THE LOWDOWN ON LOW-DOSE ENDOCRINE DISRUPTORS

Endocrine-disrupting chemicals are among the most complex environmental health threats known today. By mimicking natural hormones such as estrogen and testosterone, these chemicals can interact with the body's endocrine system and exert toxic effects that may lead to reproductive and developmental abnormalities or cancer. In October 2000, the National Toxicology Program (NTP) and the NIEHS convened an independent panel of experts from academia, government, and industry to evaluate the evidence for low-dose effects and dose–response relationships for endocrine disruptors. "This was a highly experienced panel," says NIEHS toxicologist Ron Melnick, chair of the panel organizing committee. "Members had broad experience in areas including molecular biology, reproductive and developmental toxicology, and statistical and mathematical modeling. Many of them were professors and department chairs from some of the leading biomedical research institutes in the country." The panel's conclusions were released 14 May 2001 in the *National Toxicology Program's Report of the Endocrine Disruptors Low-Dose Peer Review.*

Low-Level Exposures and Public Health

The peer review panel was assembled at the request of the U.S. Environmental Protection Agency (EPA), which is now validating test methods to screen 87,000 chemicals for hormonal effects [see *EHP* 107:A458–A460 (1999)]. In assessing public health risks from endocrine disruptors, the EPA faces some difficult challenges. Unlike traditional toxicology, in which the dose always starts out from zero, exposure to endocrine disruptors adds incrementally to what's already present in the body as naturally occurring hormone. In some instances, the natural hormone is associated with a certain degree of risk. Estrogen, for example, is known to cause breast cancer in humans. "The question is, what happens to the risk when exposure to endocrine disruptors drives hormone levels higher than what they normally would be?" explains George Lucier, chair of the peer review panel.

For this specific review, the EPA's main concern was to resolve questions concerning the effects of low-dose exposures. Humans are usually exposed to these chemicals at extremely low levels. Yet the standard tests used by the EPA to evaluate reproductive and developmental toxicity (contained in the agency's August 1998 document *Health Effects Test Guidelines OPPTS 870.3800: Reproduction and Fertility Effects*) often fail to consider the impact of doses lower than those producing no evidence of overt adverse effects, described as the no-observed-effect level, or NOEL. For this review, the panel evaluated evidence of biologic changes due to exposure to endocrine disruptors at doses below the NOEL. Melnick says biologic changes were emphasized over adverse effects because in many cases the long-term consequences of altered endocrine function have yet to be fully characterized.

The organizing committee identified 59 studies for review. These studies investigated chemicals with a wide range of hormonal properties, including bisphenol A, diethylstilbestrol (DES), ethinyl estradiol, nonylphenol, octylphenol, genistein, methoxyclor, 17β-estradiol, and vinclozolin. Melnick says the main criterion for study inclusion was an "evaluation of multiple doses extending reasonably far into the low-dose region." The raw data from 39 of the studies underwent statistical reanalysis by a subgroup of experienced, impartial statisticians.

"This was an incredibly stringent review," says Lynn Goldman, professor at The Johns Hopkins University in Baltimore, Maryland, and former assistant administrator at the EPA. "In some cases, we found the initial statistics were not properly conducted. Overall, the review process was much more intensive than anything I've seen for publication in a journal," she says. "There was complete transparency at every step of the way for all the investigators and the public. Comment from industry and environmental groups was always welcome."

Nonmonotonic Dose Response

A key outcome of the review was verification that some endocrine disruptors exhibit dose–response relationships described as nonmonotonic, meaning that within a certain dose range, a chemical's effects on a given end point actually become greater as the dose is reduced. The dose–response curves can be shaped like a U, with a high response at both low and high levels of exposure, or like an inverted U, with the greatest response at intermediate dose levels. According to Frederick vom Saal, a professor in the Division of Biological Sciences at the University of Missouri in Columbia, nonmonotonic curves challenge the EPA's standard assumption of linear or threshold dose responses, which holds that toxic effects always lessen as the dose is reduced toward zero.

