingredient identified as unsafe by CIR, hydroxyanisole, is still used.

Safety advocates see evidence of any harm in any use as reason enough for a ban. "Ingredients suspected of causing cancer shouldn't be used in cosmetics," says spokesman Kevin Donegan of the Breast Cancer Fund, a San Francisco–based nonprofit that promoted the California bill.

F. Alan Andersen, director and scientific coordinator of CIR, counters that the dose creates the danger. "We don't subscribe to the notion that if there's ever an adverse effect, [a chemical] must not be in a product people use," he says. "It doesn't make sense to us to apply the precautionary principle. Instead, we use a risk assessment approach, and the wide margins of safety that we have found for chemicals such as phthalates using this approach assure us that actual use of cosmetics is safe."

The law drew fierce opposition from individual companies and the Cosmetic, Toiletry, and Fragrance Association (CTFA) as it worked its way through the California legislature. "CTFA supports strong federal regulation by the FDA," says Kathleen Dezio, executive vice president of public affairs and communications for the association. "For this reason, CTFA has generally opposed state-specific legislation that would undermine this national approach and lead to an unworkable state-by-state patchwork of rules . . . or unjustified, extreme requirements that are well beyond those placed on any other category of food, beverages, drugs, or consumer products." She adds that CTFA has met with the DHS and "pledged our cooperation in accomplishing the requirements" of the law.

Some manufacturers have already ceded to public pleas for safer products. In the past two years, almost 350 of them signed a pledge promoted by the Campaign for Safe Cosmetics, a coalition of health and environmental groups, to use no chemicals linked to cancer or birth defects. Industry leaders L'Oréal and Revlon broke new ground last year when they promised that products they sold in the United States would meet more stringent European Union standards. In 2004 Europe enacted a ban on suspected carcinogens, mutagens, and reproductive toxicants in personal care products.

"We're definitely seeing a shift in the attitude of manufacturers," Donegan says. "They're starting to see the benefits of removing anything that could cause cancer." **–Cynthia Washam**

Radu Razvan/ShutterStock

RADIATION

Tanning Trippers Get UV High

It has long been suspected that cutaneous endorphins are produced during exposure to UV light. Now, research published in the April 2006 issue of the *Journal of the American Academy of Dermatology* suggests that frequent users of tanning beds may become addicted to them. Moreover, blocking the effects of these endorphins could lead to withdrawal symptoms.

"This might explain why some people appear to be hooked on sunbathing and why frequent users of tanning beds say they experience a positive mood change or are more relaxed after a session," says coauthor Steven Feldman, a professor of dermatology at Wake Forest University School of Medicine.

Feldman's team thought that blocking this potential endorphin rush might cause such people to lose some of their tanning enthusiasm; what they didn't expect was for some to develop withdrawal symptoms.

The subjects included eight frequent tanners (who used tanning beds 8 to 15 times per month) and eight infrequent tanners (who used them up to 12 times per year). The researchers administered either a placebo or 5, 15, or 25 mg of naltrexone, a central and peripheral opioid receptor blocker; such blockage causes withdrawal symptoms in opioid drug–addicted people but not in nonaddicted people. The subjects were then asked to lie for 10 minutes on each of two tanning beds, one a true UV bed, the other rigged not to deliver UV light. Afterwards, the subjects, who were blind to the test conditions, were asked to describe which session made them feel best.

With the placebo and the 5-mg naltrexone dose, the frequent tanners showed a clear preference for the UV bed—and more strongly so than the infrequent tanners. But this preference fell away with the 15- and 25-mg doses of naltrexone, "suggesting that light-induced endorphins are reinforcing [frequent tanners'] behavior," says report coauthor Mandeep Kaur, also a dermatology professor at Wake Forest University School of Medicine.

Further evidence of this was seen when half of the frequent tanners developed nausea and jitteriness with the 15-mg dose. "These are common [opioid drug] withdrawal symptoms," explains Feldman, "and they were bad enough for two subjects to drop out." Although there were no further problems at the 25-mg dose, Feldman says these results suggest that frequent tanners suffer some degree of dependency on endorphins.

"Clearly tanning is not as addictive as smoking," remarks Robert Dellavalle, an associate professor of dermatology at the University of Colorado Health Sciences Center. "Just look at the prevalence of smoking in middle age—twenty percent in the UK and the United States. In contrast, there is a steep drop-off in the prevalence of tanning as people age."

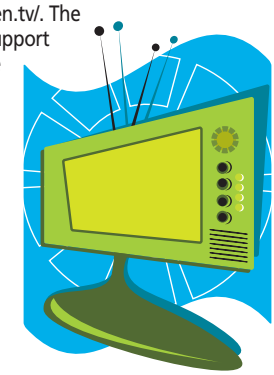
Still, says, Feldman, although it's not time for the Drug Enforcement Administration to raid beauty parlors, "these results do raise questions about the safe use of tanning beds." **–Adrian Burton**

Sun-sations. A new study shows why tanning bed rays feels so good to some people: UV light produces endorphins to which frequent tanners may become addicted.

Clockwise from left: Dragan Trifunovic/ShutterStock; Kelly Resener/ShutterStock; Joseph/ShutterStock

Now Broadcasting: green.tv

green.tv, the world's first Internet-based broadband channel dedicated to environmental issues, started broadcasting in March 2006 from its website at http://www.green.tv/. The channel, developed with support from UNEP, is also available as a podcast on iTunes. green.tv will carry films from around the world, produced by NGOs, community film makers, public sector agencies, and environmentally minded corporations. The site features seven subchannels focused on air, land, water, climate change, people, species, and technologies. Each subchannel will run a feature film, a news item, and a story for children. The channel's first offerings include films from Water Aid, the Sierra Club, the Eden Project, the Women's Environment Network, Farm Africa, and others.

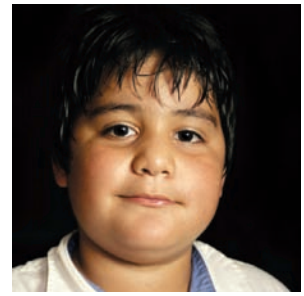


UNEP Promotes Sustainable Building and Construction

In February 2006 UNEP announced the launch of the Sustainable Building and Construction Initiative to promote environmentally friendly practices in the construction industry. Three of the world's largest construction companies—Lafarge, Skanska, and Arcelor—have signed on to the effort. The construction sector employs over 100 million people worldwide and contributes 10% of the global gross domestic product. Yet the industry also plays a serious role in problems such as climate change, waste generation, and depletion of natural resources. The new initiative will address these issues, and also lobby for laws and building standards to support sustainable practices.

Pediatric Environmental Health in Argentina

WHO statistics show that approximately 33% of diseases affecting children under age 5 are linked to environmental risk factors. To address this threat, the Argentine and Buenos Aires governments have set up new "pediatric environmental health units" in Buenos Aires and several provinces of Argentina. The units are made up of pediatricians, nurses, social workers, teachers, and others who work as a team to uncover and remediate risk factors in children's environments, often at the request of a referring physician. The units have the authority to work with schools, public works, and neighborhood residents if they believe a specific hazard exists. The units will also train other professionals within the hospitals where they are based and conduct research on child environmental health issues.



WOMEN'S HEALTH

Endometriosis and PCB Exposure

Endometriosis may be related to exposure to persistent pollutants such as polychlorinated biphenyls (PCBs), according to research published in the May 2006 issue of *Chemosphere*. This gynecological disorder linked to infertility afflicts 10% of U.S. women of reproductive age. Researchers measured blood PCB levels in women undergoing laparoscopy for suspected endometriosis or other gynecological conditions. Higher levels of PCBs were detected in women with histologically confirmed endometriosis compared with controls.

Toxicologist Elena De Felip of the Istituto Superiore di Sanità in Rome and her colleagues measured 11 PCB congeners that are most abundant in human tissue. In 80 women aged 20 to 40, the sum of all congeners was 1.6 times higher in the 40 women diagnosed with endometriosis than in controls. Three congeners, PCBs 138, 153, and 180, were particularly higher in women with endometriosis. These three congeners have been reported to have estrogenic activity and to interfere with hormone-regulated processes.

PCBs have been used since the 1930s, mainly in electrical equipment. Although no longer manufactured, these persistent chemicals accumulate in the food chain; today meat, fish, eggs, and milk are chief sources of PCBs. But diet seems unable to explain the difference in PCB levels detected in the two groups of women, since "the dietary habits of the women were basically the same," says De Felip.

De Felip suspects that differences in how women detoxify and eliminate PCBs from the body may explain the disparity. These processes are mediated by polymorphic enzymes; therefore, she says, differences in toxicokinetic activity may represent the basis for the higher concentrations detected in women with endometriosis and may also be related to higher or lower susceptibility to that condition.

Studies of PCBs and endometriosis face several limitations. Researchers typically measure only a few widespread congeners that are selected because of their toxicological activities, including an association with cancer shown in animal models. "So we're only getting part of the picture," says Germaine Buck Louis, chief of the Epidemiology Branch at the National Institute of Child Health and Human Development.

In a study described in the January 2005 issue of *Human Reproduction*, Buck's team measured 62 congeners in 84 women undergoing laparoscopy. Levels of 4 antiestrogenic congeners were 3.77 times higher in women diagnosed with endometriosis than in controls. "We don't fully understand the role of estrogenic and antiestrogenic PCBs," she says, but complex interactions of many PCBs as well as other chemicals may be involved in developing endometriosis.

Recent advances in PCB detection methods allow more congeners to be measured at lower concentrations. "Women with endometriosis may have low levels of a particular congener not found in [other women]," says Louis. Moreover, breastfeeding reduces PCB levels in women, so women without endometriosis may have lower blood levels because they become pregnant and breastfeed more often. "There's no ideal comparison group for endometriosis studies," says Louis, and "there are no easy answers." **–Carol Potera**

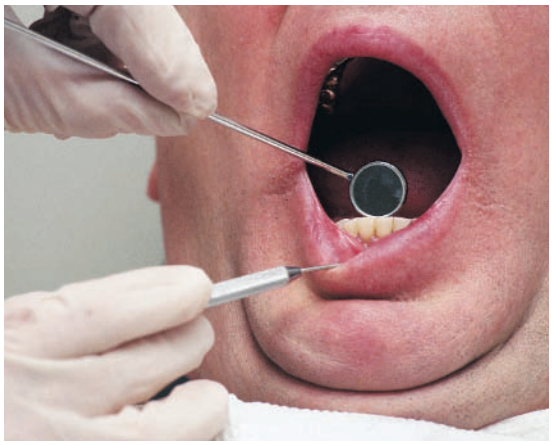
CHEMICAL EXPOSURES

No Dental Dilemma for BPA

Among the many uses of bisphenol A (BPA) is the manufacture of resin-based dental composites and sealants. Recently a team of researchers from the CDC sank their teeth into questions about whether BPA monomer leaching from sealants could be harmful to people. The results of their human study, presented in the March 2006 issue of the *Journal of the American Dental Association*, suggest that although leaching does occur, sealants are still a safe means of preventing dental cavities.

Low-level exposures to BPA monomer in pregnant rodents, at a level that humans could potentially receive from dental sealants, have been shown to disrupt reproductive development in their fetuses, and concerns have emerged about the possibility of human health effects from dental exposures. Scientific exploration of this question has yielded inconsistent results, says Renée Joskow, first author of the March paper. Much of this is due to limitations in laboratory detection and translation of animal studies to human health effects, as well as insufficiently addressing the parameters of exposure in a clinical dental setting.

The CDC team, led by Joskow (now of the U.S. Public Health Service) and Dana Barr, looked at 14 nonsmokers receiving their first resin-based sealants as part of their routine dental care. Each subject received one of two brands of dental sealant manufactured by two well-established dental equipment and material supply firms. Then their saliva and urine were tested for BPA.



BPAhhh. New data show that exposure to bisphenol A in dental sealants is likely an insignificant source of risk.

Getty Images

All the patients had BPA in their saliva and urine, even before treatment. For patients receiving Helioseal F sealants, saliva BPA doubled immediately after treatment and returned to baseline within 1 hour. Urine BPA more than tripled 1 hour after treatment and returned to baseline within 24 hours. For patients receiving Delton LC sealants, saliva BPA increased nearly 126 times immediately after application and was still 23 times higher after 1 hour. Urine BPA jumped 10 times 1 hour after treatment and was still elevated 24 hours later. Both levels eventually returned to baseline.

