Endocrine-Disrupting Chemicals Probed as Potential Pathways to Illness

Richard Trubo

SAN DIEGO—For the last 60 years, humans have been exposed to an increasing number of synthetic compounds in the environment. In fact, more than 80 000 chemicals are used commercially in the United States, found in products ranging from toys and detergents to pesticides and food packaging. Accumulating evidence that some of these widely used chemicals may have hormonelike effects on the body is heightening concerns about their potential long-term health risks, particularly when developing fetuses and neonates are exposed.

At a day-long symposium at the Endocrine Society meeting here in June, leading investigators shared the latest findings on the effects of these synthetic chemicals—as well as of naturally occurring substances such as phytoestrogens—that have been reported to have hormonelike activity and may be associated with an increased susceptibility to disease and dysfunction. The potential danger is greatest for "the fragile fetus" (a term coined more than a decade ago by University of California at Berkeley endocrinologist Howard Bern, PhD) and the neonate, which are particularly sensitive to perturbations by endocrine-disrupting chemicals.

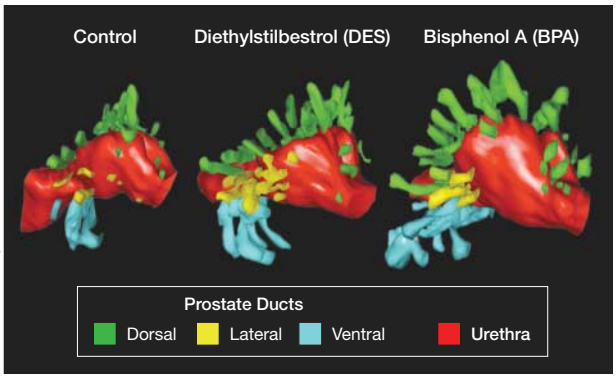
But such effects are often not apparent for years. "Exposure to chemicals with endocrine-disrupting activity during critical stages of differentiation may have permanent consequences that may not be expressed or detected until later in life," said Retha R. Newbold, MS, head of developmental endocrinology studies in the Laboratory of Molecular Toxicology at the National Institute of Environmental Health Sciences. There is now greater recognition, she added, that the developing organism may be especially vulnerable to endocrine-disrupting chemicals because of such factors as deficiencies in DNA repair mechanisms, detoxification enzymes that are not completely functional, and a blood-brain barrier that is still being formed.

EXPOSURE TO PHTHALATES

Phthalates, a ubiquitous group of chemicals used in hundreds of products, including soft vinyl plastic toys, shampoos, soaps, nail polish, vinyl flooring, and pharmaceuticals, are being scrutinized for potentially harmful effects during development. Shanna Swan, PhD, an epidemiologist and professor of obstetrics and gynecology at the University of Rochester School of Medicine and Dentistry, described her recent study of the effects of phthalates in humans, the first to show a significant relationship between maternal exposure to phthalates and adverse reproductive development in male offspring.

The scope of human exposure to phthalates had previously been shown in data from the National Health and Nutrition Examination Survey (NHANES 1999-2000), in which more than 75% of urine samples tested had measurable levels of phthalate metabolites (Silva et al. *Environ Health Perspect*. 2004;112:331-338). Research at the Harvard School of Public Health had provided human data showing a dose-response relationship between urine levels of phthalate metabolites and sperm motility and concentrations (Duty et al. *Epidemiology*. 2003;14:269-277).

Swan and her colleagues evaluated women who had been recruited to participate in a multicenter pregnancy cohort study (the Study for Future Families). Focusing on 85 eligible mother-son pairs, the researchers determined the presence and levels of nine phthalate metabolites in the urine of mothers during late pregnancy. Then, in the male offspring of these women, researchers measured the so-called anogenital distance (AGD)—the distance from the center of the anal opening to



National Academy of Sciences

Mouse studies show differences in patterns of prostate ductal development after fetal exposure to substances with hormonelike effects (*http://www.pnas.org/cgi/doi/10.1073/pnas.0502544102*).

the anterior base of the penis or the clitoris. The AGD, she said, is a sexually dimorphic measure; in rodents, it has been found to be a sensitive indicator of masculinization, with AGDs about twice as long in males as in females.

Findings by Swan's group showed a significant inverse association between human AGD and concentrations of four phthalate metabolites. The higher the levels of these four metabolites in maternal prenatal urine, the shorter the infant's relative AGD. Eleven of 12 boys with the highest combined phthalate exposure had AGDs below the 25th percentile for age and weight. A shorter AGD was associated with incomplete testicular descent and smaller penile volume. These findings, she said, are similar to those seen in rodent studies and suggest that endocrine-disrupting compounds may play a role in the undervirilization of males.

