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ENVIRONMENTAL HEALTH PERSPECTIVES

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Happening Vocab: Genetics

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STUDENT EDITION

If we are going to live so intimately with these chemicals—eating and drinking them, taking them into the very marrow of our bones—we had better know something about their power.
Rachel Carson
Silent Spring (1962)

CANCER

Save Our (Young) Skins!

Traditionally an adult disease, melanoma—the deadliest form of skin cancer—is on the rise in both children and adults around the world. In the United States, the overall rate of increase across the population was 2.8% per year between 1981 and 2001, according to data from the National Cancer Institute's Survey of Epidemiology and End Results. People under age 20, a group in which melanoma is rare, have faced an overall 1.1% annual increase in disease incidence over the same period. But the rate among 10- to 24-year-olds has increased by 3.0%, according to research in the 20 July 2005 issue of the *Journal of Clinical Oncology*.

Julie Lange, an assistant professor of surgery and oncology at Johns Hopkins University School of Medicine in Baltimore, says, "Part of the apparent rise may be that cases ten or twenty years ago were not as likely to be reported to a tumor registry." Reporting is more complete today, she says, and in some areas outpatient cases are now routinely reported along with inpatient cases. Improved reporting methods are not the whole story, though. "The incidence probably truly is increasing—it's a fairly consistent finding," Lange says.

Melanoma in children occurs so rarely that annual rate increases are measured in fractions of cases per million, so relatively small numbers of new cases can produce substantial percent changes in incidence. "From a public health burden point of view, saying it has increased from five cases to six cases per million children over a decade is more appropriate," explains Ahmedin Jemal, the American Cancer Society's program director for cancer occurrence.

The picture across the full human population is complex. "What we are seeing in adults, at least in Australia, is that amongst the older generation, their rates of melanoma are still climbing. We're seeing the effects of their sun exposure fifty, sixty, seventy years ago," says David Whiteman, a senior research fellow at the Queensland Institute of Medical Research in Brisbane, Australia. Increased attention to sun exposure seems to be working. "Amongst the younger [adult] cohort—the under-fifties and particularly the under-forties and younger—we're seeing that their rates of melanoma are not as high as previous birth cohorts at the same age."

Sun exposure and experience of blistering sunburns have been identified as important risk factors for adult melanoma. "Because we believe that UV exposure increases melanoma risk in adults, we are assuming that the same is true for children—whether there are other important factors for kids today, no one knows," Lange says.

Burning youth. Despite better awareness of the risks of sun exposure, melanoma is climbing among young people, a heretofore largely unaffected group.

Whiteman's group did a case–control study of childhood melanoma in Queensland in the 1990s to look for other such factors. "We were very interested in . . . exposure to pesticides, exposure to other chemicals, other environmental factors," he says, "but we really found no differences in [those] exposures." The group did find, however, that children with melanoma had more large noncancerous moles, heavier facial freckling, and less ability to tan compared to children without melanoma; they were also more likely to have a family history of the disease. These findings appeared in the January 1997 issue of the *International Journal of Cancer*.

Factors not yet investigated may also play a role. The Harvard Nurses' Health Study, a long-term prospective study of risk factors for chronic diseases in women, has shown an association between orange juice consumption and melanoma in adult females. The investigators hypothesize that a photosensitizing compound in oranges may contribute to risk, says Diane Feskanich, an assistant professor of medicine at Harvard and an investigator on the study. However, a parallel study in men, not yet published, did not find the same strong association. "Whether there are photosensitizing foods is an open question," she says. "Certainly there are drugs that warn you 'don't go out in the sun if you're taking this.'"

Awareness of the risks of sun exposure has improved, according to Lange. "The population in general is more aware today of the potential danger from the sun than twenty or thirty or forty years ago," she says. The same is true in Australia, which has among the world's highest incidence of the disease. "The current generations of children are probably getting less sun exposure and fewer episodes of sunburn," says Whiteman.

But better awareness of the major risks has not necessarily translated into complete protection of children. Even grasping the extent of older children's exposure to the best-known risk factor, UV light, can be difficult. Despite prevention messages, many teenagers and young adults still want suntans. "The use of indoor tanning facilities is common among teenagers," Lange says (in a 2003 survey, 47% of white girls aged 18 or 19 had used tanning beds three or more times). "Teenagers practice a lot of risky behaviors, and exposure to UV light is one of those behaviors." **–Victoria McGovern**

Corbis

CHILDREN'S HEALTH

Child Survival Gets TV Boost

A recently launched campaign known as Rx for Child Survival gets into top gear 1–3 November 2005 with the help of a six-part TV series on global health narrated by actor Brad Pitt. The series, *Rx for Survival—A Global Health Challenge*, which was produced by WGBH/NOVA Science Unit and Vulcan Productions and will be aired by PBS stations nationwide, aims to help Americans better understand global health problems. The series also highlights the plight of children under age 5, of whom more than 6 million die every year in the developing world from diseases that could be prevented or treated for just a few dollars.

Hope for better things. The Rx for Child Survival campaign is a bid to better child health worldwide using simple tools such as insecticide-treated netting to combat malaria-bearing mosquitoes.

The TV series is part of a multimedia project that also includes the efforts of *Time* magazine, Penguin Press, NPR, and an interactive website (http://www.pbs.org/wgbh/rxforsurvival/campaign/index.html). Says Paula Apsell, senior executive producer for WGBH/NOVA, "With the power of television to extend our message into eighty-six million living rooms each week, one of the most visited dot-org websites in the world, the local reach of three hundred forty-eight member stations across the U.S., and a far-reaching impact campaign forged on the precept of partnership, PBS is in a unique position to help Americans learn more about the world's most pressing issues and to show them ways to do more to make the world a better place."

With funds initially provided by the Bill & Melinda Gates Foundation and The Merck Company Foundation, the campaign's goal is not just to inform but also to encourage Americans to donate and raise money for public health interventions in the world's poorest countries. Some 88 cents of every dollar raised will be spent in the field to provide vaccines against measles and tetanus, insecticide-treated netting to prevent malaria, vitamin supplementation, oral rehydration packs for diarrhea (which kills 1–4 million children per year), antibiotics, and antimalarial agents.

The actual field work will be performed by CARE and Save the Children, humanitarian organizations with delivery infrastructures already in place. The initial recipient countries will be Afghanistan, Mali, Mozambique, Nepal, Nicaragua, Sierra Leone, and Vietnam.

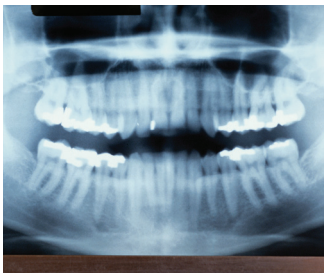
"Simple and affordable tools exist to save six million young lives lost each year from preventable causes like diarrhea and pneumonia. But these tools are not reaching the children who need them most," says Charles MacCormack, CEO of Save the Children. This is because humanitarian groups cannot afford to buy and distribute them. "Through Rx for Child Survival, Americans can help these children survive and thrive," says MacCormack.

Why another fundraising initiative? The answer is simple, says Jorge Alvar, head of the World Health Organization Department of Communicable Disease Control: "Children are dying, and we can prevent much of this tragedy at just a few dollars per head. This campaign, which aims to inform and mobilize Americans—citizens of the world's richest nation—could be of great help in that task." **–Adrian Burton**

Top to bottom: Photodisc; Save the Children; Corbis; Chris Reuther/EHP

Mercury's Afterlife?

A report released by the New England Zero Mercury Campaign says that dental fillings in cremated corpses emit about 2.5 tons of mercury each year, with the amount expected to double by 2025. Dental amalgams are 50% mercury by weight. Although the use of mercury-containing amalgams is declining steadily, 34 tons of mercury are still used for dental purposes each year. The EPA has met with the American Dental Association to discuss ways to further reduce the use of mercury in fillings.



More people today die with some or all of their teeth in place. In addition, more people are choosing cremation. The April 2005 report is online at http://www.cleanwateraction.org/mercury/pdf/NEZMC_ReportCard_DentalMercury.pdf.

Climate Change Hits the Road

Cities are the biggest consumers of electricity and therefore the primary generators of the greenhouse gases that cause global warming. Now the British Council, Great Britain's international agency for promoting education and cultural relations, has launched its US$7-million two-year ZeroCarbonCity campaign to educate people in 100 cities across 60 countries about climate change. The campaign, begun in March 2005, teaches how decisions at all levels, from urban planning to personal choices people can make every day, can contribute to or help mitigate the effects of climate change. A traveling photographic exhibition is visiting all 100 cities as part of the campaign. Related debate transcripts and publications are available online at http://www.britishcouncil.org/zerocarboncity.htm?mtk=8.

Personal Products Keep Organic Label

People who prefer to buy organic cosmetics, dietary supplements, and pet food can breathe a sigh of relief—in August 2005, the USDA ruled that the use of the "USDA Organic" label is permissible on those products. The ruling reverses an earlier decision that putting the green and white label on such items went beyond the original intent of the labeling program, implemented in 2002. Following the original ruling, the Dr. Bronner's Magic Soaps organic body care company and the Organic Consumers Association filed a suit against the USDA, a move seen as the leading factor behind the reversal.

Genomics

HapMap Complete

The International HapMap Project, a consortium of researchers and funding agencies from the United States, Japan, China, Nigeria, Canada, and the United Kingdom, is set to release a dramatically enhanced version of its haplotype map. The newly revised HapMap will be formally introduced on 26 October 2005 at the annual meeting of the American Society of Human Genetics in Salt Lake City. This information will provide researchers with an effective shortcut to map the genes contributing to particular diseases and drug responses.

The HapMap currently characterizes a total of 4 million common DNA sequence variants known as single-nucleotide polymorphisms (SNPs). With the HapMap, scientists are better able to investigate the genetic components of many complex disorders, such as asthma, cancer, and obesity. Mark Daly, an associate member of the Broad Institute, a research collaboration of universities, research centers, and hospitals in Cambridge, Massachusetts, says the HapMap shows where common SNPs are located on human DNA, and how they are distributed among populations in different parts of the world. "The HapMap allows us to accelerate our understanding of genetic variation and its relationship to disease," he says.

Most SNPs are inherited in blocks, or haplotypes, on the chromosome. Each haplotype typically carries "tag" SNPs that characterize the haplotype as a whole and thus can be used to predict the identity of the other SNPs in the same block. For example, if researchers found that a certain tag SNP showed up consistently in studies of bipolar disorder, that tag could provide some indication of the other nearby SNPs on the chromosome—SNPs that may act in concert to exert some effect on the individual phenotype. Researchers can then look more closely at those neighboring SNPs to see whether and how they contribute to a given disease. The HapMap project has identified 250,000–400,000 such tag SNPs.

Phase I of the project, which was completed in March 2005, characterized 1 million SNPs in the genomes of 269 individuals from four sampled populations: the Yoruba people of Nigeria, Han Chinese from Beijing, Japanese people from Tokyo, and a group in Utah with ancestry from Western and Northern Europe.

In Phase II, the HapMap increased the SNP density characterization in these populations to 4 million. According to Daly, this expanded number encompasses the vast majority of common SNPs thought to exist in human beings.

Lisa Brooks, program director of the Genetic Variation Program at the National Human Genome Research Institute, says that researchers will seek to validate the current HapMap's findings in additional populations, including African Americans, Mexican Americans, and others. "Phase II has given us a better genomewide HapMap," she explains. "This is a wonderful resource for mapping genes affecting complex diseases." **–Charles W. Schmidt**

Legislation

NYC Adopts Pesticide Laws

In response to the growing evidence that chemical pesticide use has potential human health consequences, New York City has adopted two new laws that aim to reduce exposures to toxic pesticides. The pesticide phase-out under these laws, signed in May 2005, will be complete by November 2006.

Under the NYC Pesticide Reduction Law, city agencies and their contractors must phase out the use on city property of pesticides that are known or suspected to cause cancer or developmental effects, and must adopt less toxic alternatives for pest control. Under the Neighbor Notification Law, the city must opt into a state law requiring that commercial lawn pesticide applicators provide 48 hours' advance notice to adjacent neighbors before spraying pesticides on lawns, trees, and shrubs.

"These bills put New York City at the forefront of the national effort to move pest control in a new direction, away from poisons and towards prevention," says Laura Haight, senior environmental associate at the New York Public Interest Research Group, one of the organizations that spearheaded community-based campaigns for the laws.

Pesticides are extensively used in densely populated cities. Cockroaches, mice, and rats thrive in multifamily dwellings, where excessive moisture, structural cracks and crevices, abundant food sources, crowded apartments, and overstuffed closets provide nutrition and shelter for pests. In the New York City metropolitan area—which in the late 1990s accounted for more than a quarter of the total pesticide use in the state—these conditions are magnified by the sheer size of the urban center, where more than 8 million people live in 800 square kilometers.

USDA Agricultural Research Service, Chris Reuther/EHP

"One of the most important potential effects from both laws may be the reduction of exposures to pesticides in schoolchildren," says Claire Barnett, executive director of the Healthy Schools Network, an advocacy organization that helped push the laws through. It is expected that these laws could potentially reduce exposure to pesticides for over 1 million children in the city's 1,500 public schools, as well as hundreds of thousands of other residents.

What made the NYC Pesticide Reduction Law feasible is that there are effective alternatives to pesticide use, says Barbara Brenner, principal investigator of an NIEHS-funded study at the Center for Children's Environmental Health and Disease Prevention Research at Mount Sinai School of Medicine. Data published by Brenner's group in the October 2003 *EHP* showed that reducing the breeding habitats for pests and using agents like boric acid that are nontoxic to humans effectively reduced cockroach infestation in an inner-city environment.

Says Brenner, "Cockroach, mouse, and rat infestation is a very real and serious problem in both indoor and outdoor environments throughout New York City. . . . However, traditional chemical pesticide spraying has not controlled the problem, bringing with it health risks and hazards of its own. Recognition of this dilemma by New York City government represents official recognition of both the problem and the need to now use proven least-toxic methods."

City council member James Gennaro, who cosponsored both bills, says, "The active participation of community organizations and scientists were both vital to the success of this landmark legislation. . . . Frankly, I don't believe this legislation would be law today without the involvement of these two essential groups." He adds, "I firmly believe that this legislation will have tangible health benefits for large numbers of New York City residents." **–Luz Claudio**

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The Endocrine Society and the Society for Endocrinology

The endocrine system encompasses the thyroid gland, the hypothalamus, the pancreas, the adrenal cortex, the thyroid, the parathyroid, and the male and female reproductive glands. Two large and well-established societies, The Endocrine Society and the Society for Endocrinology, serve the practitioners of this field. Both groups have established websites to keep their members and the general public aware of newsworthy events and developments in the field, and to educate those laypeople wishing to learn more about the subject.