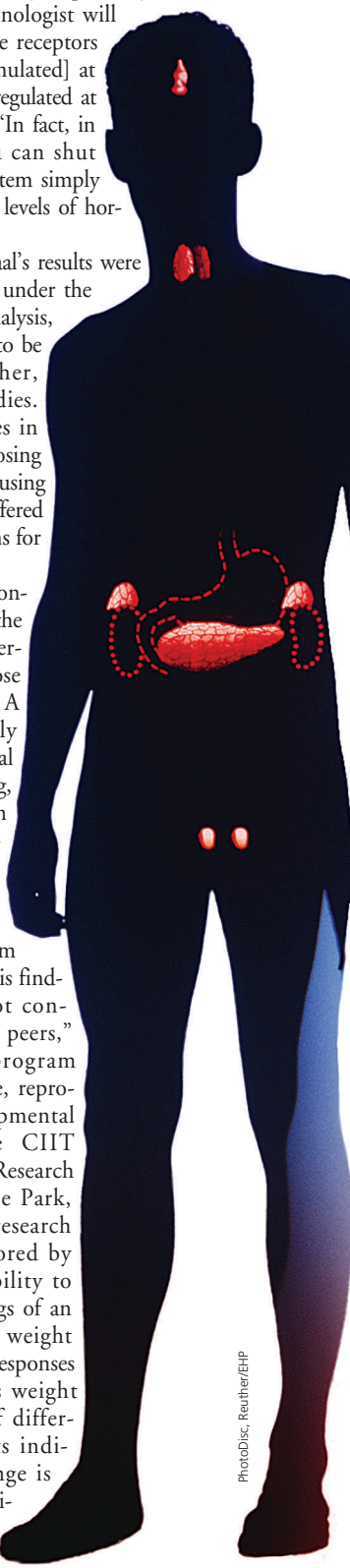
One chemical found to exhibit nonmonotonic relationships is bisphenol A, an estrogenic plastic used in products including baby bottles and water cooler jugs. Due in part to its high visibility in the marketplace, bisphenol A was the subject of its own separate subpanel review. Studies conducted by Vom Saal showed that low-dose exposure to bisphenol A in mice produces prostate enlargement with an inverted U–shaped dose–response curve. These findings have helped fuel the concern of environmentalists that exposures to the chemical might enhance the risk of prostate cancer, in addition to other illnesses.

Vom Saal wasn't overly surprised by his results. "Any endocrinologist will tell you that hormone receptors are up-regulated [stimulated] at low doses and down-regulated at high doses," he says. "In fact, in clinical therapy you can shut down a hormonal system simply by treating with high levels of hormone."

Although Vom Saal's results were shown to be credible under the panel's statistical reanalysis, they were found not to be reproduced in other, equally credible studies. Interstudy differences in animal strain, diet, dosing regimens, and even housing conditions were all offered as possible explanations for the discrepancy.

Based on the inconsistency of the data, the panelists were not persuaded that a low-dose effect of bisphenol A has been conclusively established as a general or reproducible finding, an admission seized on by the plastics industry, which insists low-dose exposure to the chemical is safe.

"I believe Dr. Vom Saal is convinced of his findings, but he has not convinced his scientific peers," says Paul Foster, program director of endocrine, reproductive, and developmental toxicology at the CIIT Centers for Health Research in Research Triangle Park, North Carolina, a research organization sponsored by industry. "The inability to reproduce the findings of an increase in prostate weight [or any pathological responses associated with this weight change] in mice [of different strains] and rats indicates that this change is not robust, nor a universal phenomenon likely to have implications for human health risk assessment."



PhotoDisc, Reuther/EHP

But Lucier cautions that the panel's statement on bisphenol A shouldn't be taken out of context. Vom Saal's data are of high quality, he says, and the evidence for a low-dose effect can't be discounted. Taken as a whole, the data for bisphenol A and other chemicals reviewed by the panel indicate nonmonotonic, linear, and even threshold responses are all possible outcomes of low-dose endocrine disruptor exposure. The fact that biologic effects were noted in the low-dose region below the NOEL for some data sets, he says, suggests that the EPA should review its current testing protocols to see if changes are required.

Multigenerational Studies at Issue

Another of the panel's most important conclusions concerns the ability of EPA testing protocols to accurately evaluate reproductive and developmental effects. Experts suggest that endocrine disruptors pose the greatest risk during fetal development, which is regulated by hormones at specific levels. Hormonal alterations due to maternal exposure in pregnancy could lead to effects such as reduced cognitive function or cancer that might not be evident for months, even years. The EPA's current method for evaluating these effects is the multigenerational reproduction study, in which animals are exposed at critical periods of sexual differentiation and their second-generation offspring evaluated at 21 days after birth.

