

areas and gaps that the conference identified as priorities for the 21st century.

Among exciting future research projects to emerge from the conference, Kenneth Olden, director of the NIEHS, unveiled his plan to create the Environmental Genome Project, a broad, multicenter effort to learn how genetic variances in individuals and populations account for differences in susceptibility to diseases with environmental triggers. The project would sequence about 200 known environmental disease susceptibility genes from five main categories: genes controlling the distribution and metabolism of toxicants, genes for the DNA repair pathways, genes for the cell cycle, genes for the metabolism of nucleic acid precursors, and genes for signal transduction systems controlling expression of genes in other classes.

## New Questions on Genomic Instability

Cancer researchers have long been puzzled by the discordance of two related observations: that perhaps as many as eight distinct genes must be somehow mutated or altered in order for cancer to occur, and that such mutations appear to occur very rarely and are normally patched up quickly by DNA repair enzymes. The question, then, is how can it be that, out of the 100,000 or so genes in the human genome, the precise mutations needed to transform a normal cell into a cancer cell occur so frequently.

The answer may lie in the relatively new field of genomic instability. Recent research from several laboratories in the United States and abroad indicates that cells exposed to certain carcinogens, particularly radiation, appear to enter a state in which the rate of mutation increases and may stay elevated for 50 or more cell generations following exposure. This state of increased genomic instability may provide the conditions under which cells accumulate the number of mutations necessary to progress to cancer.

John Little, chairman of the Harvard School of Public Health's department of radiobiology, was one of the first researchers to see evidence of genomic instability. In the early 1990s, Little found that some irradiated cells that appeared to have escaped the effects of radiation retained a susceptibility to unexplained gene mutations. Not long after, Eric Wright of the National Research Council in England found a similar elevated rate of chromosomal abnormalities in the progeny of irradiated cells.

Relationships between these two types of genetic disruption may help elucidate this phenomenon. Recently, Little selected slow-growing HPRT gene mutants from cells that had been allowed to divide 25 times after

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### On the Air

For the first time in 10 years, the EPA is revising and updating the Clean Air Act, placing stronger limits on ozone and particulate matter emissions. The proposed rules, which the EPA plans to formalize in June 1997, are designed to reduce the concentration of smog-forming ozone in the atmosphere and to limit emissions of particulate matter smaller than 2.5 microns in diameter (PM<sub>2.5</sub>), a pollutant particularly harmful to human health.

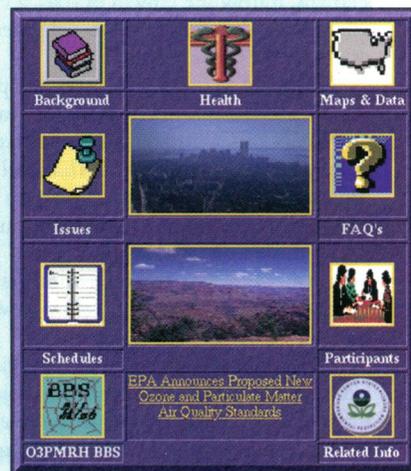
By the EPA's own estimation, nearly 122 million Americans live in counties with air quality that will be considered unsatisfactory under the new ozone standard. This number does not include additional counties that will be out of compliance with the new PM<sub>2.5</sub> rule.

The EPA maintains a site on the World Wide Web that deals specifically with issues surrounding the proposed air standards, located at <http://ttnwww.rtpnc.epa.gov/html/ozpmrh/faca-home.htm>. The site, which is maintained by the Subcommittee for Development of Ozone, Particulate Matter, and Regional Haze Implementation Programs, connects users to resources ranging from EPA press releases to original scientific data. The subcommittee is a part of the Clean Air Act Advisory Committee that the EPA established in 1990 to assist the Office of Air and Radiation on policy and technical issues associated with implementation of the Clean Air Act in that same year.