The site of The Endocrine Society, http://www.endo-society.org/, features quick links so that information and materials can be pulled up by either subject or visitor's role (e.g., clinician, student, volunteer worker). Among the 16 subjects featured are cardiovascular function, diabetes/insulin, genetics/genomics, male reproduction, and female reproduction. Also gathered in one area of the homepage are quick links to the society's five publications, membership information, a member directory, and a subscription page.

The Endocrine Society's homepage features two news sections, one of news in the general media, the other of updates within the society itself. Also available from the homepage is information on the society's annual meeting, other society events, and related external events. Visitors can also select a quick link to The Hormone Foundation, the public education affiliate of the society, which offers basic information about the endocrine system, its function, and its associated diseases and disorders.

In the Press Room portion of the site is information on the America Weighs In campaign. This program focuses on educating the media, policy makers, and the general public about the role that endocrinologists play in researching and treating obesity. The Press Room also features a link to *The Endocrine Edge*, a free monthly online newsletter geared toward the public with the latest news from the society and the field of endocrinology.

The Society for Endocrinology site, http://www.endocrinology.org/, provides information about the Bristol, England–based organization and its programs. The society is affiliated with five journals, all accessible from a page on the website. The society also publishes a free quarterly newsletter, *The Endocrinologist*, which contains society news, general news, and feature articles.

The website also offers listings of grants and fellowships, society conferences, training courses sponsored by the society, and a calendar of events. The society sponsors a number of travel grants and has five research grant programs. There is also a page on books of interest to those in the field, which includes ordering information and short descriptions.

In 1997, the Society for Endocrinology established a committee for endocrinology nurses. This committee organizes conferences and an annual training course, and publishes the quarterly *Endocrine Nursing News* newsletter with reports from members and meeting notes. Past issues are available on the society website through the Endocrine Nurses link. Another subgroup are the Young Endocrinologists. Formed to support endocrinologists for up to six years after they receive their Ph.D., this group runs educational courses, provides career advice, and organizes special sessions at the Society of Endocrinology and British Endocrine Society meetings. **–Erin E. Dooley**

Taiwan Touts Trash Sorting

Taiwan, with the acreage of Belgium but twice the population, has 200 landfills. In two years these will be full, leaving the island nation dependent on some 20 trash incinerators that emit pollutants such as dioxin. To help curb the flow of waste into the incinerators, new recycling laws have been enacted that fine residents almost US$200 for not sorting their trash properly.

The laws, now in force only in Taipei, will be implemented across Taiwan by January 2006 in an effort to cut the number of trash incinerators to five within 20 years. Over 90% of Taipei residents are reportedly complying with the new rules.

Pandemic Prevention

Officials from 192 countries agreed upon revised regulations for notifying the WHO of all major disease outbreaks and suspected bioterrorism events at a May 2005 meeting. Until now, only outbreaks of cholera, plague, and yellow fever had to be reported to the organization. The regulations, which come into effect in 2007, also require that the WHO assist member countries in responding to such threats and in fostering greater international cooperation in outbreak response. The health ministers and other officials who signed the regulations hope the new system will help contain outbreaks of infectious diseases such as SARS and influenza before they spread globally.

Less Gummy Gum

There's no doubt about it: chewed chewing gum is hard to clean up if it ends up anywhere except in a trashcan. In the United Kingdom alone, over US$260 million is spent each year by municipalities on gum cleanup, and the methods used include abrasive chemical cleaners, pressure washing, and scraping. Now the University of Manchester in England and the company Green Biologics are developing a biologically based cleaner, TP-GUM™, that is cost-effective and nontoxic. The new product, which uses enzymes to break down the chemical structure of chewing gum at low temperatures and pressure, is easier to use and less damaging to surfaces than conventional gum cleanup methods.

Clockwise from top right: Thinkstock, Chris Reuther/EHP, Photodisc, The Endocrine Society, The Society for Endocrinology

Children's Centers Study Kids and Chemicals

Many studies in recent years have documented that whether they're used to spray in the kitchen or spray in the field, pesticides have a way of getting into almost all human environments. Pesticide exposure isn't a great idea for adults, but it poses a particular concern in regards to children. These smallest humans, who spend a lot of time close to the floor and with their hands in their mouths, can encounter much higher doses relative to their body weights. And because they are still growing and developing, children are often more vulnerable to adverse effects of these and other environmental exposures. Likewise, the developing fetus may be especially vulnerable to the effects of pesticide exposure *in utero*.

In 1998, the NIEHS joined with the U.S. Environmental Protection Agency (EPA) to create eight centers across the country where scientists study environmental influences on children's health. Today there are 11 centers. Several of these centers, including those at Columbia University and Mount Sinai School of Medicine in New York City, the University of California (UC), Berkeley, and the University of Washington (UW) in Seattle, have focused their efforts on pesticide exposures—how they occur, and the effects they cause *in utero* and during early childhood. These centers have also studied exposures to other environmental toxicants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and environmental tobacco smoke.

These studies are showing that children in certain communities have elevated exposures to toxicants early in their development and that some of these exposures can lead to slightly stunted fetal growth, shorter gestation, and suboptimal neurodevelopment, as well as to predisposition to diseases such as asthma. Additional studies are showing that the potential for damage from these chemical exposures may be affected by genetic susceptibility of both the child and the mother. Thus, interactions between genes, the environment, and the timing of exposure can all contribute to a later susceptibility to develop diseases and disorders.

Columbia University

"Early-life exposures, even occurring in the womb, appear to be important determinants of that child's respiratory health and development later on," says Frederica Perera, director of the center at Columbia University and a professor of public health. "We have enormous opportunities to prevent these diseases and conditions." At Columbia University, researchers have set up a cohort study to analyze exposure to pesticides and PAHs during pregnancy and very early childhood, a time of susceptibility that Perera says has not been adequately studied in the past.

Since 1998, nearly 700 pregnant Dominican and black women from Washington Heights, Harlem, and the South Bronx have enrolled in the study. Researchers take urine samples from mothers during pregnancy and blood samples from their babies at birth, sample ambient air in the mothers' environment during pregnancy, and administer questionnaires and biomarker assessments repeatedly over the child's early years.

Perera and her colleagues found that all the women in their cohort—and, therefore, their developing babies—were exposed to PAHs from vehicle exhaust and to at least one neurotoxic pesticide during pregnancy. In the February 2003 issue of *EHP* they reported finding that high PAH levels in a mother's air samples correlated with having a smaller baby at birth. In papers published in the *American Journal of Respiratory and Critical Care Medicine* between 2000 and 2002, the team further reported that high prenatal exposure to certain PAHs was related to an increased likelihood that children would show asthma precursor symptoms and allergic responses to cockroach, mouse, and dust mite allergens at 2 years of age.

"We also see evidence that [PAH] exposures can influence cancer risk," Perera says. Prenatal exposure to PAHs was associated with DNA abnormalities in the babies' blood. This type of permanent genetic alteration has been linked to increased risk of cancer in children and adults. Also, PAH-induced DNA damage in the babies, in conjunction with exposure to secondhand tobacco smoke, was associated with significantly lower weight and smaller head circumference at birth—both signs of potential future developmental and learning problems.

The pesticide exposures of mothers and children in these urban communities occurred mainly due to insect and rodent infestations in poor-quality housing. *In utero* exposure to two organophosphate pesticides, chlorpyrifos (then the most widely used pesticide in New York City) and diazinon, resulted in an average birth weight reduction of 6.6 ounces, says Robin Whyatt, an associate professor of clinical environmental health sciences and co-deputy director of the Columbia center.

In 2000, however, the EPA announced that chlorpyrifos and diazinon would both be banned as household pesticides, and "the levels of pesticides in air during pregnancy

Home is where the exposure is. A myriad of exposures in low-income urban housing—including vehicle exhaust and pesticides used in homes—contribute to conditions ranging from cancer to low birth weight.

Ireneusz Skorupa/iStockphoto

and in the blood of both mothers and newborns were sharply reduced," says Perera. By the time samples were taken in the spring of 2001, researchers no longer saw an association between organophosphate exposure and low birth weight.

Columbia researchers are also involved in a number of other studies, including an intervention project to reduce toxic pesticide use in public housing, says Perera. Residents are taught integrated pest management techniques, including removing pest food sources, sealing cracks and crevices, and using low-toxicity pesticides such as baits, gels, and boric acid. The families involved are also given lidded trash containers, pest-proof food containers, trash bags, and cleaning supplies.

Mount Sinai School of Medicine

At the Mount Sinai center, scientists are also using a pregnancy cohort of about 400 New York women of different ethnicities who gave birth at Mount Sinai Hospital from 1998 to 2003. During this period, the study focused on pesticide exposure and how genetic variations in the paraoxonase 1 (PON1) enzyme—which detoxifies organophosphate pesticides in the body—modify response to pesticides.

Maternal blood samples were taken during the third trimester, and PON1 activity was assessed. In the March 2004 issue of *EHP*, the Mount Sinai researchers reported finding that infants exposed to chlorpyrifos *in utero* were born with smaller head circumferences, but only if their mothers also had low levels of PON1 activity, says center scientist James Wetmur, a professor of microbiology and human genetics.

In 2003, these studies of development and genetic susceptibility moved away from organophosphate pesticides, largely because levels of these chemicals dropped after the EPA ban on residential use, says Mary Wolff, director of the Mount Sinai center and a professor of community and preventive medicine and oncological sciences. Researchers at Mount Sinai are now focusing on *in utero* exposures to endocrine-disrupting chemicals often found in plastics such as phthalates and phenols such as bisphenol A.

The center preserves biologic samples from all cohort members for future studies, Wolff says, so researchers will be able to analyze phthalate and phenol levels in maternal prenatal urine samples and correlate these levels with birth outcomes and with subsequent growth and neurodevelopment. Wetmur and his team are also switching gears to search for enzymes that metabolize phthalates and phenols, as well as for genetic variation in these enzymes that might affect birth or growth outcomes.

In a separate study in East Harlem, center researchers have found that integrated pest management is effective at controlling cockroaches. In addition to reducing or eliminating exposure to toxic pesticides, the long-term cost of this method—including building repairs—is lower than standard chemical-based pest control, making it available to lower-income residents. According to a report in the October 2003 *EHP*, "The costs of adopting building-wide integrated pest management in a typical East Harlem apartment building were calculated to be $46–69 per unit in the first year (including repairs) and $24 per unit per year in subsequent years," compared to $24–46 per unit per year, not including repairs, for traditional chemical-based control. In coming years, this intervention project will next look at how the built environment affects exposures to endocrine-disrupting chemicals.

Mothers, babies, and chemicals. Researchers are studying whether variations in the enzymes that metabolize the phthalates found in some plastic bottles correlate with later birth and growth outcomes.

Getty Images

Another study to find evidence of health effects of PCBs showed that early-life exposure to these chemicals in animals can affect neuroendocrine development. Led by neuroendocrinologist Andrea Gore, then at Mount Sinai and now at the University of Texas at Austin, researchers discovered that these chemicals directly influence brain cells called gonadotropin-releasing hormone (GnRH) neurons. These neurons control reproduction in all vertebrates, and disruption in their growth or activity can lead to fertility problems, Gore says. In the October 2002 issue of the *Journal of Neuroendocrinology*, Gore and colleagues reported that a more estrogenic PCB mixture, Aroclor 1221, stimulated GnRH expression, while the less estrogenic Aroclor 1254 had both stimulatory and inhibitory effects, depending on the transcript measured.

University of California, Berkeley

As at Columbia and Mount Sinai, researchers at UC Berkeley are conducting a prospective cohort study of pregnant women and their children. Most women and children enrolled in this study—about 600 pairs total—come from low-income Mexican immigrant farmworker families who live in California's Salinas Valley.

The first goal of the study has been to understand levels and routes of exposure to pesticides and other environmental contaminants among pregnant women and children, says Brenda Eskenazi, the center director and a professor of maternal and child health and epidemiology. Researchers have collected samples of urine, breast milk, blood, and house dust. They are determining the relationship of urinary pesticide metabolites in pregnant women and children with levels of pesticides in house dust, parental occupation, and nearby agricultural pesticide applications. They are also videotaping young children to identify behaviors that may expose them to environmental chemicals.

They've found that pregnant women in their cohort show abnormally high urinary levels of organophosphate pesticide metabolites, with about 15% of them likely exceeding the maximum cumulative exposure levels advised by the EPA. Organophosphate metabolites were higher

in 6-month-old babies if the children lived with an agricultural worker. These metabolite levels were also significantly correlated with season, with urine collected in the summer showing the highest concentrations of pesticides. Levels of pesticide metabolites in urine rose as the children passed 6 months, likely because their activity levels—especially hand-to-mouth behavior—increased as they grew older.

A second goal of the study is to examine the health effects of these pesticide exposures in the children of exposed mothers. As in the Columbia and Mount Sinai studies, children in this cohort will be followed through at least age 7 to determine whether prenatal and childhood exposures have altered their cognitive development, growth, or respiratory health. UC Berkeley scientists have already found that high maternal organophosphate exposure during pregnancy correlated with shorter gestation duration, but no associations were found between organophosphate exposure and infant birth weight, length, or head circumference. A UC Berkeley center study published in the March 2005 issue of *NeuroToxicology* showed that newborns whose mothers had high levels of pesticide metabolites during pregnancy were more likely than other babies to have abnormal reflex functioning soon after birth.

The UC Berkeley center's projects also include a randomized intervention study to see what types of preventive measures best discourage pesticide transmission from farmworkers to their children, Eskenazi says. Other projects include examining pesticide levels in amniotic fluid and breast milk, monitoring ambient pollen and mold levels, and studying mechanisms of pesticide and allergen effects on neural and immune functions.

Waiting for the future. A study of Mexican immigrant farmworkers will follow children through at least age 7 years to monitor possible effects of prenatal and childhood exposure to pesticides.

More than one way in. Through pathways both expected and surprising, children of farmworkers have higher pesticide exposure than the general population.

