ENVIRONMENTAL ESTROGENS

New Yeast Study Finds Strength in Numbers

The notion that modern industrial society is producing hormonelike pollutants that can interfere with human reproduction has become a hot topic in the media and within Congress in recent months. A widely promoted book, *Our Stolen Future* (see review on p. 1444), with a preface by Vice President Al Gore, put the theory high on the public agenda—and drew a strong response from some researchers who pointed out that the pollutants don't have nearly the clout of natural estrogens in the body and, thus, may have no significant impact on humans. Now, a paper in this issue (p. 1489; also see Perspective on p. 1451) is likely to add fresh fuel to the debate. A team of researchers from Tulane University in New Orleans, using a novel screening system based on genetically engineered yeast cells, reports that a mixture of two weakly estrogenic chemicals can be far more potent than the individual compounds.

The findings are causing scientists to take a fresh look at the controversy. "It's a very striking result," says Wade Welshons, an endocrinologist at the University of Missouri. "It doesn't forge a direct connection between developmental estrogen problems and these chemicals, but it's a very important red flag." Others caution, however, that more work must be done to pin down whether the mechanism found in yeast cells has any relevance to humans. "These are very interesting observations, but they raise more questions than they answer," says Jack Gorski, a biochemical endocrinologist at the University of Wisconsin.

The Tulane research addresses one of the hottest controversies in toxicology: Do estrogenlike compounds in the environment—for example, pesticides, the plastics ingredient bisphenol-A, and some polychlorinated biphenyls (PCBs)—contribute to such ills as breast cancer, a possible drop in human sperm counts, and a rise in testicular cancer (*Science*, 15 July 1994, p. 308)? Some researchers have linked spills of such chemicals with reproductive abnormalities in wildlife, but the debate centers on whether the low levels present in the environment are sufficient to harm humans.

Strong synergy. Tulane's Collins, Klotz, McLachlan, and Arnold test combinations.

Two years ago, Tulane environmental endocrinologist John McLachlan, then scientific director of the National Institute of Environmental Health Sciences, and collaborators came upon a possible clue. The group was able to make male turtle embryos develop into females by painting the eggs with estradiol—the body's main estrogen—or certain estrogenic PCBs. At moderate doses they achieved this effect only when they combined two PCB compounds; the same PCBs applied individually were ineffective. So 6 months ago, when McLachlan teamed with molecular endocrinologist Steve Arnold and University of Florida reproductive physiologist Louis Guillette to set up a yeast system to screen for environmental estrogens, they decided to test various mixtures of the compounds.

The system consists of yeast cells engineered to contain genes that code for the human estrogen receptor and a "reporter" protein that the cell makes when an estrogenlike compound binds to the receptor. The culture turns blue when a chemical binds to the receptor, and the intensity of the color reflects how strongly the receptor is activated.

Tests on four pesticides believed to be only very weakly estrogenic—the pesticides dieldrin, endosulfan, toxaphene, and chlordane—yielded little or no response, as expected. (All but endosulfan have been banned in the United States, but they persist in the environment, sometimes in combination.) When the chemicals were paired, however, the activity shot up by a factor of 160 to 1600. "It was really quite astounding," McLachlan says. The group also found a fivefold synergistic effect in the yeast cells with a PCB mixture that had reversed the sex of the turtle eggs. And they showed that their results were not specific to the yeast system by getting comparable effects with PCBs in human endometrial cells.

Although the various combinations of pesticides were only 1/500 to 1/15 as potent as estradiol itself, McLachlan says his group worked with "levels [of environmental estrogens] actually achieved in some systems," such as the turtle eggs and PCBs in the serum of a group of women with breast cancer. The results, he says, "at least provide a mechanism where low levels of weak-acting environmental estrogens could have a greater-than-expected effect."

Other researchers emphasize that the results must be verified in various animal species to establish whether they are relevant to wildlife or people. The yeast-cell system "is a good controlled experimental system. But these are the first observations from the system," says toxicologist Michael Gallo of the Robert Wood Johnson Medical School in New Jersey. "[Now researchers] have to move into different phyla and ratchet down on the molecular explanation." McLachlan's team is now studying the estrogen receptor's binding pockets in search of a molecular mechanism.

Toxicologist Stephen Safe of Texas A&M University, a vocal skeptic of the notion that environmental estrogens are linked to human health effects, agrees that the findings "are really interesting and may have environmental significance." But he says the data do nothing to undermine a major criticism of the hypothesis: that many synthetic and natural environmental estrogens, including some in plants, are actually "antiestrogenic"—they block or reduce the activity of estrogen receptors—and could cancel out even powerful synergistic estrogenic effects. "We have to look at the opposite side of the coin," Safe says. McLachlan acknowledges this possibility and says his group has begun testing antiestrogenic chemicals and estrogenic/antiestrogenic combinations.

For now, the findings will stimulate more studies of chemical cocktails—an area largely overlooked in recent research on endocrine disrupters, which has focused on individual compounds. And if the results do hold up in various animal species, scientists may need to revise their current assumption that the effects are additive. "The safety margin may be a lot smaller than has been anticipated," says toxicologist John Gierthy of the New York State Department of Health. It could also "make testing extremely complex," he adds.

Indeed, the results may need to be taken into account by an Environmental Protection Agency (EPA) advisory panel now being formed to come up with in vitro test strategies to screen for environmental estrogens that pose the greatest potential threat, says Lynn Goldman, head of the EPA Office of Prevention, Pesticides, and Toxic Substances. Legislation pending in Congress would require EPA to begin screening such chemicals within 2 years. The Tulane findings could have "enormous policy implications" for EPA, says Goldman. "Obviously," she says, "these systems are more complex than we had imagined."

–Jocelyn Kaiser