For a basic introduction to the EPA's national ambient air quality standards (NAAQS) and the process through which they are revised and implemented, users should follow the Background link on the home page. The EPA Announces Proposed New Ozone and Particulate Matter Air Quality Standards link is connected to pages that describe the newly proposed standards. Some of these pages contain press releases and general information on when and how the standards will be implemented, including how the EPA will monitor ozone and PM<sub>2.5</sub> levels. Other pages discuss the health effects of air pollutants and describe what areas of the country will be affected by the new rules. The site also provides links to directions for commenting on the revised standards, including mailing instructions, a toll-free telephone number, and several e-mail links.

Under the Maps and Data link on the home page, maps show which areas of the United States would be out of compliance under different averaging time scenarios for the proposed PM<sub>2.5</sub> and ozone concentration standards (for example, whether the EPA bases ozone exceedances on average concentration over one hour or eight hours). The data used to create these maps can be downloaded from the EPA site by clicking on icons near the maps. The Regional Haze and Visibility section at the bottom of the Maps and Data page shows where in the United States ozone pollution has significantly decreased people's ability to view and enjoy the landscape.

Other links on the home page connect users to resources that provide a more in-depth view of the revision process. The Issues link is the doorway to a collection of original papers used by the EPA in drafting the new standards. Some papers discuss how boundaries should be drawn separating different compliance zones, while others discuss implementation dates and the economic incentives and sanctions that will be used to enforce the new PM<sub>2.5</sub> and ozone limits. The Schedules link on the home page provides meeting dates and a timeline for the activities of the Subcommittee for Development of Ozone, Particulate Matter, and Regional Haze Implementation Programs, while the Participants link lists the members of each subgroup involved in the NAAQS revision process.



radiation exposure. Over the following 20 cell generations, 10–40% of the offspring of these HPRT mutant clones contained chromosomal aberrations, compared to only 2–3% among progeny of non-HPRT mutants.

“The classical view of gene mutations is that each is independent, and that the probability of there being two multiple mutations in a cell is just a computation of the frequency of each,” Little says. “Our results could mean that, among cells exposed to radiation, there’s a range of induced instability. Perhaps, during the growth of cells, a change takes place that makes some daughter cells more unstable than others.”

When Andy Grosovsky, associate professor of environmental toxicology at the University of California at Riverside studied genomically unstable cells that failed to express the thymidine kinase gene on chromosome 17, he found two different causes: a point mutation in the gene, and a chromosomal deletion or recombination resulting in loss of heterozygosity and slow growth of the cell.

“This would indicate that instability is likely to have an effect on genes that are sensitive to inactivation by loss of heterozygosity,” says Grosovsky. “Many tumor suppressor genes, such as the gene for retinoblastoma and the *p53* gene, are recessive, and could be inactivated in this way.”

Thea Tlsty, director of molecular pathology at the University of California-San Francisco, has been investigating the role of *p53*, a gene already implicated in many cancers, in genomic instability. The *p53* gene is responsible for shutting down cell growth during DNA repair, and Tlsty has shown that *p53*'s absence is associated with increased mutation and chromosomal abnormality.

“Think of the cell like a car,” says Tlsty. “In a cell that has a mutation in *p53*, there’s no way to put the brakes on. So normally, for example, if a cell gets irradiated, *p53* puts on the brakes and the cell repairs the lesions caused by radiation. If the damage gets repaired this way, you’re okay, but if you don’t have brakes, you’re in trouble.”

“It’s a very interesting phenomenon, and the emphasis is on ‘phenomenon,’ because the underlying mechanisms aren’t at all understood,” says Richard Pelroy, the radiation effects branch program director at the National Cancer Institute’s division of cancer biology. In 1996, the NCI and NASA jointly earmarked \$2 million for further study of this genomic instability, and 34 grant proposals are currently under consideration.