One of the panel's main concerns is that the 21-day period of evaluation is too short. The risk of missing developmental effects if studies are terminated too soon is exemplified by the research of Retha Newbold, a biologist with the NIEHS Laboratory of Toxicology who has studied DES, an estrogenic hormone once used to prevent miscarriages that wound up causing cancer in the adult daughters of treated women. Newbold has performed studies in which mice were exposed at 1–5 days of age to DES and then allowed to grow for 18 months. In her studies, Newbold found significant increases in uterine tumors among the treated mice, which she says wouldn't have been detected with the standard multigenerational assay. Uterine tumors were also observed in similar studies with genistein, which is an estrogenic component of soybeans.

Echoing the opinion of the panel on this issue, Goldman says, "I think we're going to have to revisit the standard multigenerational test to ensure that the end points, the animal models, and the ages of evaluation are appropriate for assessing development effects from endocrine disruptor exposure."

Although evidence of low-dose biologic effects was confirmed by the review, panel members were careful to note that the toxicologic significance of these findings is unknown. "Take the issue of prostate enlargement with bisphenol A exposure," says Goldman. "I don't think we understand the significance of that finding. Where do we go with this? How is it going to impact the way in which we regulate bisphenol A? This is a new area, and we don't have many precedents to rely on. We just don't have years of decision making based on this type of data."

The panel's conclusions raise serious questions about the extent to which current EPA testing protocols are able to accurately evaluate endocrine-disrupting effects. The panel notes that extended dose ranges are not typically used, and end points such as cancer and neurodevelopmental effects are rarely considered in multigenerational assays. No specific recommendations as to how the process might be improved were made. But the panel's report clearly states that the EPA will need to revisit current test methods for evaluating reproductive and developmental toxicity to determine if changes are needed for endocrine-disrupting agents. **–Charles W. Schmidt**

New Mouse Genomics Consortium

The NIEHS announced on 3 May 2001 that five research centers comprising the Comparative Mouse Genomics Centers Consortium have been selected to develop mouse lines with genetic variations that may be key to understanding the effects of environmental stressors on the body. The mice, protocols, assays, assessment criteria, and data generated through projects using them will be made available to scientists around the world to help determine how such gene variations make some individuals more sensitive or resistant to environmental exposures. The centers will develop new mouse models to study the processes that repair environment-damaged DNA and regulate cell life cycles. The consortium is part of the Environmental Genome Project, begun by the NIEHS in late 1997 to identify genes and alleles that affect individual response to environmental exposures.

Pooling the established resources of this diverse group of scientists will ease the transfer of emerging materials, data, and technology and is intended to encourage discoveries that will help scientists better understand the complex interaction between the environment and human health. "This is a very comprehensive program, with over ninety investigators based at the centers," says José Velazquez, the NIEHS program director for the centers. Information gathered through research at these centers could not only help scientists better predict health risks to sensitive individuals, but could also help policy makers draft public and environmental health regulations that have a more scientific basis and that are more cost-effective to better protect those affected.

The centers will be governed by a steering committee responsible for providing general direction and guidance to consortium programs. A main focus of the centers will be to develop technologies such as three-dimensional protein imaging, which could be used to predict the function of the variations; whole-mouse imaging using PET scanning, which could more quickly detect tumor formation; microarray gene expression analysis; proteomics; and a whole new suite of bioinformatics tools to integrate the emerging data. The consortium will also explore modeling strategies, including *in vitro* DNA variant assessment (which is used to determine which allelic variants to model) and construction of transgenic vectors, using state-of-the-art molecular biological approaches for gene targeting.

One major goal of this project, says Velazquez, will be the launch of a new bioinformatics Web site in late 2001 that will integrate the available gene and protein data for approximately 200 genes that function to repair environmental damage and regulate cell life cycles. In addition, the consortium's Web site, which should be available to the public by November 2001, will display the models under development and bioinformatics tools to mine the available data. Both sites will be available through the Environmental Genome Project home page, located at **http://www.niehs.nih.gov/envgenom/home.htm**.

The participating centers are The Albert Einstein College of Medicine of Yeshiva University (the Bronx, New York), the University of Washington (Seattle), the University of Cincinnati (Ohio), the University of Texas Health Science Center at San Antonio, and the University of Texas M. D. Anderson Cancer Center (Smithville). Over the next five years, the centers will receive $5–6 million in grants per year. Each center will focus on separate sets of genes determined to play a role in aging and the development of cancer and other diseases. **–Erin E. Dooley**