University of Washington

The UW center also is measuring the extent of pesticide exposure in agricultural communities. Building upon previous UW center research in the Yakima Valley, center researchers have found that children of orchard workers can be exposed to pesticides that are transported on the clothing, boots, and skin of their farmworker parents, says center director and professor of environmental health Elaine Faustman. These studies also linked children's exposure with specific agricultural crops, which will be detailed in upcoming unpublished papers. Such findings will allow the UW center to intervene more effectively in preventing the occupational take-home pathway for pesticide exposure in children.

UW scientists have also developed a laser-based method that allows them to monitor pesticide spray drift in real time. They've shown that pesticides can volatilize unexpectedly in certain conditions, especially in extreme heat—so even though time has elapsed since a crop spraying, it still may not be safe for children to go near the fields. These results should influence EPA recommendations for safety near agricultural fields after pesticide application, Faustman says.

A major part of UW research has focused on genetic susceptibility to the neurotoxic effects of organophosphate pesticides. Using data gathered by the UC Berkeley center, UW researchers have shown that people with certain forms of the PON1 gene break down chlorpyrifos more efficiently than people with different forms, although all forms detoxify diazinon at the same rate.

However, knowing which genetic variant a person has does not tell you what level of PON1 is present in the blood, says UW research professor of medical genetics Clem Furlong. Knowing the activity levels of the enzyme is important in determining how well a person will metabolize organophosphates and the potential for health impacts from organophosphate pesticide exposure.

"I think it's extremely important to emphasize that, because epidemiologists continue to try and estimate risk only by doing genotype," Furlong says. "You really need to look at the functional status of individuals." It takes nearly a year for infants to begin making the amount of

Top to bottom: Brandon Clark/iStockphoto; Greg Nicholas/iStockphoto

PON1 they will have as adults, and this may lead to increased vulnerability to exposure during this time, says Furlong. Maternal PON1 can provide some protection *in utero*, but "if you have a mother with extremely low PON1 levels, this is a serious concern—there's no ability of that fetus to protect itself," he says.

In animal studies, UW researchers have examined the mechanisms through which pesticides cause neurotoxicity. They've found that different pesticides can have very different influences on cell proliferation, differentiation, and death during brain development, and all of these effects are dependent upon dose and time of exposure during development. For example, in the March 2004 issue of *Toxicological Sciences* the team reported that chlorpyrifos induced apoptosis in primary cortical neurons cultured from embryonic and newborn rats. Currently, says Faustman, center researchers are expanding studies in mice to see how the combination of exposure and genetic susceptibility affects behaviors in the animals.

Where to Go From Here

The research coming out of these children's centers over the past seven years has revealed that there are still far more unknowns than knowns, says Nina Holland, an adjunct professor of environmental health sciences at Berkeley and member of the UC Berkeley center. There are also discrepancies between some of the findings emerging from different centers. For example, the Columbia center's report in the July 2004 issue of *EHP* that *in utero* exposure to chlorpyrifos or diazinon resulted in an average birth weight reduction of 6.6 ounces contrasted with a UC Berkeley study in the same issue, which found no adverse relationship between fetal growth and any measure of *in utero* organophosphate pesticide exposure (in fact, that team found increases in body length and head circumference associated with some exposure measures). But the overall finding, Holland says, is that "we have to pay much more attention to potential effects of pesticides on very young children."

Center researchers are translating their experimental results into interventions, educational materials, community forums, press releases, and newsletters that can be used by parents, health care providers, and policy makers to improve the environmental health of local children. For example, the Columbia center has established a community educational campaign called "Healthy Home, Healthy Child." Through this program, they have

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Headliners | Smoking

NIEHS-Supported Research

The Role of the Parent in Deterring Child Smoking, as Seen by Rural Native American and White Parents

Kegler MC, Malcoe LH. 2005. Anti-smoking socialization beliefs among rural Native American and white parents of young children. Health Educ Res 20(2):175–184.

Studies suggest that there are differences between the races in parental "anti-smoking socialization"—that is, how parents influence their children's expectations regarding the feasibility, acceptability, and consequences of smoking cigarettes. For instance, black parents are more likely than white parents to set ground rules regarding tobacco use for their children, and are less likely to assume that teens will inevitably experiment with smoking. Now Lorraine Halinka Malcoe and NIEHS grantee Michelle C. Kegler of Emory University have compared antismoking socialization beliefs among rural white and Native American parents. Better information on how beliefs vary racially could help shape more effective ways of teaching parents to deter their children from smoking.

Teen smoking rates vary significantly between racial and ethnic groups. According to data from the Centers for Disease Control and Prevention for the year 2000, 31.8% of white high school students reported smoking in the past 30 days. Hispanic students were next at 22.6%, followed by Asian Americans at 20.6%, and blacks at 16.8%. Data on smoking among Native American teenagers are not as readily available, but some studies have indicated the rate among Native Americans overall is comparable to or higher than that of whites. In 2000, 36% of adult Native Americans smoked, compared with 24.1% of white adults.

The study showed that Native American and white parents were similar in their antismoking socialization beliefs with one exception: Native American parents were less likely to believe that schools are better than parents at teaching children about the dangers of smoking. Less educated parents were more likely to believe that strictly forbidding children to smoke only makes them want to smoke more. Consistent with earlier results, parents of both races had less stringent beliefs and a lesser sense of parental efficacy compared to black parents.

Methods to bolster antismoking socialization beliefs of less-educated parents may be important in preventing children in low-income rural communities with high smoking rates from beginning to smoke. Although limited in size and scope, this study provides evidence that future research should focus on ways to increase parental communication of antismoking beliefs and assessment of whether such interventions result in lower rates of smoking onset. **–Jerry Phelps**

surveyed parents and caretakers of children in Harlem, Washington Heights, and the South Bronx to determine what these people are most concerned about. Then they've compiled tip sheets on topics such as air pollution, tobacco smoke, nutrition, pesticides, and lead poisoning, and they've distributed these on the street and at community health fairs and public forums. Center researchers have also trained staff at community centers to deliver health workshops to many different types of local groups, such as parent–teacher associations, churches, after-school programs, and foster care agencies. They also send summaries of their findings—in English and Spanish—to all the mothers involved in these cohort studies.

One important focus for the future is the National Children's Study, according to Nsedu Obot Witherspoon, who is executive director of the Children's Environmental Health Network. Slated to begin enrolling in the fall of 2007, the proposed study will follow 100,000 children from preconception or early pregnancy through adulthood, examining the effects of many different environmental exposures on various health outcomes. Leaders of the study include the NIEHS, the National Institute of Child Health and Human Development, the Centers for Disease Control and Prevention, and the U.S. EPA.

Researchers from all of the children's centers and from the National Children's Study should be able "to work hand in hand and will provide a wealth of information we would have otherwise never had," Obot Witherspoon says. "It's going to be phenomenal." **–Melissa Lee Phillips**

BEYOND THE BENCH

Virtual School

In today's world of high-speed interconnection, technology in the classroom helps keep students interested and engaged in the learning process. Taking advantage of this favorable avenue of instructional opportunity, the Community Education and Outreach Program (COEP) of the NIEHS Center in Molecular Toxicology at Vanderbilt University, in conjunction with the university's Center for Science Outreach (CSO), has developed an innovative interactive videoconference teaching program known as "Virtual Scientist in the Classroom." The program creates a direct connection between Vanderbilt University faculty and students all over the country, allowing university researchers to lecture on environmental health topics related to the work they are performing in their own laboratories.

"Through the center's involvement with outreach and education, we are able to provide reliable, up-to-date, and cutting-edge science to classrooms throughout Tennessee and the U.S.," says Bradley Hawkins, the COEP director. "In addition, the students are able to interact with our researchers in a manner that was not available just a few years ago."

The program relies on volunteer faculty with diverse research interests—neuroscience, diabetes mellitus, biomedical engineering, physics, molecular toxicology, and chemistry, for example—who create their own presentations and conduct the sessions in real time in the CSO virtual learning studio (all presentations are taped and archived for future multi-classroom sessions). The topics for presentations to date have included how medicines are developed, how chemicals damage DNA, and the importance of micronutrients. The format of each session is left to the discretion of the expert presenters, and may include anything from PowerPoint slides to movie clips, live virtual tours of lab facilities, even real-time electrocardiograms. One physics professor presented the theory of relativity in character as Albert Einstein.

The sessions of 30–45 minutes can be presented to one school at a time or to multiple site audiences. Scientist–student interaction is a main component of the sessions; questions and feedback from students are expected and encouraged. By using a communications bridge capable of connecting to multiple sites within a videoconference session, the researchers open up the world of scientific discovery to students in classrooms all over the state of Tennessee and beyond. When the program was created in 1999, it primarily reached out to middle and high school students in Tennessee, but has grown to include videoconferences to children in 75 schools in 20 states.

Typically four to six sessions on varied topics are offered each semester. Teachers can find complete descriptions of each session online at http://www.vanderbilt.edu/cso/ and can register there for each session. The sessions are free for Tennessee students, although a charge is applied for out-of-state schools. Once teachers have registered, they can download supplemental lesson material and will receive e-mailed confirmation and detailed instructions for participation. In a new feature, researchers answer questions that arise after each session, and the 1- to 2-minute video response also is archived on the site.

The continued commitment and enthusiasm of the contributing faculty members is a cornerstone of the program, and helps keep the videoconference sessions relevant and timely. "I believe that as researchers we need to take an active role in helping to educate and inform the public about issues related to adverse health outcomes upon exposure to poisons, to educate the public about sources of poisons in food and air, and the mechanisms by which they affect our health," says Michael Aschner, a professor of pediatrics and pharmacology who has presented on the subject of chemical insults to the brain. Hopefully, he says, educational outreach programs can help bridge the gap between public understanding and public perception of toxicology. **–Tanya Tillett**

M(aven)TV? Vanderbilt University specialists use the Internet to connect kids with science straight from the lab.

Vanderbilt University CSO

Are EDCs Blurring Issues of Gender?

Although scientists have postulated a wide range of adverse human health effects of exposure to endocrine-disrupting chemicals (EDCs), the nexus of the debate is the concern that prenatal and childhood exposure to EDCs may be responsible for a variety of abnormalities in human sexuality, gender development and behaviors, reproductive capabilities, and sex ratios. Scientists today are asking hard questions about potential human effects: Do EDC exposures impair fertility in men or women? Can they cause sexual organ malformations, stunted reproductive development, or testicular or breast cancer? Do fetal exposures to EDCs alter sex phenotypes? Do they change later gender-related neurobiological characteristics and behaviors such as play activity and spatial ability? Could such exposures even be involved in the etiology of children born with ambiguous gender?

EDCs include a spectrum of substances that can be loosely classified according to their known or suspected activity in relation to sex hormone receptors and pathways. The most-studied and best known are the environmental estrogens, which mimic estradiol and bind to estrogen receptors (ERs). ER agonists include the pesticide methoxychlor, certain polychlorinated biphenyls (PCBs), bisphenol A (BPA; a high production volume chemical used to make polycarbonate plastic), pharmaceutical estrogens such as diethylstilbestrol (DES) and ethinyl estradiol, and phytoestrogens, which occur naturally in many plants, most notably in soybeans in the form of genistein and related substances. There are a few known ER antagonists, or antiestrogens. Antiandrogens, or androgen receptor (AR) antagonists, include the fungicide vinclozolin, the DDT metabolite *p,p′*-DDE, certain phthalates (a group of chemicals used to soften polyvinyl chloride plastics), and certain other PCBs. And there are other types of EDCs that affect particular endocrine targets. The various EDCs differ greatly in their potencies relative to natural hormones, and in their affinity for target receptors. Some have been shown to act via non–receptor-mediated mechanisms, for example by interfering with hormone synthesis.

In many well-documented cases of high-level fetal exposures to known EDCs such as DES, certain PCBs, and DDT, the answer to the question of whether exposure is associated with gender-related effects is clearly yes. But high-level exposures such as these are relatively rare and isolated. The debate today centers on low-dose exposures—generally defined as doses that approximate environmentally relevant levels—and the idea that low-dose intrauterine exposure to some EDCs during certain critical windows of development can have profound, permanent impacts on subsequent fetal development and adult outcomes.

Critics of this idea maintain that thus far there is no credible evidence to suggest that low-dose exposures cause any adverse human health effects. But if low-dose exposures were confirmed to be the threat that proponents of the concept insist they are, public health would clearly be at risk, regulatory agencies' risk assessment approach would need to be revised, and certain common chemicals—including some that are massively produced and economically important—would likely disappear from the marketplace.

In a June 2000 *EHP* review article on human health problems associated with EDCs, Stephen Safe, director of the Center for Environmental and Genetic Medicine at Texas A&M University, concluded that "the role of endocrine disruptors in human disease has not been fully resolved; however, at present the evidence is not compelling." Frederick vom Saal, a developmental biologist at the University of Missouri–Columbia, disagrees, particularly in light of the research that's been presented in the years since that review. "The jury is *not* out on human effects," he says. "In terms of the amount of information we have in animals and the amount of information we have in humans, clearly there is a huge difference, but that's a lot different than saying the jury is out on whether

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EDCs influence humans." One thing both scientists might agree on, though, is that right now there are still more questions than answers.

A Delicate Process

The endocrine system, comprising the hypothalamus, pituitary, testes, ovaries, thyroid, adrenals, and pancreas, is one of the body's key communications networks. It regulates the function of specific tissues and organs by secreting hormones that act as precise chemical messengers. Development and regulation of the reproductive system is one of the major functions of the endocrine system.

Sex determination and development begin early in gestation, with the differentiation of the embryonic gonad into either testes or ovaries. If the *Sry* gene is present on the Y chromosome, it will, when activated, trigger a complex cascade of hormonal events that ultimately results in the birth of a baby boy with all of the requisite male equipment in place and functioning properly. In the absence of the *Sry* gene, the end product of the process will be a baby girl. The female phenotype is considered to be the "default" pathway for mammalian reproductive development.

Differentiation and development of the sexual organs continues throughout gestation under the guidance of the various sex hormones (such as estrogen and testosterone) produced by the endocrine system. For males and females alike, the entire process of reproductive development is exquisitely sensitive to minute changes in levels of the sex hormones, particularly during certain critical windows of development.