Barr believes the patients' baseline BPA came from background exposures from environmental sources such as water and food packaging. These, she suggests, could be "a more chronic low-level source of exposure" than dental sealants. Barr adds that in her view, although point-source exposure from dental sealants might approach levels that induce health effects in rodents, "[it] is not the most significant source of exposure in humans." Moreover, she holds that exposure to BPA from dental sealants, already variable and short-lived in the body, could be easily reduced further by having the patient spit frequently in the hours after application. **–Julian Josephson**

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Cure Autism Now

In 1995, parents of children with autism joined together to form the nonprofit organization Cure Autism Now (CAN). Since then, its membership has grown to include clinicians and scientists committed to accelerating the pace of biomedical research in autism. CAN raises and distributes funds for research on the causes, prevention, and treatment of autism, as well as education and outreach. As a resource for everyone interested its work, CAN has a website located at http://www.cureautismnow.org/.

So far, CAN has committed over $25 million to research funding and has established and continues to support the Autism Genetic Resource Exchange (AGRE). Clicking on the Research link at the top of the CAN homepage takes visitors to an overview of the CAN science program, which includes six initiatives that the group believes will yield the most effective treatment for individuals with autism.

The Genomics Initiative focuses on gene mapping and microarray work. CAN's goal is to identify several genes involved in autism within the next three years. Closely related to the Genomics Initiative is the AGRE, an open gene bank with a large collection of immortalized cell lines and DNA samples gathered from families with more than one autistic child. Available on the AGRE page is a link to research updates published since 2001.

The goal of the Innovative Technology for Autism Initiative is to stimulate development of products that provide realistic solutions to the issues encountered by those with autism, their families, educators, health care specialists, and researchers. The initiative offers multiyear grants, fast-track "bridge" grants, and educational programs. It also sponsors a workgroup within which investigators can meet, share, and collaborate, and which also serves to actively bring new investigators into the field.

One major hurdle that autism researchers are working to overcome is the lack of any biomarker for diagnosis. The CAN Biomarkers Initiative has yielded two preliminary findings of possible autism biomarkers—one a novel protein in the urine of children with autism and some of their unaffected relatives, and the other a distinct lipid profile that was seen in 20 AGRE samples. CAN has launched a study in an effort to replicate and confirm these results.

In the past few years, new findings on neuroplasticity, the ability of the brain to grow and change throughout life, have led to significant breakthroughs in the treatment of stroke and dyslexia through a process called neural retraining. To apply these same ideas to the treatment of autism, CAN has established the Neural Retraining Initiative. The initiative's first project, led by Michael Merzenich of the University of California, San Francisco, is working to design, produce, and test nonpharmaceutical tools and techniques, including one to prevent the emergence of full-blown autism in at-risk infants.

CAN has also awarded several grants through its Environmental Factors in Autism initiative to study the neurotoxicity of mercury and how it may factor in the development of autism. Thimerosal, which contains ethylmercury, has been widely used as a preservative in vaccines and other health and medical products, and has been raised as a potential contributor to autism. **–Erin E. Dooley**

Unlimited Mileage from the Drive-Thru?

A company offering rental cars powered entirely by biodiesel opened its doors in Los Angeles in February 2006. The cars get 400 to 800 miles per tank on 100% biodiesel made from recycled cooking oil. Bio-Beetle Eco Rental Cars first started in Hawaii in 2003 with a single car, and now offers 16 at that location, while the LA location is starting with 4 vehicles. Company founder Shaun Stenshol hopes to open two more U.S. locations by the end of the year. Other biodiesel rentals may not be far behind: Enterprise is pilot-testing an offering of biodiesel Jeeps in Portland, Oregon.

Goldman Environmental Prize 2006

For 17 years, the $125,000 Goldman Environmental Prize has been awarded to activists dedicated to effecting environmental change in their home countries. The six winners for 2006 are:

- Yu Xiaogang, of China, who created groundbreaking watershed management programs while documenting the socioeconomic impact of dams on Chinese communities. China's central government now considers social impact assessments for major dam developments.
- Anne Kajir, of Papua New Guinea, who uncovered government corruption that allowed rampant illegal logging of the region's largest remaining intact parcel of tropical rain forest. As a novice lawyer, she successfully defended a Supreme Court appeal forcing the logging industry to pay damages to indigenous land owners.
- Tarcísio Feitosa da Silva, of Brazil, who led efforts to create the world's largest area of protected tropical forest regions in a remote area of northern Brazil that was threatened by illegal logging.
- Craig E. Williams, of Kentucky, who convinced the Pentagon to halt plans for burning old chemical weapons that had been stockpiled around the United States.
- Olya Melen, of Ukraine, who used the legal system to temporarily halt the construction of a massive canal through the rich wetlands of the Danube Delta.
- Silas Kpanan'Ayoung Siakor, of Liberia, who revealed evidence that former Liberian president Charles Taylor used profits from unchecked logging to pay for a 14-year civil war. The revelation led the UN Security Council to ban the export of Liberian timber, part of wider ongoing trade sanctions.

Top to bottom: Cure Autism Now; Goldman Environmental Prize

A Meeting of the Minds on Mice

If genetics research is ever to fulfill its promise of revolutionizing medicine, genotypes must be linked to phenotypes—that is, individual genomic characteristics must be identified and associated with outcomes in the forms of disease susceptibility and/or development; individual responses to drugs, infectious agents, or environmental exposures; or other individual characteristics such as behavioral tendencies. Why does the person who never smoked develop lung cancer, while the three-pack-a-day smoker remains healthy? Why do some people become addicted to drugs, while other users are never hooked? Why does a particular medication work wonders in some people, but not work at all in others? These and countless similar questions represent the enormous challenge still facing researchers as they strive to make personalized medicine a clinical reality.

The answers to many of these questions may yet be discovered in the genomes of mice, our diminutive mammalian relatives. That's certainly the hope and belief of the members of the mouse genetics community, 180 of whom gathered 6–9 May 2006 in Chapel Hill, North Carolina, for the fifth annual meeting of the Complex Trait Consortium (CTC), a loosely woven international organization tightly knit in its dedication to elucidating human characteristics by identifying their genetic counterparts in mice.

The "complexity" of complex traits derives from the fact that they are polygenic—multiple genes interact to cause these conditions, and the genes involved may not interact additively. Ninety-three reports presented at the CTC meeting updated progress in the hunt for the multiple genes and quantitative trait loci (or chromosomal "hot spots") associated with a wide variety of complex traits such as heart failure, tumor resistance, obesity, drug and alcohol addiction, and schizophrenia. Sponsored by the NIEHS, the UNC–Chapel Hill, and Agilent Technologies, the conference brought together a diverse group of mouse geneticists, molecular biologists, statisticians, and bioinformaticists from 10 countries.

The CTC is all about collaboration and interaction. "It's unquestionably the best meeting that I go to every year," says Abraham Palmer, an assistant professor of human genetics at the University of Chicago. "The opportunities to communicate with other geneticists working in other fields and with the people who develop our methodology are critically important, and accelerate by months or even years the rate at which the field can move forward," he says.

Karlyne Reilly, a principal investigator in the Mouse Cancer Genetics Program at the National Cancer Institute, agrees. "It brings together a wide variety of science around techniques and how you solve the problems that are common to these different areas," she says. "I always come away with new tools to play with, that I can apply to my own research."

Building a Better Mouse Line

The CTC is presently at the midpoint of building a resource that should prove enormously valuable in the effort to associate genotypes with phenotypes. The Collaborative Cross (CC) is a carefully planned and controlled mouse recombinant inbreeding program that began in 2005 with eight genetically heterogeneous strains. Upon its expected completion in about four years, 1,000 lines closely modeling the breadth of human genetic diversity will have been generated. According to conference keynote speaker Jean-Louis Guénet, a professor emeritus of mouse genetics at the Institut Pasteur in Paris, it will be "one of the most important pages in the book of genetics of the future."

Armed with several powerful new bioinformatic and biostatistical tools being developed specifically to take full advantage of the resource, the CC will enable researchers to hunt far more precisely and efficiently for the multiple genes and quantitative trait loci that constitute complex traits, and will allow the community to share and integrate their raw data sets far more effectively.

"The idea is to accumulate as much diverse data as possible for relatively fixed strains, what we call the 'genetic reference population,'" says Robert Williams, a professor of anatomy and neurobiology at the University of Tennessee Health Science Center and one of the founders of the CTC. "The hope is that everybody will use their own tools—their own methods and their own phenotypes—but the Collaborative Cross will provide a way to bind those results together by using the same animal resource."

According to conference co-organizer David Threadgill, an associate professor of genetics at UNC–Chapel Hill, the goal is for the CC to evolve to become "the central resource for experimental mammalian biology." With a fixed genetic reference population and common tools, he says, "it will be the resource that everybody turns to," because every piece of data collected through the CC will be immediately comparable to any other piece of data in the database.

CC Riding

The CC will enable a so-called systems genetics approach, as opposed to the traditional, laborious effort to identify one gene at a time. As Guénet points out, diseases that are the consequences of the alteration of a single gene—one example is cystic fibrosis—tend to be marginal in terms of frequency. However, polygenic diseases tend to be much more widespread, he says: "Next door to you, you probably have someone with asthma, dermatitis, or autoimmune disease. . . . So we have to work hard to understand the genetic determinants of these complex diseases, and presumably what we are going to learn from the mouse can be transposed to the human being, because we share ninety-eight percent of our genes with the mouse."

Williams shares Guénet's optimism about the tremendous potential of the CC to shed useful light on common human diseases. "You have to understand the function of the gene and its products in a complex milieu, in a mouse or human—not only a mouse or human, but many different mice and many different humans," he says. "We think [the CC] will provide the resource to do that." He adds that the ability to conduct experimental population-based research with the CC should allow much more comprehensive exploration of the genetics associated with gene–environment interactions.

That exploration will also be enhanced by the completion of a mouse genetics initiative undertaken by the NIEHS and Perlegen Sciences to identify the genetic variants in 15 diverse strains of laboratory mice, including SNPs (single-nucleotide polymorphisms), indels (insertions/deletions), and haplotypes (blocks of related SNPs). The database, a project of the recently established NIEHS Center for Rodent Genetics, is scheduled to be unveiled in September 2006, and is anticipated to be a rich and robust source of information for the mouse genetics community.

Signs of early but significant progress in the CC initiative were among the highlights of the meeting. Conference co-organizer John E. French, an NIEHS research physiologist, is encouraged by results emerging from pilot studies. "There's at least been a proof of principle established that it's going to be a very effective tool," he says. "We are only seeing the beginning evidence of that—there's a long way to go—but some of the promise has been identified and, I think, validated." According to Williams, the pilot project is now of sufficient size (two recombinant inbred sets, LXS and BXD, with 80 member strains) that "it provides the community with a good flavor of what this will look like when we have an order of magnitude more strains than we do now."

Threadgill is excited by the flavor that's already emerging. "The major things that are starting to come out are the results of integrating data sets, integrating genetic

variations, and integrating gene expression patterns," he says. The new knowledge that's coming out of that—the identity of new genes that are potential master modulators of genetic networks, and how those may actually also be very important for mediating disease processes—speak to the remarkable potential that will be realized when the CC is completed.

A Case in Point

Research results presented by Palmer on his group's work at the University of Chicago illustrate the broad outlines of the types of studies being undertaken by mouse geneticists. Palmer and colleagues are investigating the genetic underpinnings of susceptibility or resistance to drug addiction; given today's working definition of "the environment," recreational drug use is fast becoming a xenobiotic exposure of great interest. An understanding of the genotypic differences between addiction susceptibility and resistance could lead to new targets for therapeutic drugs or preventive interventions.

The team selectively bred mice to have very high or very low sensitivity to locomotor stimulation, a particular behavioral effect of methamphetamine that is a characteristic animal response to drugs of abuse. They then measured the expression of more than 14,000 genes in a region of the animals' brain known to be involved in response to the drug. Ultimately, they arrived at a candidate gene that was found to be very differentially expressed in the high- and low-sensitivity mice—casein kinase 1 epsilon (*Csnk1e*). It was a gene already known to be involved in locomotor stimulant response of animals to various drugs. But the question then became, was it important in humans?

