Sex Matters

Exploring Differences in Responses to Exposures

Leading researchers across the spectrum of toxicology gathered at the NIEHS on 15 October 2002 for an in-depth roundtable discussion on the biology of sex differences in environmental health. Cosponsored by the NIEHS and the Society for Women's Health Research, an advocacy group located in Washington, D.C., the one-day conference examined how sex interacts with environmental exposures to yield sometimes-different health effects in men and women.

A primary goal of the conference was to outline how a person's sex influences his or her environmental health—teasing out how biological and societal differences in men's and women's lives impact their health. "Environmental health lies right in the middle of the great tangled hairball of research," says Sherry Marts, the society's scientific director. "Sex is a crucial biological variable that needs to be looked at at all levels and in all organ systems. Sex matters."

The conference was one of a series sponsored by the society to help establish an agenda for understanding the influence of sex and gender across the life sciences. The series expands on a 2001 Institute of Medicine report, *Exploring the Biological Contributions to Human Health: Does Sex Matter?*, which the society helped initiate and sponsor. Other series roundtables have addressed sex differences in cardiovascular health and disease, immunity and autoimmunity, and prenatal development. "We want to encourage that sex differences be part of research, all research, integrated into everything," explains society president Phyllis Greenberger.

Understanding Sex Differences

The first half of the October roundtable focused on traditional and newly developed scientific tools and how they can enhance understanding of sex-specific responses to environmental exposures. James Huff, an NIEHS senior investigator, discussed bioassays in chemical carcinogenesis. The picture is complicated. "Roughly half the chemicals we test have a tumor effect at one site in one sex in one species," Huff reported. Many cancer differences in animal bioassays, he said, likely reflect differences in the sex-hormonal biological context as it may be impacted by environmental exposures.

Although highly effective in developing preventive health strategies, animal-based bioassays also have weaknesses, especially the time and animal facility costs involved in whole-organism work. Expanding these assays to cover effects in both sexes would increase costs further by increasing the number of animals involved. However, these bioassays clearly signal sex differences in response to environmental exposures, and have led to better biologic understanding of such differences.

Mary Jane Cunningham, director of discoveries at Molecular Mining Corporation, a Kingston, Ontario–based data analysis and predictive modeling technology company, discussed methods that may overcome those barriers. She described gene expression microarrays and advanced data mining and analysis methods allowing rapid detection and comparison of the effects of environmental exposures. "It takes only three to four days to obtain the expression of ten thousand or more genes using arrays, and only a matter of minutes to analyze the data," she said. "These technologies are available now. However, when the cost of the arrays is reduced or [they] become more efficient, you can afford to allow for testing of both sexes." (Others are not so optimistic, however, and believe that microarray technologies will take enormous funding, resources, and time to develop and validate.)

Kent Hunter, an investigator at the National Cancer Institute, described work using quantitative trait genetics, a breeding approach that untangles processes controlled by multiple genes to elucidate how environmental factors can affect development of sex-specific cancers. He has found that how individuals differ in one trait—expression of the tumor suppressor *Atm*—may help determine whether breast cancer stays dormant or metastasizes. Complicating the picture is the fact that *Atm* is sensitive to caffeine, a common environmental exposure for both men and women. The interplay between environmental factors and individuals' genes is key, Hunter said: "The exposure is critical to development of disease. You can be susceptible to a disease, but if you're not exposed, you won't develop the condition."

Brent Palmer, a University of Kentucky associate professor of environmental biology, complicated the picture further with a discussion of endocrine disruption, showing that even without genetic factors, sexually distinct environmentally induced effects can propagate to subsequent generations. Endocrine disruptors, he said, can both mimic and thwart the actions of steroid sex hormones. These compounds can have profound effects, especially during critical periods of fetal and childhood development, because they mimic or antagonize the effects of sex hormones. Sexual development and adult fertility may be affected, as well as processes such as immune function and development of cancer. Further, because they can affect reproduction and germ cell development, their effects can be multigenerational, with germ cell damage in a parent emerging in later children.

Other discussion centered on the concept that genetics are involved in only 5% or less of cancers. Thus, says Huff, the environment represents a much greater determinant in environmentally associated disease.

Discussants agreed that there is no technology that will soon answer the question of how the sexes differ. Nevertheless, efforts should be made to evaluate the effect of environmental agents on both sexes, and to elucide the effects that environmental agents, including pharmaceuticals, have on sex-specific health problems.

How Sex Matters

The second half of the conference focused on specific diseases. Allen Silverstone, a professor of microbiology and immunology at the State University of New York/Upstate Medical University in Syracuse, highlighted immunostimulation and -suppression by environmental agents. Ellen Silbergeld, a professor of epidemiology at the Johns Hopkins Bloomberg School of Public Health, brought up the prospect of environmental factors as "coconspirators" in autoimmune disease—not causing diseases by themselves, but helping diseases arise when they otherwise might not. She gave the example of mercury, which accelerates the development of lupus erythematosus, a disease that occurs 8–10 times more often in women than in men. In mouse studies, the metal stimulated the disease even when present at concentrations 50- to 100-fold lower than those typically judged toxic.