In papers published in the *Journal of Animal Science* throughout 1989, vom Saal demonstrated this sensitivity in a series of mouse experiments. These studies showed that in multiple-birth species it was possible for adjacently positioned male and female fetuses to transmit tiny amounts of hormones to each other, with pronounced phenotypic consequences. "We found that a difference of about a part per billion of testosterone and about twenty parts per trillion of estradiol [endogenous estrogen] actually predict entirely different brain structures, behavioral traits, enzyme levels, and receptor levels in tissues, hormonal levels in the blood—there is nothing you look for that . . . doesn't differ in these animals," says vom Saal.

Such a delicately timed and precisely controlled process presents a myriad of opportunities for perturbation from exposure to EDCs. These chemicals mimic hormones, and can disrupt differentiation and development in a wide variety of ways, by duplicating, exaggerating, blocking, or altering hormonal responses. The developing fetus and early neonate may lack the protective metabolic mechanisms present in adults that help detoxify and break down chemicals, maintaining homeostasis in the system. Also, tissues are rapidly dividing and differentiating in the fetus, and such a high level of cell activity is vulnerable to disruption of normal development. With such small body mass in the fetus and child compared to an adult, exposure levels may be amplified in terms of relative dosages reaching target tissues. And sometimes, exogenous EDCs may show very low binding to plasma hormone-binding proteins and thus roam the body in an unbound state, with unknown effects.

Much of what remains to be discovered about the impacts of EDC exposures on the fetus relates to a new concept called the developmental origins of health and disease (until recently known more commonly as the fetal basis of adult disease). "People are just now recognizing that this is indeed a possibility," says NIEHS scientist Retha Newbold, a pioneer in the study of endocrine disruption who has spent decades researching the effects of exogenous estrogens, particularly DES. "Developmental exposure to low doses of EDCs may not lead to malformation or to anything you can look at and immediately recognize as a problem," she says. "But it still could have long-term effects, such as alterations in metabolism, alterations causing cancer later on, or alterations causing infertility."

Evidence of Effects

Reproductive and developmental abnormalities linked to EDC exposures have now been documented in birds, frogs, seals, polar bears, marine mollusks, and dozens of other wildlife species. For example, alligators in Lake Apopka—one of Florida's most polluted lakes due to extensive farming activities around the lake, the presence of a sewage treatment facility, and a major 1980 spill of pesticides

Watching wildlife. Research has documented reproductive and developmental abnormalities linked to EDC exposures in wildlife species such as alligators and polar bears, although what these results mean for humans is still unknown.

Left to right: Digital Vision; Gaurawa Kumar/Stockphoto

including DDT and DDE—have been shown to have been "feminized." That is, zoologist Louis J. Guillette, Jr., and colleagues first reported in the August 1994 *EHP*, the males have shortened penises and low levels of testosterone, while the females have excessive levels of estrogens. Sex reversal (in which an animal of one sex matures with the reproductive organs and capabilities of the other sex) and skewed sex ratios (in which there is an unusually greater proportion of one sex than the other) have been seen in several fish populations, particularly colonies living in close proximity to pulp and paper mills and sewage treatment plants. Other reports have shown reproductive effects among wildlife resulting from exposure to EDCs excreted into the water supply by women taking birth control pills.

Many of the adverse outcomes seen in wildlife populations have been replicated in laboratory experiments, confirming the role of EDCs in their occurrence. Among the papers reporting such confirmation were a May 1997 article in *EHP*, in which Guillette, D. Andrew Crain, and colleagues replicated alterations in steroidogenesis (the production of sex hormones) in alligators. More recently, in the December 2004 issue of *EHP*, Jon Nash and colleagues showed that long-term laboratory exposure to environmental concentrations of the pharmaceutical ethinyl estradiol caused reproductive failure in zebrafish.

According to a report on EDCs published in volume 75, issue 11/12 (2003) of *Pure and Applied Chemistry* by the Scientific Committee on Problems of the Environment/International Union of Pure and Applied Chemistry (SCOPE/IUPAC), more than 200 animal species are either known or suspected to have been affected by these chemicals. "The weight of evidence for endocrine disruption in wildlife is really overwhelming," says Joanna Burger, a professor of cell biology and neuroscience at Rutgers University who cochaired the SCOPE/IUPAC project.

The SCOPE/IUPAC report was less definitive on the extent of human effects of endocrine disruptors. "It is too early to reach firm conclusions about whether human populations are seriously at risk from potential exposures to [EDCs], and further vigilance is clearly required," the authors wrote. "However, it is somewhat reassuring that after substantial research in the past decade, there have been no conclusive findings of low-level environmental exposures to [EDCs] causing human disease."

The report further notes, however, that "[c]hemical interferences with steroid biosynthesis and metabolism can produce adverse health effects, even though the inducing agent would not be detected as an [EDC] using receptor-based test systems. This is an important area of study because some examples of [endocrine disruption] occurring in animals derive from exposure to inhibitors of steroidogenic enzymes such as 5α-reductase and aromatase. Some such agents are known to be active in humans and are used successfully in the treatment of a range of human hormonal conditions." The authors suggested that evaluation of such effects will require integrated screening that incorporates *in vitro* and *in vivo* technologies.

A comprehensive report issued in 2002 by the World Health Organization's International Programme on Chemical Safety, titled *Global Assessment of the State-of-the-Science of Endocrine Disruptors*, reached similar conclusions. The report stated that "although it is clear that certain environmental chemicals can interfere with normal hormonal processes, there is weak evidence that human health has been adversely affected by exposure to endocrine-active chemicals. However, there is sufficient evidence to conclude that adverse endocrine-mediated effects have occurred in some wildlife species." Citing the fact that studies to date examining EDC-induced effects in humans have yielded inconsistent and inconclusive results, the group wrote that, although that explains their characterization of the evidence as weak, "[that] classification is not meant to downplay the potential effects of EDCs; rather, it highlights the need for more rigorous studies."

The *Global Assessment* further states that the only evidence showing that humans are susceptible to EDCs is currently provided by studies of high exposure levels. There is, in fact, clear evidence that intrauterine EDC exposures can alter human reproductive tract development and physiology. The most thoroughly characterized example is DES, the synthetic estrogen prescribed to millions of pregnant women in the United States and elsewhere from the 1940s to the 1970s to prevent miscarriage. The drug is known to have caused a rare form of vaginal cancer in thousands of daughters of women who took DES, as well as a variety of adverse reproductive tract effects in both the daughters and sons of those women.

The DES situation could be seen as a worst-case scenario for prenatal EDC exposure—the deliberate delivery of a potent estrogenic chemical in high doses. Viewed another way, it has provided researchers a rare opportunity to study the effects of prenatal EDC exposure in a relatively controlled fashion, with a well-defined population and well-characterized exposure to a single potent agent.

Over the course of her research, Newbold has developed a mouse model of DES exposure that has proven extremely useful in studying the effects of DES and other environmental estrogens, particularly those outcomes that may be manifested only later in life. "With the experimental model, there are a lot of questions we can ask with DES that will tell us about the weaker environmental estrogens," she says. "We can change the timing of exposure and the amount of exposure, and we can look at different target tissues."

The animal model has replicated numerous abnormalities reported in DES-exposed humans, and has also predicted some human outcomes. "We have published documentation [see, for example, the October 1985 issue of *Cancer Research* and volume 5, issue 6 (1985) of *Teratogenesis, Carcinogenesis, and Mutagenesis*] that a number of the reproductive anomalies seen in DES-exposed mice, such as retained testes and abnormalities in the oviduct in females, were also later reported in DES-exposed humans," says Newbold.

The Phthalate Connection

But reliable correlations between animal data and human outcomes have proven elusive, particularly when it comes to showing an association between human exposures to environmental EDCs at ambient levels (that is, unrelated to spills or other acute contamination events) and adverse health effects. That may be about to change for one class of chemicals—phthalates.

Phthalates are commonly used in a wide variety of consumer products such as solvents, soft plastics, and cosmetics. The National Health and Nutrition Examination Survey showed that the majority of the U.S. population carries a measurable body burden of several phthalates. There is an extensive body of literature regarding the effects of prenatal phthalate exposure in rodents. Those effects include an association between intrauterine exposure and abnormalities in male animals in a biomarker known as anogenital distance (AGD), or the distance between the rectum and the base of the penis. AGD has been shown to be a sensitive measure of prenatal antiandrogen exposure. This pattern of genital dysmorphology has come to be known as the "phthalate syndrome."

In the first study to look at the link between AGD and EDC exposure in humans, Shanna Swan, a professor of obstetrics and gynecology at the University of Rochester, and her colleagues collected data from 85 mother–son pairs participating in the Study for Future Families, a multicenter pregnancy cohort study. The mothers' urine was analyzed for the presence of several phthalate metabolites, and the infant boys, aged 2–36 months, were examined for genital developmental characteristics, including AGD, which was standardized for weight to develop an anogenital index (AGI).

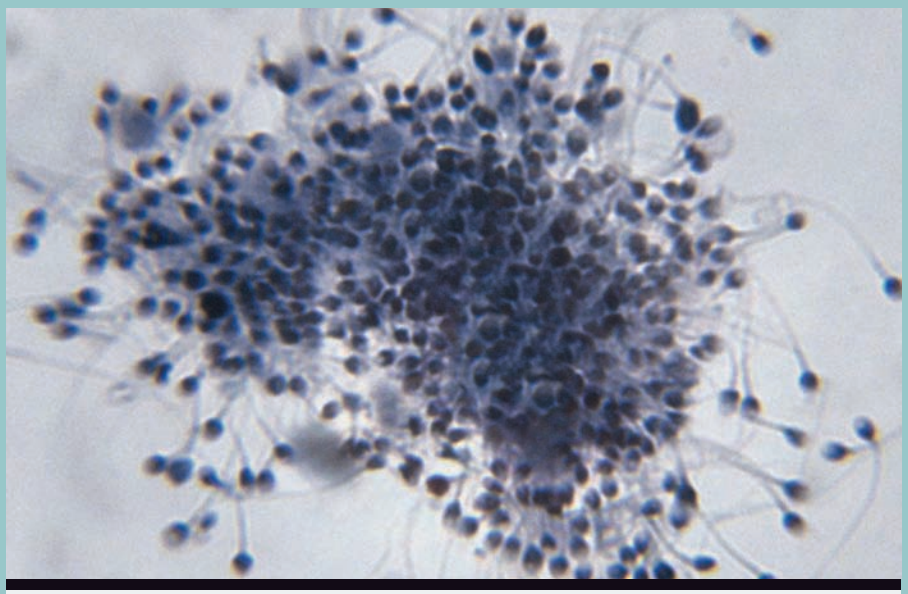
Although the researchers found no sign of frank genital malformations or disease, they did discover an association between elevated concentrations of four phthalate metabolites in the mothers and shorter-than-expected AGI in the infants, as reported in the August 2005 issue of *EHP*. And, importantly, shortened AGI was found in infants exposed prenatally to phthalate metabolites at concentrations comparable to those found in one-quarter of the U.S. female population. The boys with short AGI were also significantly more likely to have incomplete testicular descent (cryptorchidism). "We know that incomplete testicular descent is a risk factor for poorer semen quality, lower sperm counts, [impaired fertility], and testicular cancer," says Swan. Although it is obviously impossible to predict adult outcomes, she says these infants may be at risk of testicular dysgenesis syndrome (TDS) in the future.

TDS is a concept put forth by Danish researcher Niels Skakkebæk and colleagues, in which four adverse male reproductive end points—impaired semen quality, cryptorchidism, hypospadias (abnormal location of the urethra), and testicular cancer—are risk factors for each other. Says Swan, "The idea is that the development of the testis is interrupted in fetal life, and that this has consequences in adult life, as well as at birth. That certainly is something we've seen in rodents, and this study is the first evidence we've seen of TDS in humans."

Swan's study is among the first to combine a population-based, measurable, low-level EDC exposure, observed physiologic effects, and solid biological underpinnings. Even skeptic Safe says that this is the kind of study needed to begin to answer the many questions about EDCs and human health. "This looks to be a good approach, and suggests a correlation," he says. "Whether it's causal of anything and whether it holds up or not, I don't know. It needs to be repeated in different locations and with more and more integrated measurements." Swan plans to do just that, as well as to follow up on her current pregnancy cohort by measuring gender role behaviors in both the male and female children, who are now between 2 and 5 years old.

The Phthalate Esters Panel of the American Chemistry Council, a trade organization based in Arlington, Virginia, maintains that "there is no well-established and credible evidence for adverse effects [due to phthalates] in humans at environmentally relevant doses," says panel manager Marian Stanley. With regard to Swan's study, Stanley says, "It correlated some effects in infant males with some lower-molecular-weight phthalates, particularly diethyl phthalate, for which effects in rodents occur only at very high doses, and which is not considered to pose reproductive or developmental concerns by reviewing government agencies."

Stanley also points to questions about the biomarker used in the study. "The measurement that was used is something that I think is still subject to debate. You see the AG distance in rodents, and while it is a marker of something, it is certainly not a biological effect," she says. "I think the study has been overinterpreted by lots of other people [besides] the authors of the study."



A question of Y. A Swedish study of fishermen exposed to CB-153 and *p,p′*-DDE associated elevated levels of these chemicals with a higher proportion of Y-chromosome sperm, suggesting that exposure to EDCs could skew the ratio of boys to girls.

Getty Images

EDCs and Sex Ratios

Sex ratio—the proportion of male to female live births—is very constant on a worldwide basis, typically ranging from 102 to 108 male births for every 100 female births. In recent years, however, a number of reports have suggested that environmental and occupational exposures to EDCs may be altering the sex ratio within given human populations.

In one such study, appearing in the July 2005 edition of *Human Reproduction*, a group of Swedish researchers analyzed blood and semen samples from 149 fishermen to investigate whether exposure to the persistent organochlorine pollutants CB-153 (a PCB) and *p,p′*-DDE affected the proportion of Y- and X-chromosome-bearing sperm. They discovered that elevated exposure levels of both chemicals were positively associated with a higher proportion of Y-chromosome sperm. The researchers conclude that their findings add to evidence that exposure to persistent organic pollutants may alter the offspring sex ratio, with the higher proportion of Y-chromosome sperm likely tending to lead to a higher proportion of male births.