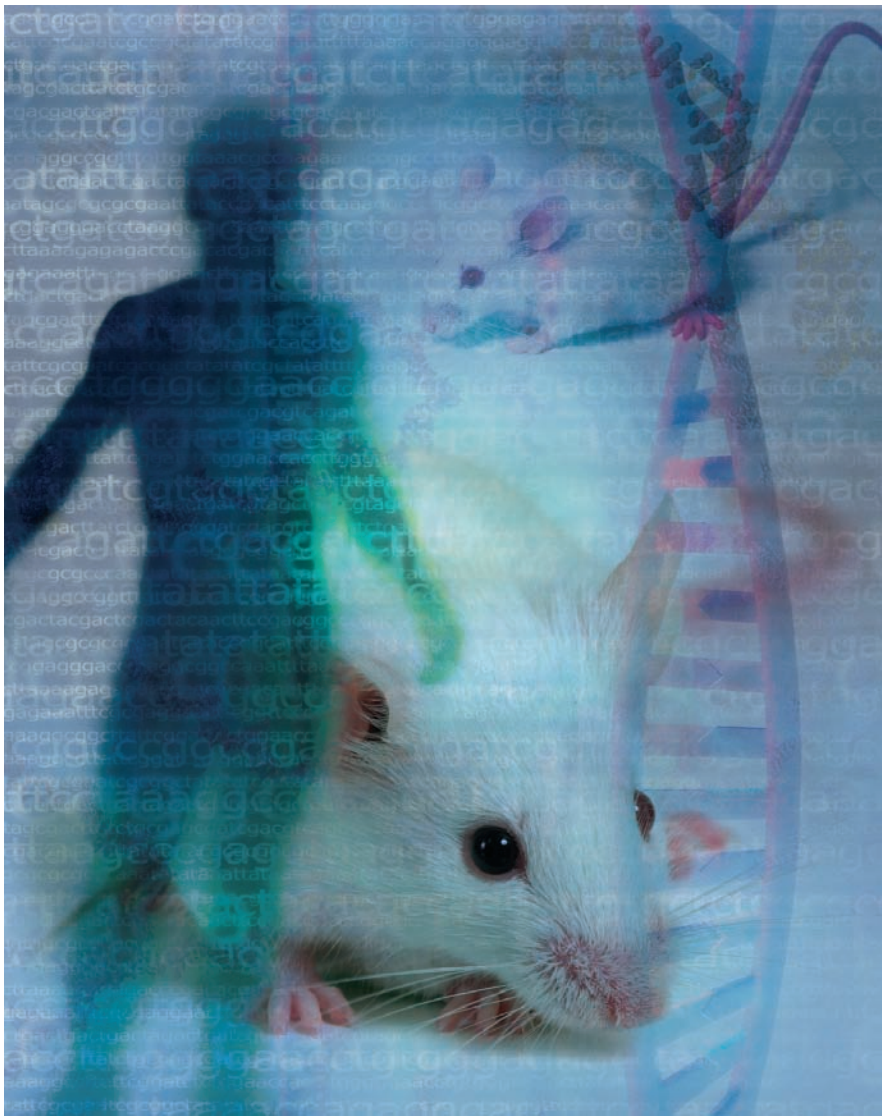
Fortuitously, thanks to colleague Harriet de Wit of the University of Chicago Department of Psychiatry, Palmer had access to DNA from a cohort of 100 healthy human volunteers. In a double-blind study, the subjects received 0-, 10-, and 20-mg doses of amphetamine in a randomized order. Responses were measured by standardized questionnaires, and were then compared to results of genotyping tests, to see whether there was a correlation between response to the drug and polymorphisms in *Csnk1e*.

"We found a statistically significant association between this gene, *Csnk1e*, and people's sensitivity to the euphoric effects of the drug," says Palmer. "So the people with one genotype 'got a buzz,' while people with another genotype didn't. We hypothesize that that may have implications for the likelihood of a person with one genotype who samples the drug to continue to use the drug, and that of course would put them at grave risk for developing an abusive relationship with the drug."

Palmer suspects that polymorphisms in *Csnk1e* may also be important in a variety of other systems whose mechanisms might be similar to that of addiction. These include the manic phase of bipolar disorder and the use of stimulants to treat attention deficit/hyperactivity disorder.

"I think we're now at a point where it's just about to become easy to go from a phenotype to identifying some of the genes that are involved in that phenotype," says Palmer. "To get all of them is going to take longer, and it's going to require further refinements in our methodology, but I actually think that the story I told is going to become a common story. . . . In the same way that molecular biology took a long time to mature, and now is unbelievably central to the way we think about the progress of medicine and health sciences, I think this field of genetics is right at that turning point."

Knowledge gained from the genomes of our mammalian cousins by groups like the CTC may provide the vital information to eventually usher in the much-anticipated era of personalized medicine. Says Threadgill, "What it really comes down to is being able to predict which individuals are going to be susceptible to certain environmental exposures or disease processes, which individuals are going to respond adversely to combinations of alleles, so that interventions and preventive medicine can be applied where they need to be applied, rather than in global fashion." **–Ernie Hood**



Culling out complex traits. A consortium of international scientists is launching new mouse research initiatives to help elucidate genetic components of complex human diseases.

PhotoDisc, Arnold Greenwell/EHP, Matt Ray/EHP

Environmental Polymorphism Registry

Banking DNA to Discover the Source of Susceptibility

Walking outside on a day when ozone levels are at "code orange" doesn't bother one person, but for someone else, it can result in chest pain, coughing, wheezing, or lung and nasal congestion. Why? Polymorphisms, tiny interindividual variations in genes, may be part of the reason. Providing a pool of information to help researchers determine how these variations interact with the environment to cause disease is the ultimate goal of the Environmental Polymorphism Registry (EPR), which is sponsored by the NIEHS and conducted in collaboration with the University of North Carolina at Chapel Hill's General Clinical Research Center.

A little prick for a big cause. The Environmental Polymorphism Registry aims to collect 20,000 blood samples that will be studied to help determine how genetic differences may result in disease.

Any North Carolinian over 18 years of age can donate a sample—about a tablespoon of blood—to the EPR. Rather than recruiting donors with a particular disease, the EPR aims to gather, over five years, samples from 20,000 people who represent the general state population. The regional nature of the effort facilitates recruitment and follow-up.

"Recruitment is monitored to ensure that the EPR population is representative of the North Carolina population," says Patricia Chulada, one of the four principal investigators of the EPR and a health scientist administrator at the NIEHS. "If we see deficiencies in certain groups, then we can increase efforts targeted to those particular groups."

This approach will help researchers find out which polymorphisms are most common. "We want to look at people's genetic material and find variations, and then go back and figure out what those variations mean," says another EPR principal investigator, Paul B. Watkins, a professor of medicine at UNC–Chapel Hill and director of the General Clinical Research Center.

Chulada and Perry Blackshear, the NIEHS director of clinical research, initiated the registry by approaching Watkins and Susan Pusek, director of training and career development at the General Clinical Research Center. Watkins says the institute—and Blackshear himself—realized that "this is an essential direction of research to understand why some people are healthy and some are sick."

There are multiple DNA registry efforts in the United States. Two major DNA banking efforts include Northwestern University's NUgene Project and the Marshfield Clinic's Personalized Medicine Research Project, both launched in 2002. International DNA banks are even more common, Chulada says. For instance, Iceland's deCODE Project has recruited more than 80,000 subjects and has published findings on genes associated with arthritis and many other common conditions.

The EPR is unique, however, because it is designed to focus on environmentally responsive genes—those that increase the risk of disease when combined with an environmental exposure. The registry was created with the express intent of facilitating clinical studies of polymorphisms in these genes. Being affiliated with the NIEHS, where scientists are already studying such interactions, makes the EPR a natural resource for these investigations.

Protecting Participants

Unlike with anonymous DNA databases, EPR donors provide their names and contact information so they can be asked to participate in follow-up studies if their DNA contains a polymorphism of interest. Participation in follow-up studies is optional, and donors can drop out of the database at any time.

Donors learn about the steps taken to ensure confidentiality in a 6-page consent form. Study interviewers at recruitment tables also discuss this information with potential donors, Chulada says.

Donors' names and other information are stored separately from samples. When a sample is collected, it's assigned a personal identification number. The code key that links the sample to identifying information is kept separate from the sample and from all other data in a computer system that's password-protected. Access to this system is limited to only a few people directly involved in the EPR. Researchers can obtain contact information for potential participants only after approval by the EPR Oversight Committee.

To receive samples, researchers must sign a material transfer agreement, in which the researcher's institution agrees to several conditions. "They can only use the samples for what they outlined in the agreement," Chulada says. "They can't give the samples to others. And they have to destroy the samples within a certain amount of time [which varies on a case-by-case basis]."

In addition, the NIH has granted the EPR a Certificate of Confidentiality, which protects researchers from being required, even by subpoena, to disclose research data or other information about an individual to an outside party such as an insurance company, an employer, or a civil or criminal court. "This is another layer of protection built into this system," Chulada says.

Stepping Up Recruitment

The EPR has already accumulated about 4,000 samples—not far behind the 5,000 collected by NUgene since its launch. The

Dona McNeil/NIEHS

EPR's goal of 20,000 samples is the minimum needed to conduct certain types of studies with adequate statistical power, Chulada says. For example, if a researcher was interested in a rare genetic variant that occurs in only 1% of the population, the variant should be present in 200 samples from a registry of 20,000. "That would give us adequate statistical power to test for a phenotypic association of low to moderate effect, depending on other factors," Chulada says.

When the EPR began, it recruited exclusively at two clinics at UNC–Chapel Hill. It has since expanded recruitment to Rex Hospital in Raleigh and is applying for approval to recruit at Duke University Medical Center in Durham. However, Chulada says, "Although recruiting at medical clinics gave us a diverse population in terms of health and other characteristics, we learned that we could increase both recruitment rates and diversity by recruiting outside of the clinic setting."

A recruitment fair held for five days at the NIEHS campus in Research Triangle Park yielded about 420 donors. "We were ecstatic with the response of the NIEHS community," Chulada says. The general public also can donate through study drives at corporations and health fairs in Research Triangle Park. Potential participants can visit the EPR website (http://dir.niehs.nih.gov/direpr/) to find out about upcoming drives.

A DNA Goldmine

John Hollingsworth, a scientist working in the Environmental Lung Disease Group in the NIEHS Laboratory of Respiratory Biology, is one of the first investigators to apply for use of EPR samples. Hollingsworth and colleagues want to identify people who have a polymorphism in a certain gene, Toll-like receptor 4 (*TLR4*), known to be important in innate immune responses.

TLR4 was first identified as a candidate gene for response to ozone by NIEHS scientist Steven Kleeberger, leader of the Environmental Genetics Group in the Laboratory of Respiratory Biology. Subsequently, Hollingsworth and colleagues have demonstrated that mice deficient in *TLR4* are protected against airway hyperresponsiveness after exposure to ozone. "We want to determine if this gene is important in people in the biologic response to inhaled ozone," Hollingsworth says. "We're trying to validate what we've seen in mice in a human cohort."

Hollingsworth calls the EPR a "goldmine." He says, "It's a perfect situation. We have a cohort willing to be genotyped, rather than doing a mass screening of people for a single project, which is what we've had to do in the past." **–Angela Spivey**

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Headliners | Autism

NIEHS-Supported Research

Misfolded Protein Presents Potential Molecular Explanation for Autism Spectrum Disorders

De Jaco A, Comoletti D, Kovarik Z, Gaietta G, Radić Z, Lockridge O, et al. 2006. A mutation linked with autism reveals a common mechanism of endoplasmic reticulum retention for the α,β-hydrolase fold protein family. J Biol Chem 281:9667–9676.

Currently, there is only very limited information available on the etiology and biological basis of the autism spectrum disorders, although mutations in the *neuroligin 3* and *neuroligin 4* genes have caught researchers' attention in recent studies. Now NIEHS grantees Palmer Taylor and Mark H. Ellisman at the University of California, San Diego, and their colleagues have determined, among other findings, that homologous mutations in the genes encoding butyrylcholinesterase (BChE) and acetylcholinesterase (AChE) cause defects in protein processing and expression similar to those seen with *neuroligin 3*, shedding further light on a potential molecular mechanism underlying autism.

The neuroligins, BChE, and AChE are members of the α,β-hydrolase fold family of proteins. The arginine-to-cysteine substitution in the *neuroligin 3* mutation was identified in a set of twins, and cell culture studies show most of the expressed protein being retained within the endoplasmic reticulum, suggesting misfolding of the protein. In addition, the small amount of protein that does reach the surface of the cell shows compromised binding affinity for its partner, β-neurexin. Misfolded proteins causing endoplasmic reticulum retention and compromised functional activity are a common consequence of mutations in cystic fibrosis and metabolic diseases with a genetic origin.