According to Swan, the pattern of genital changes observed in the boys in her study are consistent with the "phthalate syndrome" described in rodents exposed prenatally to phthalates. In addition to decreased AGD, the syndrome is characterized by testicular, epididymal, and gubernacular cord agenesis.

RISKS OF BISPHENOL A

Another substance under scrutiny is the estrogenic compound bisphenol A (BPA), which is found in products like baby bottles, food containers, and dental sealants. About 6.4 billion pounds of it are manufactured worldwide each year, and a recent report from the Centers for Disease Control and Prevention noted that 95% of urine samples in a reference population of 394 adults in the United States had measurable levels of BPA (Calafat et al. *Environ Health Perspect*. 2005;113:391-395).

While the plastics industry has described BPA as highly durable, scientists like Frederick vom Saal, PhD, contend that under everyday conditions, BPA in products like polycarbonate plastic containers and tin cans leaches into food and beverages. "A number of studies show that structural damage to a variety of organs occurs as a result of fetal exposure to very low doses of bisphenol A," added vom Saal, professor of biological sciences at University of Missouri-Columbia. "When BPA in the low parts per trillion range is in contact with human or animal cells, it will alter cell function. The range of human exposure is as much as 100 to 1000 times higher than this."

In a recent study, estrogenic chemicals including BPA (10 µg/kg per day) were fed to pregnant mice, a dose that is lower than the typical exposure in pregnant women. Researchers found that BPA was associated with prostate changes in the developing fetal mouse that are predictive of an increased risk of cancer later in life (Timms et al. *Proc Natl Acad Sci U S A*. 2005;102:7014-7019).

A separate animal study, published on May 26 in the online edition of *Endocrinology*, evaluated perinatal exposure to very low doses of BPA and the effect of this compound on pubertal development of the mammary gland. The study found persistent changes in mammary gland morphogenesis that may be suggestive of an increased risk of breast cancer. Study coauthor Ana M. Soto, PhD, professor in the department of anatomy and cellular biology at Tufts University School of Medicine, said that these findings are "particularly worrisome since the incidence of breast cancer has increased in the developed world and has increased parallel to the introduction of endocrine disruptors such as BPA into the environment."

CROSSING GENERATIONS

Endocrine disruptors may do more than pose risks in the offspring of mothers exposed during pregnancy. Michael K. Skinner, PhD, director of the Center for Reproductive Biology at Washington State University, described exposing pregnant rats at mid gestation (the time of gonadal sex determination in the embryo) to two endocrine-disrupting compounds—the antiandrogenic fungicide vinclozolin (used in wine vineyards) and the estrogenic pesticide methoxychlor (used to replace DDT). This transient embryonic exposure caused an adult disease of subfertility related to increased apoptosis of spermatogenic cells (Anway et al. *Science*. 2005;308:1466-1469).

In the vinclozolin group (which involved a dose of vinclozolin [100 mg/kg per day] that is relatively low compared with most in vivo studies but higher than environmental levels), the animals were then bred for four subsequent generations. "Surprisingly, each generation of animal had the same disease state, even though those animals were never exposed to the toxin," said Skinner. "So it appears we have endocrine receptor–induced disease that's permanent to the lineage. With an environmental toxin, we've induced a disease state that is inherited in greater than 90% of all the males in subsequent generations, even though the exposure occurred only once in the original gestating mother."

Skinner referred to this finding as "a new paradigm" for thinking about the hazards of environmental toxins. In explaining the possible mechanism for this transgenerational effect, he pointed to altered DNA methylation (attachments of methyl groups to DNA) and permanent, epigenetic reprogramming (changes in the expression of a gene or genes achieved by factors other than changes in the DNA sequence) of the male germline.

CAUTION WITH GENISTEIN?

Not all substances capturing the interest of researchers studying endocrine-disrupting compounds are synthetic. One plant compound of interest, genistein, is a natural phytoestrogen and the major isoflavone in soy products. In fact, infants consuming soy-based infant formulas are taking in levels high enough to raise red flags about possible risks of developmental exposure.

In a recent animal study published on June 1 in the online edition of *Biology of Reproduction*, investigators administered genistein to neonates in doses of 0.5, 5, or 50 mg/kg per day. In animals receiving the highest dose of genistein for the first 5 days after

birth, there were morphological changes in the ovary (including the presence of multi-oocyte follicles) that were associated with a 35% incidence of uterine tumors in animals at 18 months of age.