A study appearing in the October 2005 issue of *EHP* takes an epidemiologic approach to the issue. Constanze Mackenzie, a member of the Faculty of Medicine at the University of Ottawa, and colleagues report a distinct skewing of the sex ratio within members of the Aamjiwnaang First Nation community near Sarnia, Ontario. They found a severe decline in the proportion of boys born among the Aamjiwnaang over the last five years, and a lesser though still significant decline over the past ten years. Although no causal factors were determined, the authors note that the community is located in immediate proximity to several large petrochemical, polymer, and chemical plants, and that previous studies—such as those following the 1976 industrial accident in Seveso, Italy—have shown that exposure to contaminants such as EDCs can impact sex ratios within small communities near such industrial facilities. The authors suggest that further assessment should be pursued to identify potential exposures among community members. [For more details on this study, see "Shift in Sex Ratio," p. A686 this issue.]

How Low Do They Go?

When is a hypothesis no longer a hypothesis, but a validated scientific concept ready to drive regulatory and policy decision making? When it comes to the so-called "low-dose hypothesis" regarding the biological activity or adverse effects of low-dose exposures to EDCs, that is the key question. The issue has been debated for years, since vom Saal's group first published in the January 1997 issue of *EHP* their findings of enlarged prostate in

male mice whose mothers had been fed low doses of BPA. Today, the controversy over whether vom Saal's findings have been sufficiently replicated, and whether the U.S. Environmental Protection Agency (EPA) should revise its risk assessment process to reflect the potential for adverse effects of low-dose EDCs, is still going strong.

Some proponents of the low-dose hypothesis argue that the traditional toxicologic approach to risk assessment is an inappropriate method to assess EDCs. The current protocol assumes a linear dose-dependent response to chemical exposures, determines the lowest level at which there is an observed adverse effect, and then adds a safety factor to arrive at an official reference dose—the daily human intake assumed to be safe. Experimental work by vom Saal and others has postulated that EDCs exhibit a U-shaped dose–response curve, with biological activity stimulated at very low doses—often several orders of magnitude below current reference doses—as well as very high doses.

Ubiquitous exposure, unknown consequences. Humans are exposed to EDCs through many routes including pharmaceuticals, air pollution, pesticides, and drinking water, but the effects of environmental exposure are largely unknown.

Proponents also state that the process of endocrine disruption itself is inherently different from many other toxicologic processes, affecting a variety of highly sensitive pathways (especially in the fetus) via novel mechanisms of action, many of which are as yet poorly understood. Also, they say, endocrine-signaling pathways that mediate responses to EDCs have evolved to act as powerful amplifiers, resulting in large changes in cell function occurring in response to extremely small concentrations.

One chemical that has become a lightning rod in the debate is BPA. By vom Saal's count, there are now more than 100 published peer-reviewed studies showing significant biological effects of low doses of BPA (almost half published within the last two years) compared to 21 reporting no effect. He is convinced that widespread exposure to BPA poses a threat to human health.

Not so, claims Steve Hentges, executive director of the Polycarbonate Business Unit of the American Plastics Council: "For our purposes, what we have to know is, does BPA cause health effects in humans at any relevant dose, particularly at the levels at which people are actually exposed? When you look at all of the evidence together, and in particular look at the comprehensive studies that are designed to look for health effects, you don't find them."

The industry group also believes that the weight of evidence does not support the concept of a low-dose effect for BPA. "And it's not just us saying that," says Hentges. "Indeed, every government body worldwide that's looked at it has reached effectively the same conclusion in terms of how they regulate BPA or consider regulating it." He acknowledges that there has been quite a bit of new research activity in this area within the past few years, but states that "even though new research has been conducted, we believe that the weight of evidence has not shifted."

Where does the EPA stand on these issues? The agency's Office of Research and Development is in the midst of implementing a multiyear plan to set the EPA's agenda and goals in the area of EDC research. The plan is part of the agency's Endocrine Disruptors Research Program, a five- to ten-year research agenda it started in 2001 to look comprehensively at the science surrounding EDC exposures and effects. The integrated program was launched at about the same time that a congressional mandate, under the 1996 Food Quality Protection Act, directed the EPA to develop a screening and testing program for EDCs.

The EPA's stance is that the jury is still out on both the public health impacts of EDCs and the need to incorporate low-dose methodologies into the agency's risk assessment protocols. Elaine Francis, director of the Endocrine Disruptors Research Program, says the EPA needs to conduct a lot more research before any definitive public health statements can be made about this class of compounds. "When you look at such a diverse group of organisms that have been impacted in wildlife, and certainly laboratory rodent species," she says, "there is enough concern that we recognize the importance of developing a body of work in humans to try

All images: Getty Images

to characterize any impact [EDCs] might be having on humans."

The agency is currently funding three research grants in the area of low-dose EDC exposures, partly in response to the conclusions reached in a 2000 peer review and subsequent report on the low-dose issue held by the National Toxicology Program at the EPA's request. In the 2001 *Report of the Endocrine Disruptors Low-Dose Peer Review*, that expert panel acknowledged that low-dose effects had been sufficiently documented at that point in time for the EPA to consider revisiting its current testing paradigm.

"The general consensus was that more work needed to be done in this area," says Francis. "Since that time, we would still agree that there has not been enough information to indicate that the existing approaches are ones that would not be valid for endocrine disruptors. But we left the door open that we would need to do more research, and the best we could do at this point is to support and promote research in that area, and we've done that."

Vom Saal is of a different opinion: "In the risk assessment process for chemicals as currently conducted, the maximum tolerated dose is used as a reference, and a span of typically not more than fiftyfold in the dose range is the maximum that anyone ever uses in the studies. Studies [from the 1 January 2005 issue of *Cancer Research* and the April 2005 *EHP* show] literally millions of fold below that dose range in adverse effects . . . from BPA, and when you have that type of unbelievable discrepancy, for the EPA to come out as it recently did and state that it has no intention of testing low doses as part of the testing process [implies] that you no longer have a scientifically based process—it is an entirely politically driven process, because they are explicitly ignoring the scientific findings that are out there."

From her perspective, Newbold feels that although there is no question that EDCs have low-dose effects, more research needs to be done to document adverse effects in humans. "We spend an awful lot of time arguing whether there are low-dose effects or not. That just infuriates me," she says. "There *are* low-dose effects. There have *always* been low-dose effects. The question is, are they adverse? We don't know, and we've got to design studies to get answers to that question." She adds, "In order to take this argument to a whole other level, we're going to have to have more epidemiology studies. I know it happens with mice, but I don't know what happens with humans."

Connecting the Gender Dots

It's premature to call it a theory; at this point, it barely qualifies as a hypothesis: some observers are putting forth the proposition that prenatal EDC exposures may affect gender identity—how a person identifies him- or herself, regardless of physical characteristics. This idea presupposes two basic concepts: first, that transgenderism (in which a person experiences "gender dysphoria," a strong feeling of having been born the wrong sex) is physiological in origin, most likely due to events during prenatal neurological development; second, that intrauterine EDC exposures can and do disrupt prenatal neurological development.

A paper in the 2 November 1995 issue of *Nature*, among other reports, lends credence to the first concept. Jiang-Ning Zhou and colleagues at the Netherlands Institute for Brain Research studied heterosexual men and women, homosexual men, and male-to-female transsexuals. They reported finding a distinctly female brain structure in genetically male transsexuals (men who had gone through hormonal treatment and irreversible sexual reassignment surgery to become women). The volume of the central subdivision of the bed nucleus of the stria terminalis (BSTc), a sexually dimorphic brain area that is essential for sexual behavior, is larger in men than in women. Anatomical study results showed that BSTc volume did not differ significantly between heterosexual and homosexual men, and that BSTc volume was 44% larger in heterosexual men than heterosexual women. In the male-to-female transsexuals, BSTc volume was only 52% that of the reference males—a volume analogous to that seen in the women. The authors write that these findings "support the hypothesis that gender identity develops as a result of an interaction between the developing brain and sex hormones."

But a study by Wilson C.J. Chung and colleagues published in the 1 February 2002 *Journal of Neuroscience* complicates this picture. This group, also from the Netherlands Institute for Brain Research, reported that BSTc size differentiation between men and women became significant only in adulthood, implying that the phenomenon may be more effect than cause. The authors do point out, however, that the lack of marked sexual differentiation of the BSTc volume before birth and in childhood does not rule out early gonadal steroid effects on BSTc functions. They point to earlier animal experiments showing that fetal or neonatal testosterone levels in humans may first affect synaptic density, neuronal activity, or neurochemical content during early BSTc development, and that "[c]hanges in these parameters could affect the development of gender identity but not immediately result in overt changes in the volume or neuronal number of the BSTc."

On the other side of the ledger, in the June 2002 edition of *EHP Supplements*, Bernard Weiss, a professor of environmental medicine and pediatrics at the University of Rochester, reviewed the existing literature on sexually dimorphic nonreproductive behaviors as indicators of endocrine disruption. Weiss made a strong evidence-based case that "gender-specific regional differentiation of the brain and, ultimately, its expression in behavior are guided by the gonadal hormones," and that the process is subject to interference by drugs and environmental contaminants. He points out that sex differences in performance and behavior are not—but should be—a recognized criterion in developmental neurotoxicity testing.

So who out there is connecting these dots?

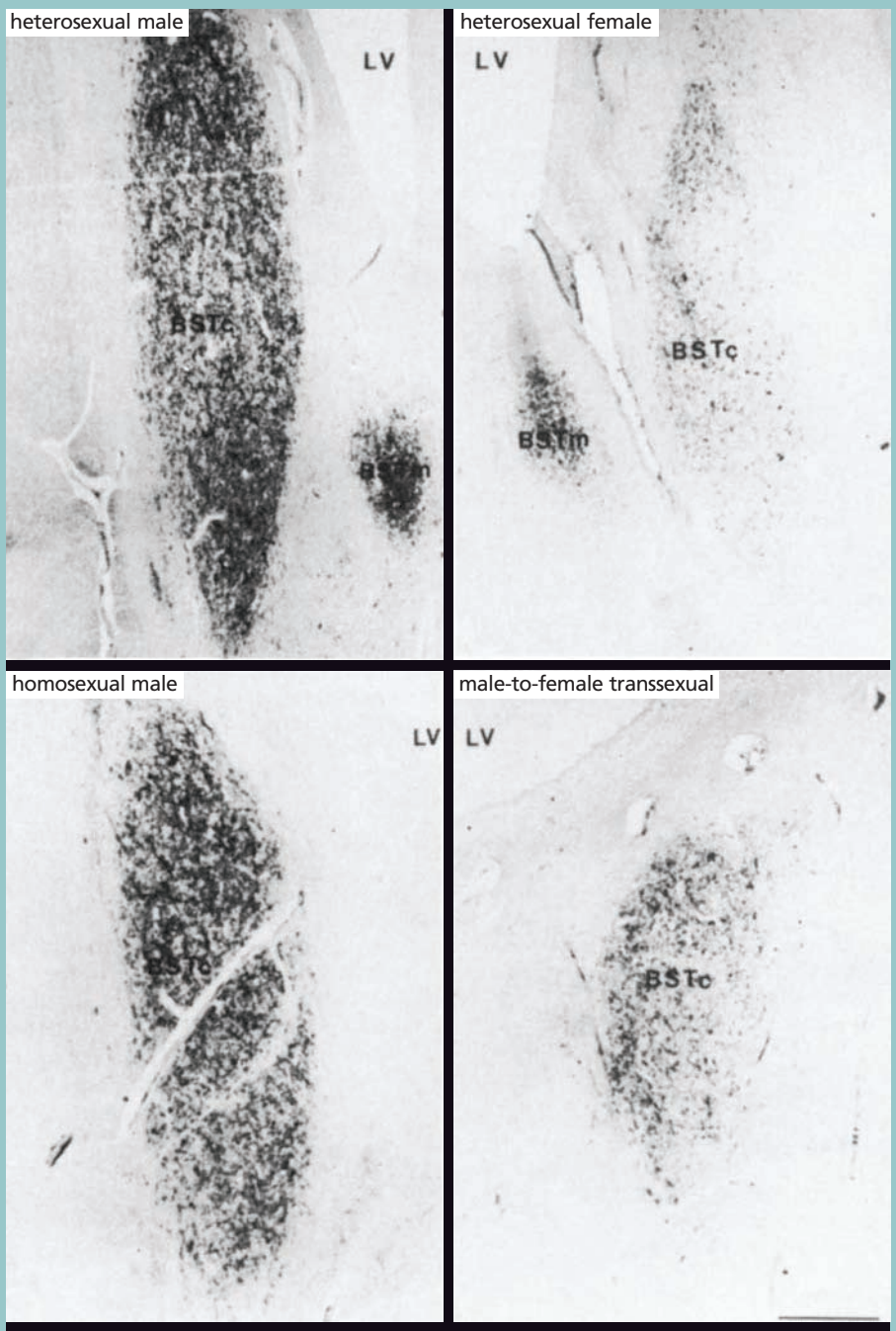
Scott Kerlin is a Ph.D. social scientist at the University of British Columbia. He devotes considerable time to monitoring the international scientific literature on DES and other EDCs as well as to researching and writing about the long-term health effects of prenatal DES exposure on males. He is himself the son of a woman given DES in pregnancy.

Kerlin recently conducted a survey study of 500 members of the DES Sons International Network, an online resource for men who know or strongly suspect they were exposed to DES *in utero*. In a paper presented in August 2005 at the International Behavioral Development Symposium in Minot, North Dakota, he reports that more than 150 respondents identified themselves as having any of a variety of gender-related disorders. Kerlin does not claim that DES causes these gender disorders, but feels that his results indicate that such outcomes should be included in research related to the potential effects of prenatal EDC exposures.

The Road Ahead

It's going to be very difficult to ever conclusively answer the basic question of whether low-level EDC exposures during development are causing deleterious reproductive or gender-related outcomes in humans. Scientists agree that one of the major challenges is to address the issue of mixtures. Typically, researchers look at the impact of one chemical at a time, but environmental exposures regularly involve an unpredictable mix of chemicals, with exposures varying widely in dose and duration. It is unlikely there will ever be a comprehensive understanding of how the many EDCs in mixtures interact with each other and with human physiology.

Convincing epidemiologic evidence of adverse effects in humans is also difficult to come by, but will be necessary to translate scientific findings into concrete actions to protect public health. Swan's study, one of the first of its kind to appear thus far, may serve as a methodological model for future investigations of low-level EDC exposures.



Reprinted from: Zhou J-N, Hofman MA, Gooren LJG, Swaab DF. 1995. A sex difference in the human brain and its relation to transsexuality. Nature 378:68–70.