In the current study, confocal fluorescence microscopy and analysis of oligosaccharide processing were used to ascertain whether the homologous arginine-to-cysteine mutation affected AChE and BChE despite their differing oligomerization states. By inserting homologous mutations in the AChE and BChE cDNAs, the investigators found that the mutation also resulted in endoplasmic reticulum retention of the two cholinesterases and enhanced degradation in the proteasome. The authors speculate that altering intracellular oxidation/reduction parameters may influence proper folding and export of these proteins. **–Jerry Phelps**

BEYOND THE BENCH

Continuing Education for Nurses on Environmental Genetics and Complex Diseases

Many people find it hard to fit professional development and continuing education into their busy work lives. Now help is just a mouse-click away for nurses seeking flexible, self-paced training in the growing field of environmental genetics. The Community Outreach and Education Core (COEC) of the Center for Environmental Genetics at the University of Cincinnati, in collaboration with the Genetics Education Program for Nurses (GEPN) of Cincinnati Children's Hospital Medical Center, has created an online Environmental Genetics and Complex Diseases educational module that introduces nurses to the principles of environmental genetics, and also teaches them how to apply those principles in nursing practice.

New age of nursing. An online continuing education module introduces nurses to principles of environmental genetics and shows them how to put such principles into practice.

Online since December 2005, the module is useful for all nurses in clinical practice, but especially targets those who work extensively with minority or medically underserved patients. The module focuses on alcoholism, lead, and asthma, three challenging public and environmental health problems in underserved communities.

"The module is designed to prepare nurses in underserved communities to identify people who are at risk for environmental genetic conditions and help those people gain access to community services that emphasize prevention and early treatment strategies," says COEC director M. Kathryn Brown.

Cynthia Prows, a clinical nurse specialist in genetics and the principal investigator of the web program, says the module organizes information into useful and manageable resources. "There is a tremendous amount of information on the Internet about genetics and about environmental health. But how do nurses who have limited knowledge in the topic areas locate the various sites, sift through all the information, decide what information is current and accurate, and then use that information for learning purposes? The answer is, most nurses don't because they don't have the time or the necessary foundational knowledge in genetics to mine the overwhelming mass of information that is accessible through the Internet."

The module developers have done that work for the nurses, and have organized the content in a way that helps nurses develop foundational knowledge in environmental genetics using high-quality resources that are applicable to their practice. Once learners create a unique username and password, they can access the module free of charge, and can re-enter it at any time at the place they last exited. Those who wish to earn 4.8 nursing continuing education contact hours after completing the module and associated evaluations pay a minimal processing fee.

The module offers nurses background information on gene–environment interactions, and teaches them environmental and sociodemographic risk factors for common diseases. It also provides screening tools and community resources for nurses treating patients with recognizable genetic and environmental risk factors. Each of the three learning tracks also offer prenatal, pediatric, and adult case studies and self-assessments with each content area.

After completing the module, nurses are able to approach their communities armed with valuable knowledge of gene–environment interactions and insight into how those interactions can affect human health. They are also equipped with a wealth of online resources that can be accessed long after they complete the training module.

"Making sense of the fast-growing literature about how the health impacts of environmental exposures through the life span are mediated by our genetics is a challenge for health care professionals," says Brown. "We hope that the vast array of resources identified in these self-paced, online modules will be helpful to primary care practitioners trying to make sense of new developments in genetic screening tests, environmental prevention strategies, and treatment options."

The module is available at http://gepn.cchmc.org/. Three additional genetics education modules currently available include Promoting Informed Decision-Making about Genetic Testing, Ethical and Social Issues Related to Genetic Testing, and Interpreting Family History. Two new modules are also in the pilot testing phase: Genetics Is Relevant Now—Nurse Views and Patient Stories, and Nurses' Role in Pharmacogenetics/Pharmacogenomics.

–Tanya Tillett

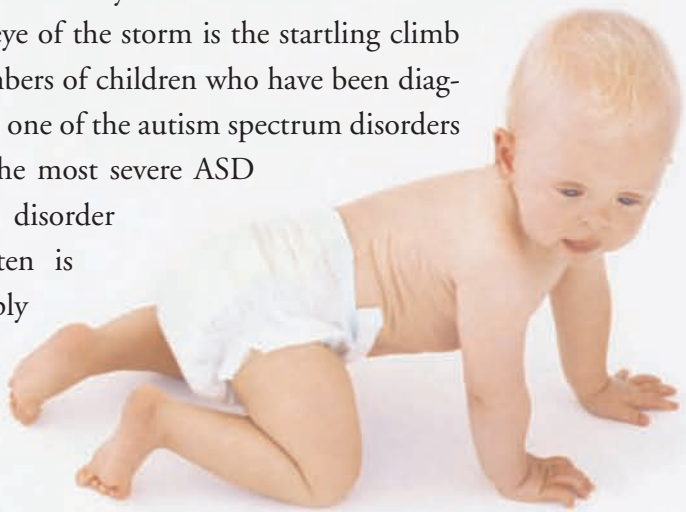
geotrac/iStockphoto

Tracing the Origins of Autism

A Spectrum of New Studies

The etiology of a medical condition might seem an unlikely subject to arouse intense feelings. Yet few medical disorders have stirred up as much passion and divisiveness among scientists and the general public as autism has in recent years. The heat of the controversy has even attracted attention from periodicals such as *The Wall Street Journal*, the *Columbia Journalism Review*, and *Wired* magazine—seemingly improbable forums for a medical debate. Why all the furor?

At the eye of the storm is the startling climb in the numbers of children who have been diagnosed with one of the autism spectrum disorders (ASDs). The most severe ASD is autistic disorder (which often is called simply "autism"); other forms include Asperger syndrome and the much rarer childhood disintegrative disorder. In the United States, the diagnosis of ASDs increased roughly 10-fold over the course of a decade, from 4–5 children per 10,000 in the 1980s to 30–60 children per 10,000 in the 1990s, according to a report in the August 2003 *Journal of Autism and Developmental Disorders*. The 5 May 2006 issue of *Morbidity and Mortality Weekly Report* describes the results of two parent surveys from 2003 and 2004, which suggested that 55–57 children per 10,000 had autism (however, an editorial note points out that, due to the nature of the surveys, parents of children with other ASDs may have reported their children as having autistic disorder).



Getty Images

Some scientists believe that much of the upsurge is the result of increased awareness of ASDs or changes in diagnostic criteria, which would suggest that the true prevalence of the disorders has been stable over time. Others disagree. "It is premature to state that there is no increase in prevalence,"

says W. Ian Lipkin, a professor of neurology, anatomy, and neurobiology at Columbia University. "None of the studies to date has been designed to definitively address the issue."

The prevalence of ASDs plays into the fundamental question of what causes these disorders. If the number of cases is truly on the rise, then it would seem likely that some change in the environment is driving up the total. That's partly what has divided scientists into opposing camps—they cannot agree on the relative importance of genetic and environmental factors in the disorders' etiology.

Alas, answering the prevalence question might not end that debate. "Even if the prevalence of autism were stable," says Lipkin, "you would not be able to rule out the possibility of an environmental trigger." That's because very little is known about the mechanisms that cause autism, be they environmental or genetic.

"The study of autism was, until recently, largely dominated by the field of psychology, where characterizing the behaviors and developing reliable instruments for diagnosis have been major areas of research over the past few decades," says Irva Hertz-Picciotto, an epidemiologist at the University of California, Davis.

Indeed, the core symptoms of ASDs—social disinterest, repetitive and overly focused behavior, and problems in communication, usually appearing before 3 years of age—have been well described. Much less research has focused on the causes of these symptoms.

Several investigations dating back to the 1970s indicate that identical twins have a much higher concordance rate of ASDs than fraternal twins, according to a report in the Spring 1998 issue of *Mental Retardation and Developmental Disabilities Research Reviews*. Those studies provide some of the best evidence that these disorders have a strong genetic component. But the identity of the genes involved, much less how they produce ASDs, has not been established. Moreover, the concordance rate for identical twins is not 100%, which suggests that at least some cases must be associated with environmental or epigenetic factors.

A few cases of ASDs have been clearly linked to environmental insults. These include prenatal exposure to chemical agents such as thalidomide and valproic acid, as well as to infectious agents such as the rubella and influenza viruses. Here again, the concordance rate is not 100%, which suggests that a genetic predisposition is necessary for chemical and microbial factors to act as triggers.

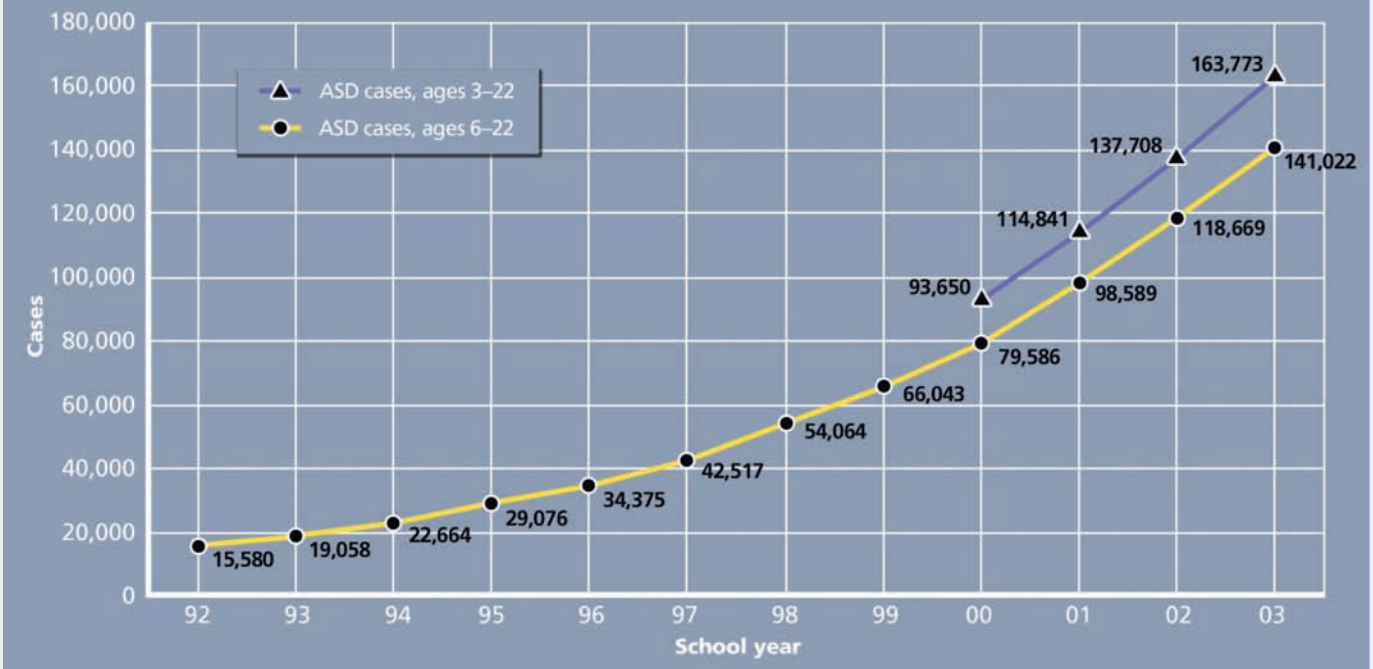
Tantalizing clues like these are prompting scientists to reconsider the research agenda for ASDs. Martha Herbert, a pediatric neurologist at Harvard Medical School, and her colleagues have been applying the methods of genomics to identify environmentally responsive genes that might be important in these disorders.

"When you realize that the widespread changes we're seeing in autistic brains may occur in parallel with or even downstream from widespread changes in the body—such as in the immune system—and that these changes may be environmentally triggered, you start looking for ways to think more broadly about genetic vulnerability. It can't be just about 'brain genes,'" Herbert says.

Some new epidemiological studies also are looking for gene–environment interactions. According to Diana Schendel, an epidemiologist and project officer for autism research at the CDC, which funds one of the projects, these initiatives will be able to examine many possible causal pathways to ASDs, including both genetic and environmental causes that may lead to the development of the disorders in different subgroups of children.

Some of these projects are already under way, whereas others will begin soon. All of the scientists involved, however, believe their research will finally provide some of the answers that everyone has been looking for.