The NCI/NASA project will focus on understanding the relationship between high

energy particles and genomic instability. Little has observed a “bystander effect” that occurs when cell cultures are exposed to high-density radiation particles, such as alpha radiation. Although only a few cells are actually traversed by alpha particles, many adjacent cells appear to exhibit signs of genomic instability for many generations afterward. Astronauts on extended space flight undergo increased exposure to heavy particles, which may likewise render cells unstable.

How instability might be communicated from cell to cell within one generation and between generations of cells is an important area for investigation. Wright has observed an increase in free oxygen radicals, normal by-products of human metabolism that can bind and break DNA, in genomically unstable cells. Several laboratories are investigating this as a possible pathway for the propagation of genomic instability.

“If it’s true that bystanders to irradiated cells are unstable, it could have a major effect on our understanding of the effects of low-dose radiation,” Little says. “This field has the potential to significantly change the way we look at cancer induction.”

## Priorities for Endocrine Disruptor Research

Concerns that some chemicals may disrupt the endocrine system causing cancer and reproductive problems prompted the creation of a federal working group in late 1995 to evaluate research needed to understand what threat endocrine disruptors pose. That group has since identified nine research priorities and inventoried federally funded studies of endocrine disrupting chemicals. This inventory identified nearly 400 projects and revealed an excessive emphasis on studies of PCBs and dioxins, said Lawrence W. Reiter, director of the EPA’s National Health and Environmental Effects Research Laboratory, who chairs the group.

Reiter spoke in Seattle at an American Association for the Advancement of Science session devoted to efforts by the White House’s Committee on Environment and Natural Resources (CENR) to develop coordinated research strategies in five areas: national environmental monitoring and research; natural disaster information and mitigation; seasonal-to-interannual climate change; North American research on tropospheric ozone; and endocrine disruptors. The first four areas include established threats to human health; not so endocrine disruptors. “The verdict is still out on how important this [area] is,” said Jerry Melillo, associate director designate for environment at the White House Office of Science and Technology Policy.

Nonetheless, evidence that domestic animals and wildlife have suffered adversely from chemicals that interact with the endocrine system, and fears that humans might also, stimulated the CENR’s attention. Its endocrine disruptors working group has identified three categories of research needs—methods, models, and measurements—divided into nine subcategories.

Concerning methods, researchers need bioassays that can identify endocrine disruptors and characterize their effects, and biomarkers that can look at both exposures and outcomes.

Scientists need models to better understand endocrine-system regulation across species and how chemicals might disrupt hormonal functions. Risk models are also needed for both exposures and outcomes, as well as models to assess biological interactions of mixtures of chemicals.

Measurement needs include better exposure-determination requirements and follow-up; multidisciplinary research and better coordination between laboratory and field work; developing sentinel species; and databases for consolidating bioassay results, looking at spatial and temporal trends in environmental chemicals, assessing field data on hormonal and endocrine disruptor levels, and tracking ongoing research.

The working group identified 394 endocrine disruptor projects funded by 14 federal agencies. The NIEHS topped the list with 93 studies, the National Cancer Institute had 59, and the EPA had 51. In all, 272 projects focused on human health, 70 on ecology, and 52 on exposure. Of the nine subcategories of research needs, four—biological interactions of mixtures, multidisciplinary research, sentinel species, and database development—account for only 6% of the projects. “Clearly, these four are under-represented,” Reiter said.

By topic, 178 studies looked at reproduction and development, 97 at carcinogenesis, 83 at neurologic effects, 37 at immunologic questions, and 98 at other issues. This ranking generally “follows the recommendations forwarded by a variety of workshops that have looked at the research in this area,” Reiter said. Seventy-one percent of the studies focused on PCBs, dioxins, and DDT and its main metabolite. A clear need exists, Reiter said, “to move away from the preoccupation” with these three chemical classes and “to begin to look at other environmentally relevant chemicals.”

Both the research needs and inventory lists are accessible on the Internet at ([www.epa.gov/endocrine](http://www.epa.gov/endocrine)).