Gender basis. In a study of the brain region known as the BSTc, which varies in size by sex, the volume of the BSTc for male-to-female transsexuals was analogous to that seen in women, leading the authors to speculate that the findings "support the hypothesis that gender identity develops as a result of an interaction between the developing brain and sex hormones."

Do we know enough now that steps should be taken in the policy and regulatory realm? Some observers, taking a precautionary approach, think that we do. For example, there are bills under consideration in the California and New York legislatures to restrict the use of certain phthalates in toys, child care products, and cosmetics, and a California bill would ban the use of BPA in products meant for use by children aged 3 years or younger. Also, the European Parliament voted in 2005 to ban the use of three phthalate plasticizers (DEHP, di-*n*-butyl phthalate, and benzyl butyl phthalate) in toys and child care items, and to prohibit the use of three others (diisononyl phthalate, diisodecyl phthalate, and di-*n*-octyl phthalate) in toys and child care items that children can put in their mouths.

Theo Colborn, a professor of zoology at the University of Florida and author of the 1996 book *Our Stolen Future*, believes the time for action is now. "In the animals, it was at the population level that we really began to realize what was going on," she says. "If we're going to wait to see population effects for all of these concerns that we have in the human population, it's going to be too late." She points out that we're already into the fourth generation of individuals who have been exposed *in utero* to chemicals that had never been used before the mid-1930s or early 1940s.

Swan agrees that there is sufficient knowledge at this point to call EDC exposures a serious threat to public health. "I don't think it's necessarily a threat to individuals," she says, "but I think that as a population we are threatened. I'm not predicting the end of the species or anything like that, but I think the increasingly alarming trends that we're seeing, in terms of couples that can't conceive or couples whose babies have undescended testicles, and so on, can have an impact on the population as a whole."

Other observers are not so sure. Harry Fisch, director of the Male Reproductive Center at Columbia University Medical Center, specializes in the diagnosis and treatment of male infertility. From his clinical perspective, other factors—including other exposures—are more important than EDCs. "The sky is not falling," he says. "A lot of times there's extrapolation from high-dose exposure to low-dose exposure. I think one of the biggest culprits for the abnormalities we see that's been totally ignored is [increased] parental age. Also, we need to look at things we're doing to ourselves before we start blaming low-level chemicals. For example, what does cigarette smoking do compared to Saran Wrap? What about the diets we eat, the high-fat intakes? Before we start blaming others, we need to look at ourselves to determine the impact of our lifestyles."

Although plastic wrap may not be responsible for human infertility, the scientific evidence fueling growing concerns about the effects of ambient environmental exposures to EDCs cannot simply be dismissed. "Vigilance is the key word here, because there are so many chemicals out there," says Burger. "Understanding the effects of chemicals is a three-pronged approach. It's being sure that we have wildlife models and people who are watching wildlife populations to see quickly if something detrimental happens. It's having really good epidemiological studies and vigilance of people in various places. And it's backing those two up with laboratory science immediately when a problem turns up, to try to ascertain the cause quickly."

Ernie Hood

Roughly 100 pharmaceuticals have now been identified in rivers, lakes, and coastal waters throughout Europe and the United States in concentrations of parts per billion to parts per trillion. The first major European studies on this topic—in journals such as volume 67, issue 1–4 (1997) of the *International Journal of Environmental Analytical Chemistry* and the November 1998 issue of *Water Research*—examined German ground and surfaces waters, and found occurrences of drugs including cholesterol regulators, analgesics, and antiseizure medications. Since that time, numerous other studies have documented the presence of pharmaceuticals, including potential endocrine disruptors, in other locales as well.

So far there is no evidence of adverse human health effects due to traces of pharmaceuticals in water. But scientists have linked certain pharmaceuticals with disturbing ecosystem changes. For example, in volume 8 (1994) of *Chemistry and Ecology*, researchers demonstrated that the feminization of fish—male carp and trout producing vitellogenin, an egg protein usually found only in females—was associated with exposure to sewage effluent now known to contain ethinyl estradiol, the active ingredient in birth control pills.

There is much concern about what is not known: ecotoxicity data are available for less that 1% of human pharmaceuticals, according to estimates published in the April 2004 issue of *Regulatory Toxicology and Pharmacology*. Today, intensive research is under way to investigate the effect of human medications on the environment.

In 1999, in response to these concerns, the European Medicines Agency (EMEA) began drafting guidance that outlined an environmental risk assessment procedure to accompany pharmaceutical companies' applications to market new drugs in Europe. The latest draft was published in January 2005, after several revisions, and the public comment period closed in April 2005. Scientists and pharmaceutical companies alike hope the guidance will be finalized later this year.

The proposed European guidance is the first to recommend long-term ecotoxicity testing for environmental risk assessment of pharmaceuticals from the outset of the proposed testing program (in contrast, U.S. Food and Drug Administration [FDA] requirements for chronic ecotoxicity testing come later in that agency's assessment). The European guidance is also the first to take into account the possibility of environmental effects from extremely low concentrations of bioactive substances, such as endocrine disruptors.

Damming the Flow of Drugs into Drinking Water

If finalized, the guidance could call for substantially more testing of new drugs than has been demanded thus far. Its implementation would also generate much-needed chronic ecotoxicity data. "The main advance in this draft guideline is that we really address this issue and get more information on the toxicity of these compounds," says Thomas Heberer, an environmental chemist at the Technical University of Berlin and coauthor of many papers on the topic, including the 1997 *International Journal of Environmental Analytical Chemistry* report.

What the Draft Guidance Covers

The draft guidance outlines the risk assessment procedure for new active pharmaceutical substances, their metabolites, and possibly excipients (the inert substances in which a drug is delivered) if they are deemed similar to chemicals with known adverse environmental effects. It does not

Top to bottom: Corbis; image100

apply to drugs already on the market. If an environmental risk is found, the guidance recommends that the manufacturer take appropriate precautionary and safety measures to limit the product's environmental impact. The guidance specifically recommends the labeling of pharmaceuticals when there is a possibility of an environmental risk, to educate people about how best to dispose of expired or unused medicines.

The guidance applies only to potential environmental risks that are a consequence of people storing, taking, and excreting medicines. The potential risks posed by the manufacture of drugs are not addressed, nor does the guidance apply to "orphan" drugs used only to treat rare diseases. Separate guidance governs medicinal products containing genetically modified organisms.

Proposed EMEA Protocols

The EMEA risk assessment protocol is a tiered process that begins with a rough calculation of the aquatic predicted environmental concentration (PEC) of the new drug. During this Phase I prescreening, substances whose PEC is deemed too low to be of concern to environmental health are ruled out for further assessment. Vitamins, electrolytes, amino acids, peptides, and proteins are exempted by the guidance because they are not tailored active ingredients (unlike, for example, a drug that interacts with a receptor) and thus are deemed "unlikely to result in significant exposure of the environment." However, the guidance does note that certain substances that are likely to cause effects at very low concentrations, such as endocrine disruptors, may need to be addressed regardless of the quantity released into the environment.

Phase II begins with Tier A testing, which aims to determine the aquatic fate and effects of the drug. Its degradability, potential to bioaccumulate, adsorption on sewage sludge, and toxicity to sewage microbial populations are evaluated from the results of standard tests also used in the FDA risk assessment. Also included in Tier A of the EMEA protocol is the long-term testing of fish, *Daphnia* (water fleas), and algae to assess the predicted "no effect" concentration (PNEC) of the new drug for each of these species. The PEC is further refined at this stage in the EMEA assessment by taking into account the pharmaceutical company's projected sales forecast for the drug.

The risk assessment is terminated if the outcome of Tier A testing results in a PEC lower than the PNEC. However, if the PEC is greater than the PNEC in either water, sediment, the sewage treatment plant, or soil (where sewage sludge has been spread as a fertilizer), this indicates a potential risk, and further Tier B testing is initiated. These tests follow the protocol in the *European Technical Guidance Document* to further investigate the risk posed by the drug to the environment. For instance, where there is a potential risk to soil, tests would be conducted to determine the drug's biodegradation in soil, its toxicity to soil invertebrates, and its acute effects on plants and soil microorganisms.

At this stage, data on the drug metabolism and excretion profile may be consulted to allow a more accurate calculation of the PEC and determine whether metabolites need to be tested. The EMEA guidance recommends that metabolites exceeding 10% of the drug residue should be assessed for environmental risk. If this round of testing indicates that the PEC of the drug will be greater than the PNEC, then pharmaceutical companies following the European approach must propose recommendations to limit the drug's impact on the environment.

There are two major differences between the proposed EMEA approach and the existing FDA approach. First, the FDA protocol turns to chronic testing only if acute testing indicates a risk or if there is an indication that the drug could bioaccumulate. The latest scientific research suggests that acute testing is not a reliable indicator of all chronic effects, however, and the EMEA document reflects this finding.

Second, the trigger concentrations of pharmaceuticals that prompt risk assessment under the FDA and EMEA guidance differ by a factor of 10 when dilution is taken into account. "The way the two guidelines express this trigger may be confusing," says Virginia Cunningham, director of environmental sustainability sciences for GlaxoSmithKline. She explains that the EMEA's trigger of 0.01 microgram per liter (µg/L) reflects a surface water concentration, whereas the FDA's 1.0 µg/L trigger reflects an "expected introduction concentration," or the concentration of a compound in sewage effluent.

The EMEA trigger of 0.01 µg/L is calculated from the maximum daily dose of the drug per patient and the assumption that 1% of the population is treated daily with the drug; this is divided by the amount of wastewater per person per day and a dilution factor of 10. The FDA trigger corresponds to a PEC in surface water of 0.1 µg/L, assuming a dilution factor of 10, and is calculated from manufacturers' sales estimates.

The consideration given to metabolites and the provision for the introduction of scientific experts into the risk assessment process—both part of the revisions to the 2003 guidance—are welcomed by scientists. "It allows for experts to be drawn into the discussion and give their opinions rather than be sticking blindfolded to a number," says Evelyn O'Brien, a scientist in the Ecotoxicology Workgroup at the University of Konstanz in Germany and coauthor of a discussion of the draft guideline published in the July 2004 *Trends in Biotechnology*.

One caution added by zoologist Theo Colborn, whose seminal 1996 work *Our Stolen Future* uncovered the dangers of endocrine disruptors in the environment, is that conflict of interests for experts working in academia but funded by drug companies must be revealed. "The important thing is," she says, "that in [the United States] they're selecting experts to do things like this on campuses where the particular department that that individual is working in oftentimes receives tremendous amounts of grant money from the pharmaceutical company. Openly admitting conflict of interest is so important."

The EMEA website notes that members of the agency's scientific committees "are not permitted to have any direct financial or other interests in the pharmaceutical industry. . . . They are required to make an annual declaration of their financial interests and also any indirect interests which could relate to the pharmaceutical industry." Colborn also hails the guidance for including excipients as well as active ingredients in the risk assessment process. For instance, phthalates such as diethyl phthalate and dibutyl phthalate, used as plasticizers in the coating of some site-directed drugs, may be a potential source of phthalates for people taking these drugs, as reported in the May 2004 issue of *EHP*.

Limitations of the Guidance

There are certain serious, though perhaps unavoidable, limitations to the guidance. One is the fact that they are not retroactive. "The only thing that [researchers] are concerned about is that the guidance only concerns those pharmaceuticals that are not yet on the market," says Heberer. "It's our main concern about this guideline, but compared to the situation in the past it's really an advance." But even if future legislation required the environmental risk assessment of drugs already on the market, the big question would be who should do the testing since the originator of a drug is often no longer the main manufacturer.

Another major problem is that monitoring may be difficult. "There are problems detecting certain substances that have been on the market for years," says O'Brien. Examples of such hard-to-detect drugs include the antidepressants known as selective serotonin reuptake inhibitors (which include Paxil, Prozac, and Zoloft). "So the analysis can be quite difficult," she says, "and that's one of the main stumbling features."

Further, it is not clear how drugs that pose risks will be handled, apart from the addition of labels to recommend appropriate disposal of expired drugs. Another emerging area of concern in North America and Europe alike is the disposal of used birth control patches and hormone replacement patches. Because pharmaceuticals can save lives, the guidance does not suggest removing them from the market even when a risk is found.

"I think there's going to be a lot of emphasis on labeling, and also on treatment processes," says Alistair Boxall, a senior lecturer at York University and Central Science Laboratory in England. "So perhaps if you've got a hospital where cancer drugs are being used, it may be that we have to start putting treatment processes on the end of the [sewer] pipes of those hospitals to remove some of the drugs."

Drug take-back programs for expired pharmaceuticals are in place in parts of Europe, so labeling drugs with instructions to return unused portions to a pharmacy makes sense. By comparison, in the United States, the Controlled Substances Act complicates such schemes because it prohibits patients from transferring controlled medicines to anyone other than a law enforcement official. However, a drug return program has recently been legislated (though not implemented) in Maine.

Another limitation, also difficult to avoid, is that the draft guidance only briefly addresses the possibility of additive or synergistic effects, noting that an assessment factor of 10 is applied to the PNEC to account for extrapolation from lab data to field impacts. "It's worth pointing out that the guidance is written as if the concern is for a single drug in isolation," says Christian Daughton, chief of the environmental chemistry branch at the Environmental Protection Agency National Exposure Research Laboratory. "But if a drug shares a common mechanism of action with other drugs, or even other pollutants, there's the possibility for additive effects."

Some scientists and drug companies are concerned that assumptions in the guidance could lead to unrealistic PECs. The initial calculation assumes the worst-case scenario: that the drug is not metabolized or degraded at all, so the full dose ends up in the environment (this is one of 30 points raised by the Pharmaceutical Research and Manufacturers of America in their comments on the guidance). But others worry that actual concentrations in the environment could be higher than the calculated PEC due to the guidance's assumed 1:10 dilution factor for sewage effluent entering rivers. In farming areas, water levels drop precipitously in dry weather when water is drawn for crops and cattle, so the 1:10 dilution factor could be too high. Colborn, a Colorado resident, says, "Most of the river water that's in this part of the West is coming from returned sewage treatment plants." O'Brien argues the same point in cities where the influx of people stretches the capacity of sewage treatment plants.

Another problem noted by O'Brien is that peak or seasonal variations are not taken into account—flu epidemics, drought, or heavy snowfall could temporarily increase drug concentrations in specific places to values higher than the calculated PEC. Colborn also comments that local use of pharmaceuticals differs, reflecting, for example, recent visits by pharmaceutical representatives telling doctors about new drugs. "To estimate that pharmaceuticals will be released homogeneously across a particular region is, I think, mistaken," she says. Daughton addressed these and related issues in greater detail in the May 2003 issue of *EHP*.