Reported Cases of ASDs in the United States and Outlying Areas



Source: graph—Fighting Autism, http://www.fightingautism.org/idea/autism.php?; data—http://www.IDEAdata.org/; CDC National Center for Health Statistics

CHARGE

The Childhood Autism Risks from Genetics and the Environment (CHARGE) project is unique among the large ASD epidemiological studies. It focuses solely on autistic disorder, and it emphasizes a search for environmental factors—including a broad array of chemicals in food, consumer products, and ambient air, as well as infectious and medical exposures—that might be linked to the disorder. The study is funded by the NIH.

CHARGE is a case–control study in which a group of autistic children aged 2 to 5 years is compared to a group of age-matched controls in a population-based study. "Because of the California Department of Developmental Services' system of Regional Centers [nonprofit corporations that coordinate health care services and support for citizens with developmental disabilities], we have a handle on enumerating a high proportion of the children newly diagnosed with autism in our defined area over a specific time period," says Hertz-Picciotto, the principal investigator of the CHARGE study. "Similarly, we can enumerate the children in the same area and time period who are not cases. We then sample from both."

The project was initiated in 2002 with the goal of recruiting 1,000 to 2,000 children. Half of the children will be autistic. The other half will make up two control groups: one group of children with developmental delays (but not an ASD) and a second group of children selected from the general population without regard to developmental characteristics.

The advantage of the case–control design is that scientists can acquire large numbers of children with the disorder. By comparison, in a cohort design researchers would need a very large sample size, given the prevalence of autism, to acquire the same number of cases.

Hertz-Picciotto expects to have enrolled nearly 700 children by August 2006, the end of the first funding period. "I've applied for another five-year grant," she says, "and I hope to be funded to enroll nine hundred in that round, which would bring us to sixteen hundred children."

The CHARGE team is looking at possible exposures during the prenatal period and early childhood. Some of the data will be gathered through comprehensive interviews with parents, but Hertz-Picciotto admits that this is not the best way to look for exposures. "You ask people questions, and their answers may be colored by the fact that they know they have a child with a condition," she says. "They may spend a lot of time thinking about what they might have done or what might have gone wrong, and they may have preconceived ideas about what caused [the disorder]. They might not be as objective." Such problems with postdiagnosis interview information are recognized as a weakness of retrospective studies.

The scientists are getting around this issue by examining each child's medical records and those of the mother during pregnancy and delivery—nonsubjective data gathered in the course of routine obstetric care. They are also collecting blood, urine, and hair specimens that will be analyzed in the laboratory.

The study has already provided some intriguing leads. "We're finding that the immune system seems to function at a lower level in autism," says Hertz-Picciotto. "That's an important clue. It could mean that whatever causes autism also disrupts the immune system, or it could be that the immune system disrupts neural development so that something goes awry in laying down brain circuitry prenatally or in the early postnatal period." [For more information on the CHARGE study, see p. 1119, this issue.]

ABC

The Autism Birth Cohort (ABC) Study, now under way in Norway, is a large prospective design that is expected to gather information on 100,000 babies. The work is being led by scientists at the Mailman School of Public Health at Columbia University, who are collaborating with colleagues at the Norwegian Institute of Public Health, with funding from the U.S. National Institute of Neurological Disorders and Stroke.

"When you want to know why some people are more at risk than others in a population, then that's best answered using a cohort design," says Ezra Susser, an epidemiologist at Columbia University and a co-investigator on the ABC project. "When we think about environmental causes of [ASDs], we're probably interested in phenomena that occur prior to birth or perhaps shortly after birth. So you want to collect prospective data from people as early as possible in pregnancy." Because ASDs are not common, the study will need large numbers of children to have enough statistical power, according to Susser.

So far the ABC team has recruited 75,000 pregnant Norwegian mothers, but Susser is hoping for more. "We've got enough to look for an environmental risk factor, but you need larger numbers for studying gene–environment interactions, which could turn out to be important," he says. It's possible the team could acquire greater numbers by collaborating with other studies. One candidate for collaboration is the Avon Longitudinal Study of Parents and Children in the United Kingdom, which is looking at the complex ways in which environmental features may relate to optimal development and health in children. But there's been no agreement yet, Susser says.

Even so, the ABC scientists are optimistic about their study. "Little is known about the natural history of [ASDs]," says Lipkin, who is the principal investigator of the project. "By starting prenatally, we're collecting detailed, critical information about environmental exposures in an unbiased fashion."

The scientists are also collecting plasma, serum, RNA, and DNA. "We have extraordinary biological materials," says Lipkin. "We can pursue biomarkers as well as exposure to toxicants and infection. We also have maternal DNA, paternal DNA, and the child's DNA [so-called trio data]; thus we can look for the appearance of novel mutations," he adds.

The ABC researchers will follow the children through time, with parents answering questionnaires about the health and social interactions of their children as they reach 6, 18, and 36 months of age. "It may be that the developmental trajectory tells us much more than a single time point can ever tell us about the pathogenesis of [ASDs]," says Mady Hornig, a physician-scientist at Columbia University who participates in the project.

Despite their enthusiasm for the project's potential, the ABC scientists feel they could accomplish much more if they only had the funding. "The pity of it is we have no money to do the biological work," says Lipkin. "We can collect the samples and do the questionnaires, but we've been unable to get funding to look for any of the environmental factors. We're collecting blood, but we won't know whether there's a biomarker until we do a biomarker analysis. We have funds to collect RNA, but in order to do the transcript profiling we need approximately four hundred dollars per sample," he says.

Lipkin adds that there's only so much that one can do with questionnaire data. "We do ask about infection and diet, but that's not the same as having a lab value that can validate what was reported, and then look at a direct correlation with the outcome," he says.

Lipkin believes that part of the problem is that searching for environmental factors goes against the current research paradigm in ASDs. "The focus is on genetic factors," he says. "Infectious diseases,

toxicology, and immunology receive short shrift. The ABC is clearly the right opportunity to pursue these other leads because we have the ideal samples to survey prenatally and postnatally," he says.

The scientists are just now receiving the responses to the 36-month questionnaire. "It'll probably be another two years before we have our first report," Hornig says. Funds are now in place to study the children at 36 months; however, the team hopes to follow them for a lifetime, according to Hornig.

CADDRE

In response to the Children's Health Act of 2000, the CDC established and funds six Centers for Autism and Developmental Disabilities Research and Epidemiology (CADDRE) to investigate potential risk factors for ASDs. The multisite approach offers a study group that is geographically and demographically more representative of the general U.S. population than a smaller regional study could provide, according to Craig Newschaffer, an epidemiologist and principal investigator at the Johns Hopkins Bloomberg School of Public Health CADDRE site.

According to Newschaffer, the CADDRE sites will use a case cohort design in which the exposure patterns of the ASD cases are compared to a random sample of children living in the same geographic area. A third study group, consisting of neurodevelopmentally impaired children who do not have an ASD, will round out the sample populations. The investigators hope to enroll a total of 650 to 900 children, aged 3 to 5 years, in each study group across all the sites, making CADDRE the largest study of its kind in the United States, says Newschaffer. A uniform protocol across the sites will allow the scientists to pool their data.

CADDRE will collect and archive blood, cheek cell, and hair samples from the children in order to investigate a broad range of potential risk factors. "We're not focused on the environment as much as CHARGE is," says Newschaffer, "but we are collecting data on questionnaires and reviewing medical records on exposure, in addition to the biosampling for exposures."

The scientists should have sufficient numbers to look at gene–environment interactions. "We are collecting DNA from the parents and the kids from each of the groups. We'll have trio data in each of the three groups, a potentially powerful design," says Newschaffer.

Studies of Environmental Factors in Autism Spectrum Disorders (ASDs)

Name of Study (Location)	**Goal**	**Study Size**	**Time Frame**	**Ages Studied**	**Funding Source(s)**
Autism Birth Cohort (Norway)	Investigate prenatal and postnatal environmental exposures that may lead to ASDs	100,000	2004–2008	Gestation–3 years	Columbia University, Norwegian Insititute of Public Health, NINDS
California Autism Twin Study (United States)	Study the behavior and learning styles of children with autism and their twins	300 twin pairs	2004–2009	Not specified	NIMH
Centers for Autism and Developmental Disabilities Research and Epidemiology (United States)	Compare environmental exposure patterns of children with ASDs, neurodevelopmentally impaired children without ASDs, and the general population	2,700	2000–2011 (to date)	3–5 years	NIH
Childhood Autism Risks from Genetics and the Environment (United States)	Investigate prenatal and early childhood environmental exposures that may contribute to ASDs	2,000	2002–2006 (possible 5-year extension)	2–5 years	NIH
Early Markers for Autism (United States)	Analyze maternal and infant blood samples for early biomarkers of ASDs	400	2004–2006	Gestation–3 years	NIMH, National Alliance for Autism Research
Markers for Autism Risk in Babies—Learning Early Signs (United States)	Study prenatal factors that may affect development of ASDs in children with at least one sibling with an ASD	unknown	2006–2011 (planned)	Gestation–unknown	unknown

Key to U.S. Funding Agencies: NIMH—National Institute of Mental Health; NINDS—National Institute of Neurological Disorders and Stroke; NIH—National Institutes of Health

Corbis

CADDRE scientists will also characterize the behavior of the children, as well as describe any comorbid medical conditions and atypical physical features. The goal is to sort out different etiologic subgroups within the autism spectrum. As Newschaffer explains, "There are a lot of possible reasons why we've had a hard time coming up with genetic and nongenetic risk factors. One of them is that autism is likely a heterogeneous condition, with different etiologies producing kids with what appear to be similar phenotypic profiles. If you don't separate out the different etiologic groups, it's going to be very hard to find an association with a gene or an exposure. If we limit our analyses to kids that have a certain profile, we're going to be able to make some informed guesses about what profiles might allow risk factors to emerge," he says. The CADDRE sites will begin recruiting children into the study in the fall of 2006.

More Studies, More Acronyms

There are several other smaller epidemiological studies in the works. In California, scientists are tapping into specimen banks that have stored blood samples taken from mothers during pregnancy and from their children at birth. The Early Markers for Autism (EMA) study employs a case–control design, with about 100 children with an ASD (primarily autism), 100 who are developmentally delayed, and 200 from the general population. "We can correlate what's happening in the mom and the baby, which is really exciting," says Lisa Croen, a perinatal epidemiologist at the Kaiser Permanente Division of Research in California and the project's principal investigator.

EMA is a multidisciplinary collaboration with epidemiologists, geneticists, immunologists, neurovirologists, and endocrinologists, according to Croen. "Because autism is so complex, it's important for all these researchers to communicate with each other. I think EMA is a model for how to do research in autism," she says. EMA is unique, according to Croen, because the study will be looking for biological markers of ASDs very early in development, during gestation, and at birth. "This allows us to focus on mechanisms that may be leading to autism rather than mechanisms that are consequences of having autism," she says.

The EMA scientists are investigating genetic and nongenetic factors, with a focus on the immune dysregulation hypothesis of ASDs. "We're measuring different kinds of immune markers, including immunoglobulin levels and antibodies to specific infectious agents, cytokines, and autoantibodies," says Croen. "We're looking for things that distinguish kids who are subsequently diagnosed with autism from those who aren't. This will help us understand the pathobiology of autism—the mechanisms that are leading to the dysregulation in development."

The three-year EMA is currently in its last year. "We still have lots of analyses to do," says Croen, "but we're beginning to write some papers. We're finding differences between the children in levels of certain proteins measured in the circulating blood collected from mothers during pregnancy. I think the study has much to contribute to our understanding of the biology of what might be going wrong."

Croen is also an investigator on the California Autism Twin Study (CATS), which expects to recruit 300 identical and fraternal twin pairs born between 1987 and 1999 in which at least one of the twins has an ASD. Comparing the twin pairs will allow the scientists to estimate the heritability of ASDs—the relative genetic and environmental contributions to the disorder. "Knowing the behavioral and developmental differences between the twins might help us understand the effects of gene expression, the *in utero* environment, and environmental triggers," Croen says.

Hertz-Picciotto is also excited about a five-year study that she and her colleagues hope to begin soon. Unlike CHARGE, the new effort, called MARBLES (Markers for Autism Risk in Babies—Learning Early Signs), will be a prospective study in which data will be gathered before the children are diagnosed. Pregnant women who already have at least one child with autism will be enrolled right at the beginning of pregnancy. The mothers will keep diaries about their symptoms and health-related events, and the researchers will collect cord blood samples and placentas.

Based on previous research, Hertz-Picciotto expects that about 1 in 10 siblings of the autistic children will also have the disorder, and perhaps 1 in 4 or 5 will be "on spectrum" with a related but less severe condition such as Asperger syndrome, or with some symptoms of the broad behavioral phenotype, such as language delays and atypical social skills. "This work is complementary to the case–control approach, and should provide us with a lot of information that will build on what we find in CHARGE. It should be a phenomenal resource," she says.