EyeWire

Mary Beth Martin, a Georgetown University Medical Center associate professor of oncology, examined the role of cadmium as an estrogen mimic that can activate sex hormone receptors. Bernard Weiss, a professor of environmental medicine at the University of Rochester School of Medicine, rounded out the session with comments on the cognitive and behavioral consequences of environmental exposures, particularly metals. Even without toxic exposures, males and females behave and perform differently on many tasks, and men's and women's brains are anatomically distinct, he said.

"Experimenters for the most part have not tended to ask why [these differences occur]," Weiss said. "It's astonishing when you realize that women suffer from clinical depression at two to three times the rate of males." To begin sorting out how toxic exposure can affect behavior, Weiss is studying the effect of endocrine disruptors on wheel-running in rats. Running in a wire wheel is a behavior both male and female rats enjoy, as judged by their willingness to seek it as a reward. Wheel-running varies with the estrous cycle in female rats; they run more as their estrogen levels rise, and their running patterns change on exposure to endocrine disruptors.

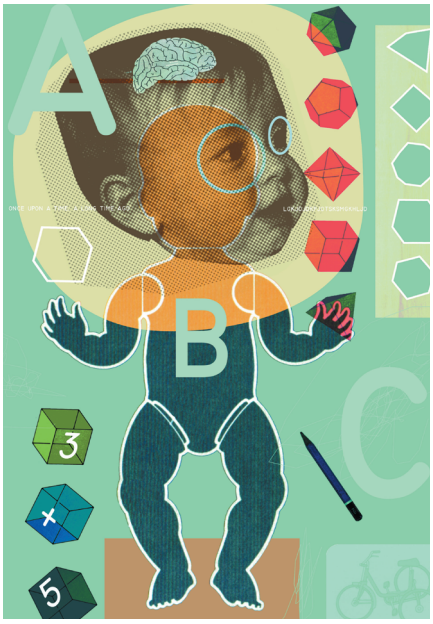
Although it is clear that men and women often respond differently to toxicants, the discussants agreed that predicting just how the sexes will respond, and when they will respond differently, has not proved simple because, Silverstone said, of the limited amount of data at this time. One approach to improving understanding of critical differences between the sexes would be to ask that grant makers require researchers to include both sexes in both basic experimental research and animal studies. "It's only been recently that when you're doing human NIH research with institutional review board approval you've had to include women," Silverstone said. Animals studies do not yet even have the same requirement.

The researchers agreed that bringing together insights from a broad group of sciences and scientists is key to better understanding sex differences, despite barriers to collaborative science, including a tax in which grant overhead is collected by each of the institutions involved in a collaborative project. Fundamental needs include more funds and more personnel to invest in whole-organism approaches and translation of laboratory results into medical practice. "We need to know in terms of clinical care what works and what doesn't," Greenberger said. "There's plenty we don't know about women. There's plenty to be learned."
–Victoria McGovern

New Thyroid Theory

How Maternal Hormone Affects Developing Brains

When does a fetus's developing brain become sensitive to thyroid hormone? What developmental processes are affected by thyroid hormone, and how do these effects occur? How can toxicologists use these insights to evaluate environmental contaminants that affect the thyroid system? These are the questions that clinicians, basic researchers, and toxicologists addressed at a recent conference, "Thyroid Hormone and Brain Development: Translating Molecular Mechanisms to Population Risk," held 23–25 September 2002 in Research Triangle Park, North Carolina. The international conference was sponsored by the NIEHS, the U.S. Environmental Protection Agency, the NIH Office of Rare Diseases, the Agency for Toxic Substances and Disease Registry, the American Chemistry Council, and the Center for Neuroendocrine Studies at the University of Massachusetts, Amherst.



Digital Vision

Neonatal thyroid hormone plays a major role in brain development. It is widely known that a lack of thyroid hormone late in pregnancy and the first months of a child's life results in congenital hypothyroidism. If untreated, this condition results in moderate to severe mental retardation, growth failure, deafness, and neurological problems. Those symptoms can be ameliorated by administering synthetic thyroid hormone before the child is three months old. After that, it's too late—the defects are permanent.

Until recently, endocrinologists believed thyroid hormone did not play a significant role in brain development before late pregnancy, says conference cochair Tom Zoeller, a biologist from the University of Massachusetts, Amherst. But a growing number of research scientists and clinicians are starting to recognize earlier windows during which thyroid hormone plays critical roles in fetal brain development. This controversial idea means that maternal thyroid hormone may play a direct role in fetal brain development—it may, in fact, act directly on the developing brain before the fetus begins making its own thyroid hormone. "We're seeing a paradigm shift to say that prenatal effects from thyroid hormone occur earlier during development than previously thought," says Zoeller.

Evidence supporting the direct impact of a mother's thyroid hormone on her child's brain development comes from epidemiological studies, patient reports, and, most recently, from basic research that uses experiments with mice to understand the molecular mechanisms for thyroid hormone action on the brain.