One worry for pharmaceutical companies is that the increased amount of testing required could translate into costly delays for the release of new drugs. About 50 new drugs come onto the market in the United States each year, and approximately a dozen of those are predicted to occur above the trigger concentration requiring them to undergo the first level, or Tier A, of risk assessment testing.

But only one new drug in the last few years has gone on to the next level to be tested for environmental risks through chronic ecotoxicity tests, according to Florian Zielinski, a chemist at the FDA Center for Drug Evaluation and Research. "In fact, in the States, almost all pharmaceuticals in the Tier A assessment will come out at under one microgram per liter," says Chris Metcalfe, a professor in the Environmental and Resource Studies Program at Trent University in Ontario, "whereas in the EU there will be a fair number of pharmaceuticals which will move from the Tier A to the Tier B as a result of their lower thresholds." British labs put about 20 new pharmaceutical products on the market each year.

Forging Ahead

Since neither the EMEA guidance nor its U.S. sister document addresses pharmaceuticals already on the market, there is much research into whether wastewater treatment can economically remove pharmaceuticals. Increased retention time within treatment plants, chlorination, ozonation, and the natural reduction of a compound's mass or concentration over time due to processes such as biodegradation all increase the removal of some drugs from wastewater; more advanced treatments such as adding activated carbon or reverse osmosis can remove even more. "But there's never a silver bullet," says Shane Snyder, research and development project manager of the Southern Nevada Water Authority. "There's always a catch."

The catch with ozone treatment is that it forms bromate, which is a regulated disinfection by-product; with chlorination, the catch is that chlorine combines with ammonia in the sewage treatment system to form chloramines, which are not strong oxidants and so cannot break down compounds such as estrogens. However, chlorination can destroy almost all the estrogens if ammonia is removed first, says Snyder. But even with the use of reverse osmosis (which removes pharmaceuticals down to parts per trillion) and the addition of activated carbon, there's the problem of what to do with the retained contaminants.

Although Europe has been at the forefront of recognizing and addressing the potential environmental hazard posed by pharmaceuticals, other countries are perhaps beginning to catch up. In the United States, for example, the Federal Interagency Task Group on Pharmaceuticals and Personal Care Products was formed in September 2004. This group comprises seven federal agencies and is chaired by the FDA. The group had its first face-to-face meeting in July 2005 to identify federal research needs and gaps. One of the questions raised was how much of the estrogen in wastewater comes from synthetic sources.

In Canada, the Environmental Impact Initiative was formed in 2001 in response to growing evidence that pharmaceutical substances are being found in the environment. The initiative, which accepted public comments through September 2005 on proposed options for regulating these substances, may result in new rules for the environmental assessment of substances in products regulated under the Food and Drugs Act, according to Health Canada. Japan is also in the process of formulating a plan for environmental risk assessment of pharmaceuticals with sales exceeding one ton per year.

In the meantime, the EMEA draft guidance is seen as an appropriate response to an emerging issue which includes possible risks not just from pharmaceuticals but also from personal care products. "What has come into the scientific literature is that most pharmaceuticals do not show acute ecotoxicity, so the whole mindset is shifting to chronic toxicity, and I think the EMEA guideline is trying to reflect that," says Cunningham. "None of the people I talk to have a problem with that."

Pat Hemminger

Getting the Lead Out of Electronics

The electronics industry is learning to do without: it is having to abandon one of its long-time staples, lead–tin solder. For decades lead–tin solder has been used to attach electronic components to printed wiring boards. However, with the body of evidence pointing to serious adverse health effects of lead, the search for a replacement has spawned intense effort in the electronics industry and in universities. Now scientists think they may have found some promising leads: solders made of alternative alloys and polymer formulations known as electrically conductive adhesives (ECAs).

The Linchpin of Electronics

Solder is the "linchpin of electronics manufacturing," says Jack Geibig, acting director of the Center for Clean Products and Clean Technologies at the University of Tennessee. "Without it, it's difficult to achieve a proper electronic connection that is durable and reliable."

Lead has been ideal for solder. In fact, says Carol Handwerker, chief of the metallurgy division at the National Institute of Standards and Technology, "The whole electronics infrastructure was designed around the melting point and physical properties of [lead]." Lead is malleable and thus easy to work with, and it doesn't fracture, she says. When lead is combined with tin in the correct proportion (63% tin to 37% lead), the resulting alloy has a low melting point of 183°C, which is another advantage, Geibig says: "If you're not operating at really high temperatures, you have more control over processes, so that the processes aren't sensitive to slight temperature variations, which are costly to control." Low temperatures also mean less strain on the equipment and materials (such as printed circuit board and components) that must be heated as part of the assembly process.

The main impetus for the industry to leave lead behind is a ban on lead in electronics imposed by the European Union. Under the Restriction of Hazardous Substances directive, as of 1 July 2006 lead must be replaced by other substances in electronic equipment. (The directive also bans mercury, cadmium, and hexavalent chromium.) Any electronic components bound for Europe are subject to the ban.

Lead is not a problem when contained in electronic equipment, says Robert Donkers, an environmental counselor for the European Commission who is based in Washington, DC. However, when electronic components are deposited in landfills, he says, people may scavenge for equipment and break it open, or the lead may leach out of landfills and into drinking water. The risk is compounded in countries that receive massive imports of electronic waste.

Sticking with the problem. Electrically conductive adhesives are one alternative to lead–tin solder being tested in the search for healthier electronics.

Gary Meek/Georgia Tech

In China, for example, unprotected workers, including many children, strip recyclables out of electronic components in a cottage industry of sorts [see "e-Junk Explosion" in the April 2002 issue of *EHP*].

Lead exposure, even at low levels, is well known for its harmful effects on children, resulting in lowered IQ. Lead also affects the ability to pay attention. Children exposed to low levels may appear hyperactive and irritable, according to the American Academy of Child and Adolescent Psychiatry. The current maximum allowable level for blood lead in the United States is 10 micrograms per deciliter (µg/dL).

Alternative Alloys

The main approach to replacing lead in solder has been to look for other metals as substitutes. Electronics manufacturers began to look for alternative metals in the 1990s, notes Handwerker, when now-abandoned proposals were being discussed in the United States to ban lead in electronics.

Ronald Gedney, a consultant for the International Electronics Manufacturing Initiative (iNEMI), a technology consortium, has been intimately involved in the search for alternatives. He says that a search by industry experts for possible replacements for lead–tin solder winnowed down 75 metal alloy alternatives to about half a dozen. "We decided the biggest benefit for the industry would be to pick one solder, concentrating our development and research efforts on one alloy and making it work," he says.

The industry eventually selected a tin–silver–copper combination as offering the most reliability and ease to work with as a replacement. The formulation—95.5% tin, 3.9% silver, 0.6% copper—is also known as SAC solder, for the first letters of the chemical symbols of each of the elements (Sn, Ag, Cu). "Tin–silver–copper appears to have at least as good reliability if not higher reliability than tin–lead," says Handwerker.

Furthermore, according to a 2005 draft report issued by the U.S. Environmental Protection Agency titled *Solders in Electronics: A Life-Cycle Assessment*, silver was "rarely encountered above the detection limit" in synthetic landfill leachate created to test the stability of electronics components. Silver—which is regulated as a hazardous material—is toxic to aquatic life.

With a melting point of 217°C, SAC solder also is closest in melting point to the conventional lead–tin solder. This does mean, however, a yet-unquantified increase in energy use. Furthermore, the higher temperature may pose problems for the electronics industry. Higher temperatures mean more stress on components and the entire manufacturing process, notes Geibig. Higher temperatures also mean increases in the time it takes to make products, because more time is required to heat and cool the products during the course of their manufacture.

SAC solder is used widely in the industry today. However, many of the components being made could not withstand the higher temperatures, says C. Michael Garner, director of materials technology operations at Intel: "That required re-engineering and getting new materials, not only for newer products but for older products. All the older products that had been in production for ten or fifteen years had to be converted over to high temperatures." He says it has taken a massive effort to integrate the new solder into production processes.

There are also short-term consequences of using the new solder. Anytime there is a change in materials, there is a learning curve in using the new materials, says Karl J. Puttlitz, who managed IBM's efforts to reduce lead in its products before he retired last year. He anticipates the occurrence of more manufacturing defects as a result of the changeover. "We can expect that at least initially the failure rates [of products] will increase," he says. In fact, he notes the industry has asked for exemptions to the EU lead ban in certain critical electronic components where lives and security might be involved, such as equipment used in hospitals, until a track record is established with consumer goods such as cell phones and digital cameras. (The EU directive does permit exemptions to the lead ban if replacing lead is technically or scientifically impractical or if negative health, environmental, or safety consequences of replacing lead outweigh the benefits of the ban.)

A Stickier Approach

A more experimental alternative to lead–tin solder is the use of ECAs. These are polymers, such as silicone or polyamide, containing tiny flakes of metals such as silver. The polymers adhere to the printed circuit boards, and the metal flakes conduct electricity.

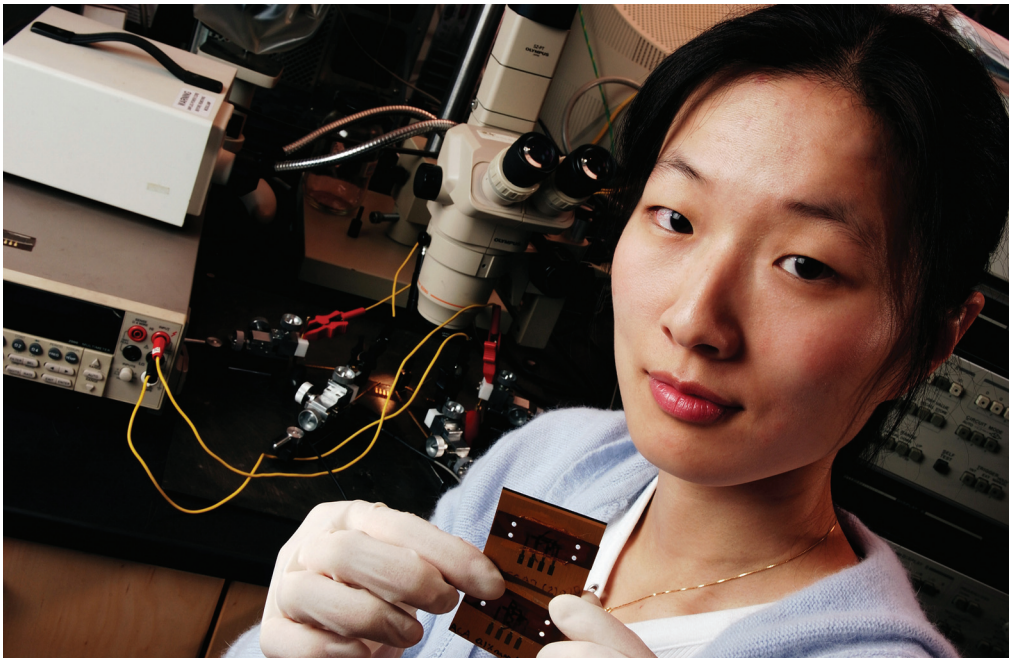
ECAs offer a range of advantages, notes C.P. Wong, a professor in the School of Materials Science and Engineering at the Georgia Institute of Technology who is regarded by many in the field as the leading researcher in this new technology. Silver's electrical conductivity is very high, and its electrical resistance is very low, he points out. "If the current-carrying capability [can be boosted], ECAs can replace solder," he says.

And there is another benefit. The temperature required to apply ECAs to circuit boards is far lower than that required for lead-based solder—150°C compared to 183°C. "You save energy, number one," says Wong. "Number two, you subject all the components to lower temperatures and thus less thermomechanical stress. That enhances their reliability."

Preliminary studies comparing parts using ECAs instead of solder, such as a Finnish study presented in 2000 at the 4th International Conference on Adhesive Joining and Coating Technology in Electronics Manufacturing, suggest that ECAs boast a much tighter bond than solders—perhaps an order of magnitude better, says James Morris, a professor of electrical and computer engineering at Portland State University. But he adds this research has to be replicated before it is regarded as valid.

The alloy alternative. Tin–silver–copper solder offers a safer solder than the lead–tin alloy, and research is continuing to address limitations on its use.

Bernzomatic



Current advances. Researcher Grace Yi Li holds samples of electrically conductive adhesives being studied at Georgia Tech's School of Materials Science and Engineering. Such adhesives may one day replace lead-based solders.

Gary Meek/Georgia Tech

ECAs are available for a small number of applications requiring low power—for instance, liquid crystal displays—though they are not ready for the marketplace in general, where greater amounts of current are needed. Wong is working to enhance their ability to carry current. He is adding molecules of dicarboxylic acid to the silver flakes, which provides a link between the flakes, allowing for efficient and quick conduction of electric current. "It looks like it can be as good as or even better than lead–tin solder. We demonstrated that it works [in a presentation at the March 2005 national meeting of the American Chemical Society], but we still need further research and development," says Wong.

Wong and his collaborators are also using another means to boost the capacity to carry current—self-assembled monolayers. These are single layers of sulfur-containing molecules known as thiols that are attached to gold pads in the electronic device. At less than 10 angstroms (10 ten-billionths of a meter) in length, the molecules chemically bind to the gold pads in the device and the board, providing a direct electrical connection.

Still more work is needed on these structures, however, because they begin to fail structurally if the component heats up above 150°C. And there are other concerns about ECAs. With time, notes Wong, the ability of ECAs to conduct electricity drops, and resistance to electricity increases. Another concern is moisture. "Water is absorbed by polymers, in general," says Morris. That can encourage corrosion, he says, and may cause other as yet unknown problems, he says.

Wong also points to the need for ECAs to become tougher so they can withstand the force of being dropped. One way to do this, says Wong, is to develop polymers that are rubberized and made more elastic, so they won't break. Finally, Garner reiterates that these materials have not been reliable for carrying moderate to high amounts of current under normal operating conditions.

Wong and Morris are optimistic that with more research and development, ECAs can be successful alternatives to lead–tin solder. And Puttlitz does see a place for them in consumer electronics such as cell phones and digital cameras, which are not "mission critical" applications where reliability is a matter of life and death as in medical monitoring equipment or aircraft electronics.