You Say You Want a Revolution

In April 2004, the U.S. DHHS issued a publication, *Congressional Appropriations Committee Report on the State of Autism Research*, describing recommendations made by a panel of expert scientists convened by the Interagency Autism Coordinating Committee (IACC). The IACC panel suggested an ambitious agenda, which included the goal of identifying environmental risk factors and their associated developmental windows within a four- to six-year period, as well as identifying genetic and nongenetic causes of ASDs and their interactions within seven to ten years.

Hertz-Picciotto, a member of the IACC panel, thinks these goals should be taken with a grain of salt. "I'm optimistic that we will have identified some environmental risk factors, and may have excluded a few others, between 2008 and 2010—but by no means will we have the final word. The genetics and the gene–environment interactions may be even tougher. Unfortunately, I don't see enough groups working on the environmental contribution to autism, so it may be slower than projected," she says.

Mark Blaxill, vice president of SafeMinds, a parent-led advocacy group, also believes that environmental risk factors don't receive enough consideration. "The CDC has not addressed the crisis in autism responsibly," he says. "They should be raising the alarm, and they have failed to do so. They should be asking why so many children are sick. Instead, they've tried to suggest a degree of doubt about the increases, and that diverts attention and funding from environmental causes."

Schendel responds, "It is clear that more children than ever before are being classified as having an ASD. It is important that we treat common developmental disorders, and especially the ASDs, as conditions of urgent public health concern. The CDC's efforts in addressing this public health concern include funding for ASD monitoring programs to understand ASD trends, funding for research into the genetic and environmental causes of ASDs, and education and outreach programs to promote early identification and timely intervention for all children with developmental problems."

Despite the promise of the new epidemiological studies, some researchers are still dismayed, as one scientist put it, that "geneticists are running the show, and ignoring the environmental aspects." What would it take for things to change? Blaxill invokes the ideas of philosopher Thomas Kuhn, who suggested that scientific revolutions occur when an old paradigm is replaced by a new one. "I believe we're in the middle of a paradigm shift," Blaxill says. "The dramatic explosion of autism rates does not fit the genetic model. It's an anomaly that will kill the old paradigm."

Michael Szpir

Other Major Environmental Health–Related Studies

Name of Study (Location)	**Goal**	**Study Size**	**Time Frame**	**Ages Studied**	**Funding Source(s)**
Agricultural Health Study (United States)	Evaluate the role of agricultural exposures in the development of cancer and other diseases in the farming community	90,000	1993–2008	Children, adults	NCI, NIEHS, EPA
Australian Multi-Centre Study of Environment and Immune Function	Examine how environmental factors influence immune diseases and how immune disorders vary by latitude across Australia	1,000	2003–2008	Teenagers, adults	National Multiple Sclerosis Society (U.S.)
Avon Longitudinal Study of Parents and Children (United Kingdom)	Determine the current problems in child health and development and how they may be prevented	14,000	1991–2010	Infant–early adulthood	UK Medical Research Council, Wellcome Trust, others
Bangladesh Vitamin E and Selenium Trial	Investigate whether vitamin E and/or selenium has a beneficial effect in reducing skin cancers and other types of cancer	4,500	2005–2010	25–65 years	NIH
Diesel Particle Exposure and Lung Cancer (United States)	Assess the association between exposure to diesel exhaust and lung cancer mortality	55,750	2001–2007	Adults	NCI
French Longitudinal Study of Children	Describe child growth at different ages, assess levels of exposure to the main environmental pollutants, and analyze the links between exposure and public health	20,000	2005–undetermined	Birth–adulthood	French government, others
GABRIEL—A Multidisciplinary Study to Identify the Genetic and Environmental Causes of Asthma in the European Community	Examine the roles of genetic and environmental factors influencing the development of asthma	40,000	2006–2009	Children, adults	European Commission
Gene–Environment Interactions in Facial Clefts (Denmark, Norway)	Use advances in molecular technologies to provide a new level of understanding for a complex birth defect trait	200,000	1998–2007	Infants	NIDCR
Genetic and Environmental Influences on Childhood Growth (Nepal)	Elucidate the roles of genetic and environmental factors influencing childhood growth and development	900	2002–2007	3–18 years	NICHD
Health Effects of Arsenic Longitudinal Study (Bangladesh)	Prospectively examine the health effects of arsenic among a population chronically exposed to the chemical through contaminated drinking water	15,000	2000–2011	18–75 years	NIH
Longitudinal Study of Australian Children	Assess emerging health and developmental concerns and their determinants in children	10,000	2003–2009	Infant–12 years	Australian government
National Children's Study (United States)	Examine the effects of environmental influences on the health and development of children	100,000	2000–2006 (funding discontinued after 2007)	Gestation–21 years	NICHD, NIEHS, EPA, CDC
NewGeneris (European Union)	Investigate exposure to chemicals in food and the environment and their connection with childhood cancer and immune disorders	600,000	2006–2001	Birth–7 years	European Community
Swiss Study on Childhood Allergy and Respiratory Symptoms with Respect to Air Pollution, Climate, and Pollen	Investigate the association between long-term exposure to air pollution and respiratory health and allergies in children	17,846	1997–2008	6–14 years	Swiss government
Singapore Cohort Study of Diet and Cancer/Singapore Chinese Health Study	Elucidate the role of diet and its interaction with genetic factors in the causation of human cancer	63,257	1999–2010	45–74 years	NCI, NIEHS
Sister Study (United States)	Learn how the environment and genetics affect the chances of getting breast cancer	50,000	2003–2013	35–74 years	NIEHS
Southern Community Cohort Study (United States)	Gain new information about the causes of cancer, heart disease, and other common illnesses	100,000	2002–2007	40–79 years	NCI
The Environmental Determinants of Diabetes in the Young (United States, Finland, Germany, Sweden)	Identify infectious agents, dietary factors, or other environmental agents, including psychosocial factors, that trigger type 1 diabetes mellitus	7,092	2004–2023	Infant–15 years	NIDDK, NIAID, NICHD, NIEHS, CDC, JDRF

Key to U.S. Funding Agencies: CDC—Centers for Disease Control and Prevention; EPA—Environmental Protection Agency; JDRF—Juvenile Diabetes Research Foundation; NCI—National Cancer Institute; NIAID—National Institute of Allergy and Infectious Diseases; NICHD—National Institute of Child Health and Human Development; NIDCR—National Institute of Dental and Craniofacial Research; NIDDK—National Institute of Diabetes and Digestive and Kidney Diseases; NIEHS—National Institute of Environmental Health Sciences; NIH—National Institutes of Health

TRI

Corroding Its Original Intent?

If knowledge is power, as the proverb goes, then the EPA's Toxics Release Inventory (TRI) is a powerful tool indeed. Firefighters and first responders used this nearly 20-year-old public database of toxic chemical emissions to identify potential contamination hot spots after the floods of Hurricane Katrina. Residents have used it to find out what kinds of pollutants are being emitted by nearby industries. Investment companies use it to evaluate whether or not to purchase a company's stocks. Even the Internal Revenue Service uses it to collect a pollution tax from companies that release ozone-damaging chlorofluorocarbons.

Given the TRI's extensive use, it should come as no surprise that an EPA proposal to streamline TRI regulations for the 23,000-plus facilities that report under the law has proved highly controversial. The EPA's plan must be reported, a move that critics say would affect the value of the TRI database for the public at large. But proponents argue that the cost savings that businesses would realize from the relief in paperwork would justify any loss of data.

The arguments matter, because the power of the TRI lies in the information it provides. Authorized by the 1986 Emergency Planning and Community Right-to-Know Act, the TRI doesn't limit emissions of the more than 650 chemicals it now covers, but merely requires that they be reported by the companies that manufacture, use, or process them. However, when residents find out what is discharged by industries in their neighborhoods, they can and have used the facts to force change.

Matt Ray/EHP

Companies have altered their practices when managers see their facilities top the list for particular chemical discharges. In fact, the myriad of uses of the TRI, and its success in influencing business practices, has surprised both supporters and opponents of the original law [see "Now That You Know," *EHP* 105:38–43 (1997)].

Between 1998 and 2004, the latest year for which data are available, the industries and federal facilities that report TRI data have voluntarily cut total on- and offsite disposal and other releases of TRI chemicals to the air, water, and land by 45%, or some 3 billion pounds. Since 1988, industries have cut releases of the 299 chemicals covered by the original law by nearly 60%. Because the TRI is so different from traditional end-of-pipe regulatory programs, which put limits on how much pollution can be released, it has drawn widespread praise. "Any program that the States, the Sierra Club and Monsanto can all praise is no doubt a true environmental success story," wrote 12 state attorneys general in their comments on the proposed TRI changes.

117,000 Comments and Counting

The proposed changes would increase from 500 pounds to 5,000 pounds the threshold at which facilities would be allowed to use a brief certification form (Form A) instead of a detailed reporting form (Form R) to report on their toxic chemical waste. This threshold is based on the amount of chemical wastes handled by the facility, not the amount released to the environment. Form R requires a complete accounting of a chemical's fate—the amount on the site; the amount released to the land, air, or water as emissions; the amount recycled or burned for energy recovery or destruction; and the amount shipped from the plant for treatment or disposal. In contrast, Form A simply certifies that a toxic chemical was used at the facility in at least the regulatory threshold amount, but provides no other details.

The EPA's plan also contains changes regarding a special subset of 20 chemicals and chemical compounds including mercury, lead, and polycyclic aromatic compounds. Previously, none of these "persistent, bioaccumulative, and toxic" (PBT) chemicals could be reported on Form A. Under the new rule, however, a company may file Form A for PBTs if 500 pounds or less is recycled, used for energy recovery, or treated for destruction. If any amount is released or emitted, however, the company must still use the detailed form. Furthermore, dioxins must still always be reported on the detailed form.

Toxic tradeoff? Changes to the TRI would mean less paperwork for companies but also less information for the public.

In a separate filing, the EPA notified Congress that it is considering changing the frequency of TRI reporting from yearly to every other year. Even though there has been considerable response to this third proposal, there has been little substantive debate. Federal law requires the EPA to warn Congress a year before beginning rule making on TRI reporting frequency, so the agency is still developing the details for this proposal.

When the EPA's proposed threshold and PBT changes were published in the 4 October 2005 issue of the *Federal Register*, they unleashed a flood of responses—some 70,000 responses by the 13 January 2006 deadline for public comments. Even after the deadline passed, the response didn't stop; more than 117,000 comments have been filed with the federal agency to date.

Twelve state attorneys general have called on the EPA to abandon the proposal, and a half-dozen U.S. senators and more than 50 U.S. representatives have also written the agency to question the assumptions of the plan. Recalling the TRI's genesis in the aftermath of the 1984 Bhopal industrial disaster, Representatives Stephen Lynch (D–MA), Henry Waxman (D–CA), and Dennis Kucinich (D–OH) wrote that the plan "is particularly troubling" in view of a recent petrochemical plant explosion in China that ultimately polluted the drinking water supply for millions of people. The congressmen noted that the EPA's own analysis showed that allowing industries to use the higher threshold of 5,000 pounds for Form A would allow companies nationwide to release a total of 246,092 pounds of benzene—without reporting the release.

Industry and small business community representatives have countered, however, that the EPA's proposals meet the intent of the law while saving companies time and money (the TRI already has a small business exemption that allows facilities with fewer than 10 employees—including farms, dry cleaners, and others—to completely skip reporting and data collection). The U.S. Small Business Administration's Office of Advocacy has been among the most vocal proponents for the changes, arguing that the expanded use of Form A is exactly the kind of incentive that will encourage good waste management.

"The current program does not reward the best environmental performers," says Kevin Bromberg, assistant chief counsel for environmental policy at the Office of Advocacy. "Under the current system, if you run a facility with perfect chemical management techniques and discharge no highly toxic chemicals, you must still fill out the long Form R. Small businesses that are top environmental performers should be rewarded through less paperwork—the short Form A."

David Meharey/iStockphoto

Saving Money, Same Data?

A change in reporting thresholds clearly changes the amount of detail available from the TRI; the question is how this change affects the utility of the inventory. For example, the EPA has stated that none of the detailed data now reported for 26 chemicals or chemical classes (such as chromium compounds) would be available under the proposed 5,000-pound limit for non-PBT chemicals. Most of the chemicals for which detailed reporting would be lost are pesticides.