"These animals had premature reproductive senescence," said Newbold. "Their ovaries were malformed and not completely functional."

According to one report, the daily exposure of human infants to isoflavones in soy-based infant formulas is 6 to 11 times higher (based on body weight) than the dose that produces hormonal effects in adults consuming soy foods (Setchell et al. *Lancet*. 1997; 350:23-27). But for now, said Newbold, researchers don't know how animal findings about genistein translate to the human population. "I think we have to be cautious," she added. "Humans are exposed to a number of estrogenic or endocrine-disrupting compounds and it makes sense to decrease those exposures as much as possible."

INDUSTRY RESPONSE

In the aftermath of the release of Swan's study of phthalates, the American Chemistry Council's Phthalate Esters Panel issued a statement asserting, "Initial analyses indicate that the [Swan] study has many weaknesses, suggesting that the study may not stand up under rigid scientific scrutiny. The authors themselves stated that the results need to be validated."

Meanwhile, a Web site sponsored by the Bisphenol A Global Industry Group of the American Plastics Council highlights a 2004 review by a panel of researchers convened by the Harvard Center for Risk Analysis—and funded by the American Plastics Council—in which the panel "found no consistent affirmative evidence of low-dose BPA effects for any endpoint."

Even so, a bill has been introduced in the California state legislature (AB 319) by Assembly member Wilma Chan (D, Oakland) that would ban the use of BPA and phthalates in toys and child-care products designed for infants and children younger than 3 years of age. Hearings on the bill are pending.

"The EPA [Environmental Protection Agency] is struggling with the idea of what levels are acceptable for each of these compounds," said Newbold. "Everybody is taking them seriously—but we need a lot more research." □

Mental Illness Takes Heavy Toll on Youth

Bridget M. Kuehn

A NEW SURVEY OF MENTAL ILLness in the United States indicates that mental illnesses tend to strike early in life and delays in treatment leave affected individuals vulnerable to debilitating symptoms during their most productive years.

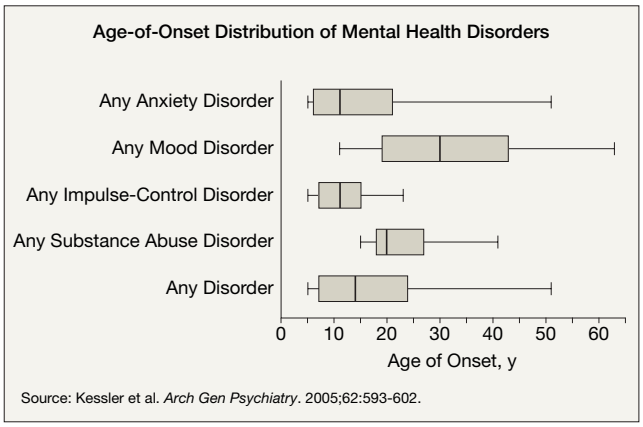
Half of all individuals who have a mental illness during their lifetime report that the onset of disease occurred by age 14 years and three fourths by age 24 years, according to the National Institute of Mental Health (NIMH) National Comorbidity Survey Replication. The nationally representative, face-to-face household survey of more than 9000 individuals aged 18 years or older, conducted between February 2001 and April 2003, is a follow-up to the 1990 National Comorbidity Study. Four articles detailing the latest findings were published in the June issue of the *Archives of General Psychiatry*.

"[Mental illnesses] really are the chronic disorders of the young," said Thomas R. Insel, MD, director of the NIMH in Bethesda, Md.

The survey looked at the prevalence of common mental illnesses, such as mood and anxiety disorders, but did not measure other less common illnesses such as autism. It also did not include individuals who were homeless or institutionalized.

The study bolstered previous findings that mental illnesses are prevalent. It found that about half of all individuals in the United States will have a mental illness in their lifetime and about one fourth will have a mental illness in a given year (Kessler et al. *Arch Gen Psychiatry*. 2005;62:593-602, 617-627). However, Kathleen R. Merikangas, PhD, senior investigator at the NIMH, noted that most cases in a given year were mild and did not require professional intervention.

"It will be the task of the next decade to discriminate between those who



Data from the National Comorbidity Survey Replication reveal the early age of onset of many mental illnesses, particularly anxiety and impulse-control disorders. The boxes show the median and interquartile range for age of onset, and the whiskers indicate the 5th and 95th percentiles.