Solder Replacement Soldiers On

Even as efforts to replace lead in solder move ahead, there still appear to be concerns about the impact that newly implemented metals will have on human and environmental health. "The alternatives to lead have not been researched as well as lead in terms of potential health and environmental impacts," says Oladele A. Ogunseitan, a professor of environmental health, science, and policy at the University of California, Irvine. "When the Europeans said industry must get rid of lead, they didn't say you must replace lead with something that is obviously safer," he notes wryly. It is important, he adds, to keep looking for lead alternatives that are environmentally benign.

Indeed, the draft *Solders in Electronics* report indicates that no metallic alternative to lead is free from environmental concerns. For instance, whereas lead may pose a greater public health problem than SAC solder, the latter uses noticeably more energy than lead–tin solder.

But the presence of today's substitutes is good enough for Donkers. "Since there are alternatives, we have chosen not to have lead in the products anymore," he says. And while he does acknowledge that there are relatively few data on the impact of the current lead solder alternatives, he asserts that "in terms of active policy, you cannot always wait till you have complete certainty, because in the meantime a lot of people get exposed [to lead]."

Harvey Black

Suggested Reading

Geibig JR, Socolof ML. 2005. Solders in Electronics: A Life-Cycle Assessment (Draft). Environmental Protection Agency, Office of Pollution Prevention and Toxics. Available: http://www.epa.gov/dfe/pubs/solder/lca/index.htm.

Li Y, Moon K, Wong CP. 2005. Electronics without lead. Science 308(5727):1419–1420.

Puttlitz KJ, Stalter KA, eds. 2004. Handbook of Lead-Free Solder Technology for Microelectronic Assemblies. New York, NY: Marcel Dekker.

Schoenung JM, Ogunseitan OA, Saphores J-DM, Shapiro AA. 2004. Adopting lead-free electronics: policy differences and knowledge gaps. J Ind Ecol 8(4):59–85.

Prelude to Intersex in Fish

Identifying a Sensitive Period for Feminization

Field studies have shown a high occurrence of intersex (the presence of both male and female characteristics) and ovotestis (the presence of eggs in the testis) in wild populations of a fish known as roach (*Rutilus rutilus*) in rivers in the United Kingdom that are downstream from wastewater treatment plants. Furthermore, studies have demonstrated that intersex males are less fertile, which may have population-level effects. However, to date, scientists have been unable to induce intersex in male fish with controlled exposures to wastewater effluents. A study conducted at The University of Exeter now shows that the sensitive period for feminization of the reproductive duct—in which the male testis forms an ovary-like cavity—may occur earlier than previously thought, and raises new questions about the conditions that lead to actual germ cell disruption [***EHP* 113:1299–1307**].

Many questions persist about the causes of and the most vulnerable life stages for various types of sexual effects induced by estrogenic chemicals in wastewater effluent. In this study, the researchers collected two different U.K. wastewater effluents and exposed wild roach at two life stages: during early life and development of the gonads (from fertilization up to 300 days post-hatch) and as adults producing germ cells following annual spawning. These adults included one group of fish that had been raised in clean water and another that had hatched and grown to maturity in the wild.

Both effluents induced synthesis of vitellogenin (an estrogen-dependent yolk precursor and biomarker of estrogen exposure) at both life stages, with the extent of this induction correlating with the steroid estrogen content of the effluent. Previous studies have demonstrated that feminization of the sperm duct to form an ovary-like cavity occurs when exposure to effluent comes during the time of sexual differentiation, which in roach occurs from 50 to 150 days post-hatch. This study showed alteration of the sperm duct with an exposure earlier in life, from fertilization to 60 days post-hatch, before any signs of sexual development appear. The alteration, furthermore, was permanent, persisting even after 240 days' maintenance in clean water after exposure.

However, no ovotestis was observed in any of the juvenile fish. There was also no evidence of ovotestis in post-spawning adult male roach raised in a clean environment and subsequently exposed to effluent. There was evidence that the wild males had previously been exposed to estrogenic stimuli, as some of males had ovotestis when the study began. The severity of this condition increased slightly during the study period, but the increase occurred across both exposed and control fish and thus appeared unrelated to the study effluent exposure.

The authors suggest possible explanations that need further study—one is that ovotestis is induced only by effluents with greater levels of estrogenic chemicals than those used in the study. The researchers evaluated the effluents for content of two chemicals previously implicated in causing intersex—steroidal estrogens and alkylphenols—and found that these levels were similar to concentrations reported in wastewater effluents in the United Kingdom and worldwide. They emphasize that chemical content and interactions ideally should be taken into account when trying to determine the conditions that lead to sexual effects.

The results of these studies raise the possibility that ovotestis may be a result either of longevity of exposure or of programming in early life that manifests itself as fish mature sexually. Previous findings from the authors support this idea by showing that the severity of intersex increases with age. The authors are further exploring these possibilities now with a laboratory study of roach that includes an environmentally relevant estrogen exposure of two years' duration. **–Angela Spivey**

Shift in Sex Ratio

Male Numbers Sink in Great Lakes Community

Sex ratio—the proportion of male to female live births—can be an important indicator of the reproductive health of a population, whether animal or human. This figure is typically fairly constant. For example, the worldwide human sex ratio ranges from 102 to 108 male births for every 100 female births; in other words, male babies make up about 50.4–51.9% of live births worldwide. Now, however, investigators have documented a significant skewing of the human sex ratio in a population located in a heavily polluted Great Lakes area [***EHP* 113:1295–1298**].

In response to concerns about a shifting sex ratio among members of the Aamjiwnaang First Nation community near Sarnia, Ontario, a team of Canadian researchers examined birth records for the group from the years 1984–2003 as part of a broader community-based investigation. The researchers discovered that, as community members had suspected, there had been a significant and precipitous shift in the sex ratio.

The expected sex ratio in Canada is 51.2% male babies to 48.8%

Chemical culprit? The Sarnia–Lambton area in Ontario is home to Chemical Valley as well as the Aamjiwnaang First Nation community, which has experienced a significant skewing of the ratio of male to female babies born in recent years, leading some to question whether environmental exposures may be to blame.

Dennis MacDonald/Alamy

female babies. For the period 1984–1992, that ratio held fairly constant among this community. In the period 1993–2003, however, male babies made up only 41.2% of live births. The five-year period from 1999 to 2003 showed an even sharper decline, with male babies making up 34.8% of live births. According to the researchers, although there is normal variation in sex ratio within populations, the deviation in this case appears to be outside the normal range.

Although there is as yet no direct evidence linking this human sex ratio decline to environmental exposures, the circumstantial evidence suggests there may be a connection. The Chippewas of the Aamjiwnaang reserve reside within the St. Clair River Area of Concern, situated immediately adjacent to several large petrochemical, polymer, and chemical industrial plants. The area is one of Canada's largest concentrations of industry. Prior soil and sediment assessment has shown that the reserve land is heavily contaminated with pollutants such as polychlorinated biphenyls, polyaromatic hydrocarbons, hexachlorobenzene, mirex, a variety of potentially toxic metals, volatile organic compounds, phthalates, and dioxins; many of these are known or suspected endocrine disruptors.

As the investigators point out, past studies have documented reproductive outcomes in wildlife populations within the same region, including reduced hatching success, altered sexual development, and changes in sex ratios. Scientific suspicion has long been focused on environmental endocrine disruptor exposures as the root cause of these effects.

The authors acknowledge that there are many other potential factors that could influence the declining sex ratio they describe. But the combination of close proximity to industrial facilities emitting known endocrine-disrupting chemicals and the documented adverse reproductive outcomes in wildlife populations in the region leads them to conclude that further investigations are warranted into the types and routes of chemical exposures—via air, water, food, soil, and sediment—for this population. A community health survey designed to explore health concerns among residents of the reserve is in progress, including information on potential covariates that may influence the sex ratio, such as parental age or smoking. **–Ernie Hood**

Lead in Cocoa Products

Where Does Contamination Come From?

Manufactured cocoa products frequently have higher lead concentrations than other foods, even though cocoa beans, the main ingredient, have some of the lowest reported lead levels for any natural food. In 2001 the Codex Alimentarius Commission, an international body based in Rome, proposed reducing the maximum permissible level of lead in cocoa products by half, to 100 nanograms per gram (ng/g) for cocoa butter and 1,000 ng/g for cocoa powder. At a March 2002 meeting in West Africa, where most of the world's cocoa supply originates, producers agreed that to reduce lead in their products, they needed research to identify the source of contamination. Now a U.S.–Nigerian research team has uncovered some of the first clues about where the lead is coming from [***EHP* 113:1344–1348**].

Lead contamination of candies has been recognized as a problem since 1820, when a British study found the poison widespread in London confectionary products. In recent years, documented lead content in candy has ranged from a mean concentration of 21 ng/g in milk chocolate bars in an Australian study to an average of 1,920 ng/g in chocolates seen in research in India. In Nigeria, a 1999 study found an average of 310 ng/g lead in cocoa powders. (For comparison, the mean U.S. lead concentration for apples is 20 ng/g, 200 ng/g for dry table wine, and 100 ng/g for canned pineapple.) Lead is known to cause anemia, muscle weakness, and brain damage, with children particularly susceptible to effects.

PhotoEuphoria/iStockphoto

Searching for the golden ticket. Cocoa beans are naturally low in lead, but cocoa products frequently are not. Now researchers are following new clues to identify the source of the contamination.

In the current study, the researchers studied the lead isotopic compositions of cocoa beans and shells from six farms in Nigeria's top three producing states to determine if soil or farm sources might be the cause of lead contamination. The team took bean and sediment samples and homogenized them to make composites for soil, beans, and cocoa bean shells for each farm. They analyzed lead concentrations using high-resolution inductively coupled plasma mass spectrometry to make preliminary isotopic measurements, followed by thermal ionization mass spectrometry measurements.

The lead concentrations for cocoa beans ranged from less than 0.103 to 1.78 ng/g, averaging 0.512 ng/g—among the lowest lead concentrations reported for any food. The average concentrations found in the cocoa bean shells, however, was about 320-fold higher (160 ng/g). Soils showed a range of isotopic compositions overlapping those of the shells.

The cocoa bean shells all had an extremely similar isotopic composition, indicating a singular source of contamination, perhaps leaded gasoline. The authors conclude that although the soil may have caused a small degree of the contamination, the narrower range of isotopic composition in the shells suggested the more singular source of contamination was the true culprit. According to the paper, cocoa bean shells are known to be very effective at removing lead from solutions. So, although they provide excellent protection of the bean inside, the shells may also serve to contaminate the cocoa beans during fermentation or drying.

Eating for two, thoughtfully. Despite the threat posed by high mercury levels in certain types of fish, new findings suggest a healthy prenatal diet most likely should include some low-mercury seafood.

The team also compared the cocoa beans with finished cocoa products and found much higher lead concentrations and greater variability in the isotopic composition among the finished products. They therefore deduced that most of the contamination occurred after the cocoa left the farm stage.

The researchers conclude that while cocoa bean shells may be one source of lead, most contamination occurs during shipping or processing of the beans and in manufacturing. Further research on those stages of the process will help to isolate the source. **–David A. Taylor**

Moms and Mercury

Fine-Tuning Fish Consumption During Pregnancy

Due to ongoing concerns that high mercury intake via fish can cause adverse neurologic effects in the developing fetus, the U.S. Food and Drug Administration now recommends that expectant mothers should limit their consumption of fish to two or fewer meals per week. But pregnant women shouldn't throw the baby out with the bathwater. A new study by a group of Harvard researchers suggests that this advice, which could result in many pregnant women eliminating fish from their diets altogether, may be denying some babies substantial neurocognitive benefits gained from important nutrients found in fish, such as n-3 polyunsaturated fatty acids [*EHP* **113:1376–1380**].

The scientists sought to determine whether fish consumption during pregnancy is harmful or beneficial to fetal brain development. To do this, they examined associations of maternal fish consumption during pregnancy, maternal hair mercury levels (a sensitive marker of organic mercury body burden) at delivery, and infant cognition at age 6 months. Study subjects were 135 mother–infant pairs who participated in Project Viva, a prospective pregnancy and child health cohort study in eastern Massachusetts.

The mothers completed questionnaires about fish consumption during their second trimester. That period of time was used to best coordinate temporally with the mercury exposure reflected in maternal hair samples, which were taken at delivery. The questions concerned how much and what categories of fish (canned tuna, dark meat, light meat, shellfish) the women ate.

Mothers consumed an average of 1.2 servings of combined fish categories per week. Their mean hair mercury level was 0.55 part per million (ppm), with 10% of the samples higher than 1.2 ppm, the current U.S. reference dose. Fish consumption was directly correlated with hair mercury levels.

Infant cognition was assessed using a test called visual recognition memory (VRM). In the VRM test, which has been shown to correlate with later IQ, the child is first shown two identical photographs of an infant's face, side by side, at a standardized distance. Then, one of the photos is replaced with a photo of another infant's face. By tracking the percentage of time the baby looks at each photo, a novelty preference score is derived, reflecting the infant's ability to encode a stimulus into memory, to recognize that stimulus, and to look preferentially at a novel stimulus.

Mean VRM score among the children was 59.8, with a range of 10.9–92.5. After accounting for characteristics such as maternal age and education level, higher fish intake was found to be associated with higher infant cognition, especially after adjusting for mercury levels, which had a dose-dependent negative impact on the infants' cognition. For each additional weekly serving of fish, the infants' VRM score was 4.0 points higher. Conversely, the researchers found that an increase of 1 ppm in hair mercury was associated with a decrement in VRM score of 7.5 points. The babies with the highest cognition scores were from mothers who had eaten more than two weekly fish servings but had mercury levels of 1.2 ppm or less.

Although the results may seem contradictory, the authors suggest that the most cognitive benefit is derived by mothers eating fish types with the combination of relatively little mercury and high amounts of beneficial nutrients. However, since the study assessed maternal fish consumption of four broad categories, there is no information presented on associations with specific types of fish. The researchers say that future studies could incorporate more detailed dietary information to help pregnant women make informed decisions about which fish meals are better or worse for their children's cognition.

Ultimately, the message behind these findings is that pregnant women should continue to eat fish, but should try to choose varieties known to be low in mercury and high in nutrients, such as canned light tuna and sardines. Finding the most appropriate balance between risk and benefit may be challenging in this situation, but given the strong associations found in the current study, making the right decisions about which fish to eat during pregnancy, and how often, may be even more important than previously suspected. **–Ernie Hood**

Digital Vision