But the EPA claims that Form A reports will remain meaningful because the public will still know that the chemical is present at a facility at levels under the proposed thresholds. "The Form A certifications for these chemicals will provide a range by which waste management quantities and practices may be estimated," the agency wrote in its proposal.

All told, the EPA estimates that the two threshold changes for Form A would save companies a combined total of about 164,000 hours a year and about $7.4 million in filing costs. The EPA's economic analysis estimates the annual savings at the facility level for each form avoided is approximately $430 for each non-PBT chemical and $790 for each PBT chemical—or between $2 and $4 per day. This savings would come at a loss of detailed information on more than 12,000 releases and disposals of chemicals around the country, which total 14 million pounds of non-PBT chemicals released to the environment—just 0.34% of the total amount released. Given the PBT chemical exception, however, the EPA proposal permits no loss of such information for releases of those chemicals into the environment.

These savings free up environmental managers to focus on solving problems instead of filling out forms, according to Jeff Gunnulfsen, manager of government relations for the Synthetic Organic Chemical Manufacturers Association, a trade group that supports the changes. "Most of our members may have one regulatory person handling many, many issues such as hazardous waste, TRI, air issues, safety, and FDA, so any burden reduction may help them focus on more pressing matters," Gunnulfsen says.

Still, official comments filed by several companies suggest that not everyone in the business world thinks the changes will save time or money. Under the law, companies must still track the same information and make the same calculations, even if they end up filing the short form. The company must be able to demonstrate to the EPA, if ever called upon, that they know their forms to be correct.

Indeed, in comments submitted in response to the *Federal Register* notice, Mark Herwig of GE Corporate Environmental Programs wrote, "An analysis of TRI data from 2003 suggests that EPA's estimated burden reduction resulting from the proposed rule could be overstated by over 50% for all facilities. . . . There are several areas of EPA's burden analysis that need improvement to accurately characterize TRI reporting burden." According to a fact sheet compiled by OMB Watch, a nonprofit government watchdog group, many other corporations have expressed similar feelings.

Sean Moulton, director of federal information policy for OMB Watch, says communities lose even if just a small percentage of the total data is lost. For example, because mining and electric utilities report extremely large emissions to the TRI, "they swamp everything," Moulton says. "In comparison to national totals, releases in Delaware may look small. But if you live in Delaware and are looking for what might affect me and my family, then Delaware is huge." He adds that many of the chemicals tracked under the TRI—such as arsenic and benzene—are dangerous even in small quantities. So focusing strictly on the relative low number of pounds lost may be a poor measure of the situation.

Mike Flynn, director of the EPA's Office of Information Analysis and Access within the Office of Environmental Information, which oversees the TRI, says the effect of the changes on communities is an issue the agency takes very seriously.

"The goal is to provide information for communities—that is an important central tenet," Flynn says. But 99% of the data would still be available, he adds, and data losses would be offset by the "clear benefits in providing incentives for these companies to cut their emissions more. This is one of the issues where we have to find the right balance."

State Program Effects

Some states have reacted strongly to the EPA proposal, partly because their pollution prevention and monitoring programs rely on the data provided by facilities for the TRI.

For example, in Washington state, if the 5,000-pound threshold is implemented for non-PBT chemicals, 40% of all chemicals now filed on Form R could be reported on Form A, which would include a loss of detail about the fate of 46,000 pounds of carcinogens, says Idell Hansen, TRI coordinator for the Washington State Department of Ecology. "We will only have the name of the chemical and the location of the facility, and we'll lose all ability to track that chemical," she says. "Under the proposed rule, we'd lose all information on eight of the top forty facilities with the greatest relative risk based on 2002 [TRI] data," including data on some of the highest-risk chemicals such as methyl isocyanate—the chemical behind the Bhopal incident.

An analysis by the nonprofit National Environmental Trust showed that roughly 900 zip codes nationwide—10% of those that are home to a TRI reporting facility—would lose all numerical toxic emissions data. The New York State Attorney General's office explored the impacts of this loss on 45,000 residents in Tonawanda, New York, a Lake Erie community surrounded by several industrial facilities. According to that analysis, changed thresholds would mean that this one community would be subject to unreported releases of 8,100 pounds of neurotoxic chemicals and 3,100 pounds of chemicals that cause respiratory problems, among other releases.

Jessica Emond, an EPA spokeswoman, says it is important to realize that even if a chemical release is not reported to the TRI, the release is almost always regulated by other environmental laws that protect air and water quality (although Moulton points out these limits frequently apply to only a single medium, such as just water or just air, leaving a loophole for releases to other media). "The EPA sets a high bar for companies," Emond says. "Even with proposed changes, this doesn't affect the amount of chemicals that a company would be allowed to release under state and federal laws."

The EPA's timetable calls for finalizing its proposed rule changes by December 2006. However, congressional action before then might preempt the agency's rule making. Three U.S. senators have asked the Government Accountability Office to examine the EPA's proposal. Additionally, in mid-May the House of Representatives approved an amendment to the Interior Appropriations Bill that would prevent the EPA from spending money to finalize the proposal until October 2008. The fate of that amendment will be decided in conference committee later this year.

Nancy Bazilchuk

Greg Allgood/Procter & Gamble

Through a glass clearly. A Maasai woman in Kenya holds glasses of polluted water and water treated with a new method to remove contaminants.

A Clear Solution for Dirty Water

Turning water into wine may be among the most venerable of miracles, but for Greg Allgood, the real miracle has been turning dirty water into drinkable water. He once wowed an audience in a Malawi village, where hundreds of inhabitants along with the country's Minister of Health watched him transform a sample of the only local source of drinking water. "There were gasps of excitement when the water turned from this horrible, muddy dark color to crystal clear and safe," he recalls.

Allgood was demonstrating PUR™, a modest-looking packet of powder that quickly turns turbid, health-threatening water into the kind of liquid most of us would pay to drink out of a bottle. PUR was developed in the late 1990s by household products giant Procter & Gamble (P&G) and shares its name—but not its technology—with home tap water filters sold by that company in developed nations. Now PUR occupies a place at the forefront of P&G's Children's Safe Drinking Water Program, a philanthropic initiative that Allgood directs.

Allgood spends about a third of his time in places like Malawi where people have limited or no access to treated, potable water sources. Worldwide, as many as 2 billion people drink water extracted from shallow wells or polluted lakes and rivers, with nothing like the municipal treatment systems that are taken for granted

in most of North America and Europe. In the few developing locales where such infrastructure might exist—and indeed, even in the richest nations on the planet—this resource can be ruined suddenly by a natural disaster like a hurricane, earthquake, or tsunami, creating an immediate, desperate, and widespread need for safe drinking water.

The Stuff of Life

Water can be the key to keeping death and disease at bay. Hydration is fundamental to bodily functions, including the ability to retain nutrients. Infants, the elderly, and immunocompromised persons are especially vulnerable to dehydration caused by diarrhea, which is in turn spawned by bacteria or viruses acquired from tainted drinking water. In African countries ravaged by HIV/AIDS, large portions of the adult population could likewise succumb to even limited numbers of parasites found in relatively clean water. "While [a healthy person] might take a couple of weeks to get over *Giardia*, it could be fatal to a person that has a reduced immune system," says Allgood. As opposed to dealing with these ailments once they appear, purifying water can keep them from appearing at all.

The CDC became interested in point-of-use treatment when cholera exploded in Peru in 1991 and spread rapidly throughout Latin America. A dependence on questionable drinking water lay at the heart of this epidemic, and the Pan American Health Organization estimated that it would take some $200 billion and more than a decade to install the necessary municipal infrastructure to alleviate the problem throughout the region. The CDC sought alternatives to help affected populations in the meantime.

Chlorine bleach was among the most widely available disinfectants, although people had difficulty gauging how much was needed to treat a given amount of water without creating an unpleasant taste or harmful concentrations. The agency therefore supported development of special bottles of dilute bleach—the bottle caps were designed to hold just the right amount of solution to safely treat one jerry can of water.

These efforts caught the attention of P&G, the leading manufacturer of bleach in many of the affected countries. But while this approach continues to be used in many parts of the world, it does not remove suspended material from the water, leaving users with water that is microbe-free but can still look dirty. So in the mid-1990s, P&G struck a formal Cooperative Research and Development Agreement with the CDC, focusing on how drinking water could be even better treated at the point of use.

Flocking Toward Solutions

P&G researchers tackled the challenge with flocculants, agents that promote molecular aggregation and can cause colloids or loose particles in a liquid to amass in clumps that sink to the bottom. Combined with large-particle calcium hypochlorite—essentially, powdered bleach—the result was PUR, a proprietary formulation that Allgood describes as reverse-engineering the municipal water treatment process.

Using PUR is like making a batch of powdered soft drink mix. Each packet of powder is designed to treat 10 liters of water. One simply tears open the packet, pours the powder directly into the water, and stirs. Within a matter of seconds, any floating material will start to flocculate into clumps that sink to the bottom. In no more than five minutes, all of the water is clear, and after standing for about 20 minutes, it will be completely disinfected. If desired, the solid remnants can be removed with the most basic of filters, such as a simple piece of cloth.

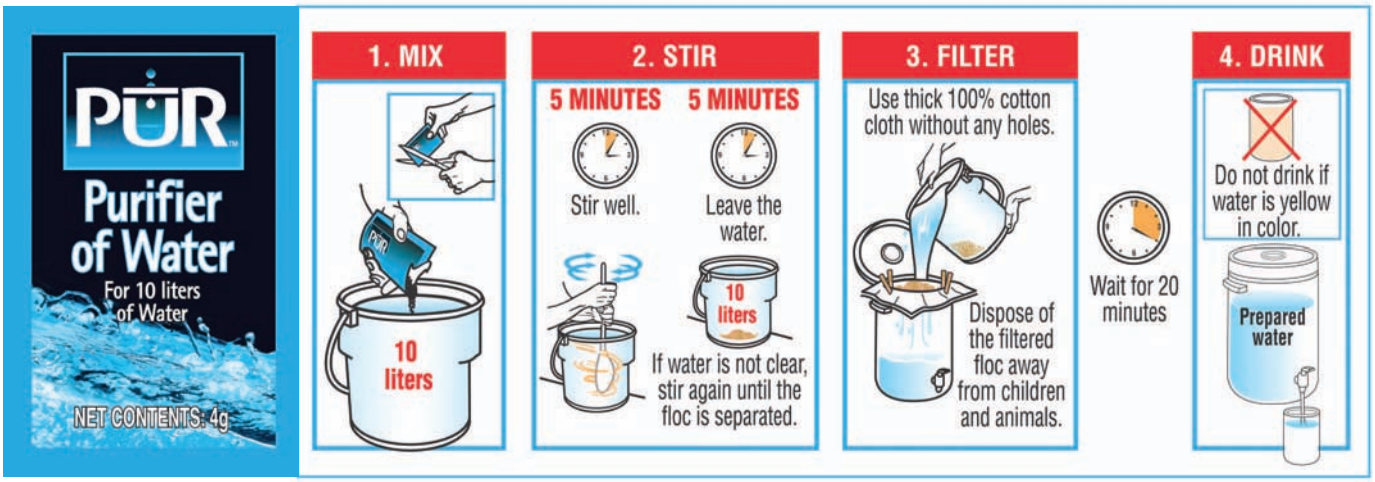
"The large particle size makes [the powder] slowly dissolve, so in essence it acts like a time-released formula of chlorine disinfectant," Allgood says. "That's important, because this product is meant to treat a huge range of waters, from clear to extremely contaminated."

Even seasoned observers, including the scientists who initially refined and tested PUR, agree that its action is nothing less than dramatic.

"It was extremely impressive, and the most impressive thing about it was its simplicity," notes John Perry, a microbiologist at Freeman Hospital in Newcastle upon Tyne, United Kingdom. He and his colleagues spent two years working closely with P&G, putting PUR through its paces in the laboratory.

"We would take a bucket of clean water and contaminate it with all sorts of things—lots of different types of bacteria, but also viruses, protozoan cysts, and they'd also put a lot of soil in it to mimic the kind of conditions that you get in the field," Perry says. "We did a very detailed analysis of what came out at the end of the process, and all of these bacteria, viruses, and cysts had magically disappeared."