The best-known epidemiological study of maternal thyroid hormone and fetal effects was published by James E. Haddow and colleagues in the 19 August 1999 issue of the *New England Journal of Medicine*. The findings of this study suggested that untreated mild thyroid failure in a mother may reduce her child's IQ scores and other measurements of intelligence, aptitude, and visual–motor skills.

Neuropsychologist Joanne Rovet of Toronto's Hospital for Sick Children looks for windows of vulnerability by studying mothers and children with thyroid disorders. After more than a decade of study, she finds that maternal thyroid hormone deficiency early in fetal development is correlated with later problems with visual attention and gross motor skills. Hormone deficiency later in pregnancy increases the risk of fine motor deficits.

Recent experimental studies have begun to explore a complex molecular mechanism that regulates concentration of active thyroid hormone and the control of gene expression in the brain. But basic science still has a long way to go to fully understand thyroid hormone action during development. For example, mice in which thyroid hormone receptors have been knocked out do not show the effects of congenital hypothyroidism, according to Fred Wondisford, chief of the University of Chicago's thyroid unit. These mice have fairly normal central nervous systems except for having profound deafness and smaller cerebellums.

Current understanding of the molecular mechanisms for thyroid hormone action suggest a hypothesis that may help to explain this apparent contradiction. Thyroid hormone receptors, in the absence of thyroid hormone, repress gene expression, according to Juan Bernal, a molecular endocrinologist at the Instituto de Investigaciones Biomedicas in Madrid, Spain. In the presence of thyroid hormone, the receptors activate gene expression. When there are no receptors, the genes are expressed at an intermediate baseline level. So knockout mice reap some of the benefits of gene expression whereas hypothyroidal mice don't. "The main role of thyroid hormone may be to amplify signals already coming from the genes," said Bernal.

The deaf knockout mice also illustrate that timing is crucial for any attempts to evaluate how thyroid hormone affects brain development. Experimental geneticist Douglas Forrest of the Mount Sinai School of Medicine in New York City explained that one must consider timing to understand why those knockout mice couldn't hear. In normal mice, early after birth, the cells that translate vibration into sound in the inner ear respond rapidly to sound. In knockout mice the early response isn't so fast. Eventually the response catches up, but by then it's too late, and this contributes to a permanent deficit in hearing. "At this meeting progress was made because people were identifying molecular mechanisms that reproduce some of the clinical data," said David Armstrong, leader of the faculty of neuroscience and membrane signaling group in the NIEHS Laboratory of Signal Transduction.

This growing understanding presents toxicologists with a challenge. They must find end points that are indicative of a transient thyroid hormone imbalance. To do this they need to "validate known end points of thyroid hormone action in the brain, such as the rate of cell division or cell death at specific times in cerebellar development, for use in toxicological studies," says Zoeller.

There are also practical implications to this growing understanding and changing perspective. Based on these results, learning disability advocacy groups are preparing to recommend preconception screening for hypothyroidism for women, according to activist Audrey McMahon of the Learning Disability Association (although some scientists question whether such screening is indicated by the science). In addition, in parts of the world where subtle iodine deficiencies affect a mother's thyroid hormone status, iodine supplementation may be more important than currently realized, according to Bernal. **–Rebecca Renner**

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More Workouts for Women May Mean Less Risk of Breast Cancer

Sternfield B, Jacobs MK, Quesenberry CP, Gold EB, Sowers M. 2002. Physical activity and menstrual cycle characteristics in two prospective cohorts. Am J Epidemiol 156(5):402–409.

Numerous studies have shown that women who exercise regularly are at decreased risk for breast cancer, compared to women who lead a more sedentary lifestyle. However, the reasons for this difference have not been fully determined. One possible explanation could be an overall healthier lifestyle among the women who exercise. Another may be that regular vigorous exercise can lead to longer menstrual cycles (and in extreme cases, usually involving competitive athletes, missed menstrual cycles). This results in decreased exposure of breast tissue to circulating estrogens and progesterone at various stages of the menstrual cycle. Because breast cancer is a hormonally mediated disease, NIEHS grantee Ellen B. Gold from the University of California at Davis School of Medicine and colleagues investigated the role of exercise-induced changes in menstrual cycle in two large cohorts of women of reproductive age. The study was also funded in part by the National Institute for Child Health and Human Development and the Semiconductor Industry Association.

The study found that physical activity was associated with increased menstrual cycle length. The magnitude of this association decreased as body mass index increased, suggesting that the greatest benefit is seen in women of average size. This finding also supports the benefits of promoting regular, even vigorous, physical exercise.

The study was limited in that it relied on self-reporting to assess the level of physical activity; some women probably overreported this factor. However, the cohorts were large enough that such measurement errors were unlikely to have biased the results, say the authors. In addition, both cohorts were ethnically and racially diverse, which may allow for greater generalization of the results to the general population of women.

To build upon these new data, the investigators say, future studies should aim at assessing the effects of exercise on both the control of the cyclical hormonal feedback that governs ovulation and the reduction of hormone levels. **–Jerry Phelps**