These results were recounted in a paper coauthored by Perry that appeared in the June 2003 issue of the *Journal of Water and Health*. Other investigators have also published findings from applications of PUR in various settings, ranging from ongoing rural development activities in Kenya and Guatemala to crises like that in Haiti following Tropical Storm



Procter & Gamble

Learning the value of health. Greg Allgood (left), developer of the PUR powder, watches as a Haitian schoolchild samples purified water as part of a school outreach program of P&G. The company will invest more than $1 million over the next two years in providing safe drinking water in Haiti's schools and clinics.

Jamie Ciesla/PSI

Jeanne in September 2004. Just a few months after Jeanne struck, various aid agencies purchased 13 million packets of PUR and transported them to parts of Sri Lanka, Indonesia, and the Maldives when they were struck by the great tsunami of December 2004.

One Option of Many

In addition to its humanitarian value in disaster relief, the product is also being marketed as a household commodity in many other parts of the world where large portions of the population lack reliable water treatment. The pricing of such a good varies widely from one market to another, based on what the local market will be thought to bear. Sally Cowal, a senior vice president with the Washington, DC–based nonprofit firm Population Services International (PSI), oversees the complex dynamics of advertising and selling PUR in different countries.

"Because we're in social marketing, we have a great belief that if you pay for something, you're much more likely to use it than if it's handed to you," she says. Of PSI's alliance with P&G, she says, "We're learning a lot from one another. They don't know particularly well how to reach the bottom of the pyramid in the countries we work in; that's what we know really well. But they know things about brands and brand management and sophisticated marketing and sales techniques that we [can] learn from them."

Neither of these organizations present PUR as a single, definitive answer to water treatment under any and all circumstances. Eric Mintz, chief of the Diarrheal Diseases Epidemiology Section of the CDC's Foodborne and Diarrheal Diseases Branch, points out that dilute bleach, membrane filters, and solar (ultraviolet) disinfection each have their appropriate niche.

"We think those all have a place, and they all have advantages and disadvantages," Mintz says. "Allowing people to choose from different options is also good." He notes that using PUR can be somewhat more expensive and cumbersome than other methods. For example, although the 13¢ needed to buy a packet of PUR in the Dominican Republic sounds cheap, this may be much more on a per-liter basis than a family would pay for the CDC's dilute bleach treatment. Plus, the PUR system requires more components—two containers, a stirrer, a filter—than most other systems. The optimal option, Mintz adds, is undoubtedly the kind of built infrastructure found in the developed world.

But Steve Luby, who heads up the CDC's work in Bangladesh, observes that much of the developing world has waited four or five decades for permanent water treatment systems to arrive. He argues that too many lives are at risk for measures such as PUR to be ignored.

"The numbers [of people at risk] are just huge, and if we wait to build infrastructure we'll lose a generation," he says. "We can do something good here, and it also gets people understanding the importance of water and the importance of *clean* water, and the need to actually invest in making water clean. We view this as a step toward community empowerment, toward central infrastructure solutions."

Tim Lougheed

Suggested Reading

Allgood G. Children's Safe Drinking Water: Notes from the Front Line [weblog]. Available: http://childrensafedrinkingwater.typepad.com/pgsafewater/.

Luby SP, Agboatwalla M, Painter J, Altaf A, Billhimer W, Keswick B, et al. 2006. Combining drinking water treatment and hand washing for diarrhoea prevention, a cluster randomised controlled trial. Trop Med Int Health 11(4):479–489.

P&G Health Sciences Institute. Safe Drinking Water [website]. Available: http://www.pghsi.com/safewater/.

Souter PF, Cruickshank GD, Tankerville MZ, Keswick BH, Ellis BD, Langworthy DE, et al. 2003. Evaluation of a new water treatment for point-of-use household applications to remove microorganisms and arsenic from drinking water. J Water Health 1(2):73–84.

Near and Not-So-Dear TRI Facilities

Prenatal Proximity and Later Brain Cancer

The most clearly established environmental risk factor for childhood brain cancer is therapeutic radiation exposure (not including diagnostic X-rays). New research now suggests that children of mothers who lived near an EPA Toxics Release Inventory (TRI) facility while pregnant may be more likely to later develop brain cancer, especially if the site released carcinogens [***EHP* 114:1113–1118; Choi et al.**].

Prenatal exposure to chemicals can have profound long-term effects, as some toxic chemicals that are stopped by the blood–brain barrier in adults may reach the fetus via the placenta. This work is the first to specifically examine brain cancer risk in children and potential exposure to TRI releases, although some previous research has suggested slight increases in risk for certain birth defects associated with such emissions.

Of the more than 650 toxic chemicals listed in the TRI, 193 are known or suspected carcinogens, according to the EPA. Fifty-five known, probable, or possible carcinogens were actually released within 2 miles of the study participants. However, it is very difficult to accurately assess exposure to TRI releases. The TRI itself shows only the type and mass of chemicals released in a given year, not where the chemicals went or precisely when they were released. Because of the uncertainty built into using these data, studies such as this must be interpreted with caution.

The study included 382 children diagnosed with brain cancer before age 10 and an equal number of cancer-free controls analyzed as pairs. Mothers of children whose brain cancer was diagnosed before age 10 years were nearly 50% more likely to have lived within 1 mile of such a site during pregnancy; the likelihood was nearly 75% higher for children diagnosed before age 5. However, when looking at risk for two major childhood brain cancer types in particular, astrocytoma and primitive neuroectodermal tumors, there was no difference.

Location equation. New data point to a mother's residence near a Toxics Release Inventory facility while pregnant as a possible factor in brain cancer risk in children.

The team used EPA Region III's chronic toxicity index, which combines total mass released with toxicity factors including carcinogenic weight of evidence and cancer potency factors. For this study, inhalation and oral cancer potency factors were included. Other potential factors, such as mothers' exposures in the workplace during pregnancy, children's postnatal exposure, and exposure through contaminated drinking water, were not taken into account. The authors therefore caution that their results are not conclusive, but should be replicated and expanded using improved exposure measures. **–Valerie J. Brown**

A Killer Smell

Mold Toxin Destroys Olfactory Cells in Mice

Mold seems ubiquitous: it permeates spaces made damp by leaking water lines, faulty roofs, or storm flooding. Although no one contests that its slimy presence is a general nuisance, its related adverse health effects have been the subject of some controversy. Now researchers at Michigan State University's Center for Integrative Toxicology have found that a toxin produced by the black mold *Stachybotrys chartarum* can damage nerve cells key to the sense of smell, at least in the noses of mice [***EHP* 114:1099–1107; Islam et al.**]. The study is the first to probe how inhaling black mold toxins affects nasal passages.

Other researchers have previously reported links between *S. chartarum* exposure and human health effects including upper and lower respiratory illnesses. There is also evidence of an association between exposure to fungi in a damp indoor environment and effects such as asthma symptoms in sensitive individuals. However, in a recent Institute of Medicine report, a panel of experts concluded that there is limited or insufficient evidence to determine whether an association exists for other suggested health outcomes such as chronic obstructive pulmonary disease, neuropsychiatric symptoms, skin symptoms, and immune diseases.

Eau de *Stachybotrys*. The mold's toxin kills olfactory neurons.

The Michigan team found that a single low dose of satratoxin G administered directly into the noses of mice selectively killed sensory neurons involved in detecting odors and sending signals to the olfactory bulbs in the brain. Satratoxins are a type of mycotoxin found in the spores and other parts of *S. chartarum*. The toxins killed the olfactory neurons by apoptosis while apparently leaving bystander cells unharmed. The mice that inhaled the fungal toxins also developed inflammation of the nasal passages and rhinitis ("runny nose" symptoms), as well as milder inflammation of the olfactory bulbs.

It is still unclear how these findings apply to humans exposed to molds. Moreover, before broader health impacts may be assessed, both the amounts of mycotoxins in the air and the nature of human exposure need to be better understood, as do the effects of mold toxins on humans' sense of smell and nasal inflammation. On first examination, however, these mouse studies suggest that exposure to airborne mold toxins may adversely affect people's ability to smell. At a minimum, the study raises new questions about the hazards of exposure to black mold in water-damaged buildings. **–Julie Wakefield**

Top to bottom: Hal Smith/AP; unknown

Potential Immunotoxic Effect of Thimerosal

Compound Alters Dendritic Cell Response *in Vitro*

Thimerosal, an ethylmercury-based compound used for decades as a vaccine preservative, has previously been linked to neurotoxic effects. New research reveals that it may also affect the immune system by altering how dendritic cells respond to biochemical signals [***EHP* 114:1083–1091; Goth et al.**].

Dendritic cells are influential primary actors in the immune system's response to infectious invasion of the body. Once activated, a single dendritic cell can direct hundreds of T cells against an infectious agent. This ability, however, depends on the dendritic cell responding appropriately to signals.

Previous studies by other researchers have indicated that thimerosal is an immunotoxicant, but its specific targets were unknown. Hypothesizing that dendritic cells might be sensitive targets, the researchers cultured bone marrow–derived dendritic cells from mice and assayed how both mature and immature cells responded to activation following treatment with thimerosal. They especially focused on the responses of inositol 1,4,5-trisphosphate and ryanodine receptors (IP_3R and RyR, respectively), which are known thimerosal targets. These gatekeepers of intracellular calcium stores are essential for signaling activities affecting dendritic cell function and maturation.

The team showed for the first time that both mature and immature dendritic cells express isoforms of these receptors, IP_3R1 and RyR1. Upon activation with the cellular energy source adenosine triphosphate, immature control cells responded with a measurable rise and fall in intracellular calcium concentration that involved RyR1 building upon the initial IP_3R1-controlled calcium release and afterward working with IP_3R1 to bring calcium down to resting levels.

Exposure to thimerosal at concentrations as low as 20 ppb altered the time course of these responses, however, and prolonged the length of time that intracellular calcium levels remained elevated. One possible consequence of these sustained calcium levels is a change in the rate and timing of dendritic cells' secretion of interleukin-6, a chemical that triggers further immune system action. Exposure to thimerosal at concentrations above 200 ppb caused immature dendritic cells to die.

The continuing use of thimerosal in some vaccines and other products warrants further investigation of possible immunotoxic effects of this compound and its constituent ethylmercury. The researchers also note that the human *RyR1* gene is highly polymorphic, an observation that raises several questions about the role of RyR1 in the immune system's genetic vulnerability to mercury. **–Julia R. Barrett**

Remember *Pfiesteria*?

Occupational Exposure Unlikely to Cause Cognitive Effects

Case reports have suggested that exposure to the dinoflagellate *Pfiesteria* may contribute to deficits in human learning and memory. Until now, however, there has been no clear, objective documentation of health effects associated with regular occupational exposure to this organism. The results of the first systematic, multiyear study of *Pfiesteria*'s human health effects now demonstrate that commercial fishermen ("watermen") likely do not face significant health risks from routine occupational exposure to the organism [***EHP* 114:1038–1043; Morris et al.**].

Pfiesteria is a common inhabitant of estuarine waters in the U.S. mid-Atlantic region in the summer and fall. In 1997, watermen working along the Pocomoke River, an estuary off Chesapeake Bay, experienced a pattern of neuropsychological deficits in association with fish kills linked to *Pfiesteria* outbreaks. Researchers studying *Pfiesteria* in a lab environment had reported similar memory and learning deficits.

Using a cohort of 88 healthy watermen with regular occupational exposure to Chesapeake Bay waters and 19 controls with minimal contact to the waters (matched to the watermen by zip code, age, and educational level), a team of Maryland researchers collected data over four summers, from 1999 through 2002. They questioned the subjects biweekly about symptoms like those reported in the 1997 episode and about their exposure to the waters and to known chemical toxicants. Subjects were tested at the beginning and end of each summer season on sensory and motor functions, attention and concentration, memory, visual functions, and verbal functions. In addition, the research team analyzed more than 3,500 water samples taken from Chesapeake Bay to monitor the presence of *Pfiesteria* and other harmful species.

Safe from *Pfiesteria*. New data suggest commercial fishermen need not fear routine exposure to the organism.

Photodisc

P. piscicida was found in water samples drawn from a number of locations in all four years of the study, and *P. shumwayae* (recently renamed *Pseudopfiesteria shumwayae*) was found in the last two years. However, the investigators found no decline in neurological function among the watermen in any year of the study.

The scientists note that unique, isolated instances of *Pfiesteria* outbreaks or unusually toxic strains of the dinoflagellate may have been associated with the marked, reversible health effects documented in the past. They point out that the present study is congruent with similar studies in North Carolina and Virginia in providing reassurance that in the absence of these conditions, watermen do not appear to face significant health risks from routine occupational exposure to estuarine waters that contain *Pfiesteria*. **–Tanya Tillett**