

Beyond Estrogens

Why unmasking hormone-mimicking pollutants proves so challenging

By JANET RALOFF

Over the past year, the news media have hammered home the message that most of the animal kingdom is bathed in a sea of pollutants that act like estrogen, the primary female sex hormone. Reinforced by photos of birds, fish, and alligators exhibiting gonadal malformations, a growing awareness and acceptance of these risks has crept into the public's consciousness.

Epidemiologists, endocrinologists, and reproductive biologists have suggested that long-term exposure to these increasingly pervasive pseudoestrogens might underlie an apparent breast cancer epidemic in women (SN: 7/3/93, p.10) and fertility impairments in men (SN: 1/22/94, p.56). (See p.47.)

But follow-up studies now indicate that the initial message was too simplistic.

Estrogenic chemicals do pose risks to people and wildlife by binding to and unlocking the genetically programmed activity of estrogen receptors dispersed throughout the body. But scientists at the Environmental Protection Agency's Health Effects Research Laboratory in Research Triangle Park, N.C., have now identified a host of pollutants that functionally mimic estrogens in animals yet act through a nonestrogenic pathway: They block the activity of male sex hormones.

Related rodent studies at this laboratory and elsewhere have demonstrated that another class of pollutants—one that includes TCDD and other dioxinlike chemicals—triggers emasculating endocrine changes through yet another mechanism.

Finally, a series of studies now under way at the University of West Florida in Pensacola is looking at the *masculinizing* effects of paper mill wastes on female fish. Ichthyologist Stephen A. Bortone found he could trigger gender-bending changes similar to those noted in the wild by exposing laboratory fish to any of five androgenic substances, including testosterone, the primary male sex hormone. Initially, this led him to assume that the water pollutant affecting fish downstream from paper mills must be some kind of environmental androgen. Now, he concedes it might be an estrogen blocker. Tests over the next year or so may help resolve the issue.

These new findings reinforce the pivotal role of animal studies in identifying pollutants with a hormonal alter ego, argues University of Missouri biologist Frederick S. vom Saal. No battery of biochemical, cellular, or tissue-culture tests exists—or is likely to come along soon—that can fully map the myriad subtle changes in development that early exposures to gender-bending pollutants may cause, he maintains. It's even less likely that scientists can predict how those changes will play out in terms of disease or fertility, he notes.

While these new data muddy the waters, they do nothing to downplay toxicological concerns associated with the pollutants. If anything, they expand the list of suspected crimes, the cast of potential troublemakers, and the sites of their potential villainy.

Over the past 3 years, studies at the University of Florida in Gainesville have illustrated some of the important trends in this developing field.

Scientists there have been working to ferret out the mechanisms by which spills of a DDT-laced pesticide along the shores of Lake Apopka poisoned local alligators. Rates of hatching and hatchling survival remain extremely low at this, the state's fourth largest body of freshwater. The young that survive possess grossly impaired reproductive systems (SN: 1/8/94, p.24).

The first clue that the endemic pesticide contamination there might have hormonal consequences came from data on the animals' sex hormones. The proportion of estrogen was much

higher than it should be, largely owing to a near absence of testosterone—even in males.

The fact that mice treated with the estrogen-mimicking drug diethylstilbestrol (DES) exhibited ovarian abnormalities resembling those in Apopka's female alligators "initially argued that these [reptiles] had been exposed to an estrogen," recalls Gainesville biologist Louis J. Guillette Jr.

But on further reflection, he decided, excess estrogen should also increase penis size in males—the opposite of what he observed in juveniles at Apopka. He says their smaller-than-normal penises suggested a deprivation of androgens, or male sex hormones. "So I started playing with the idea that the phallus phenomenon, at least, was due to an antiandrogenic effect of DDE," the long-lasting, and most toxic, breakdown product of the pesticide DDT.

Last year, Guillette brought up this antiandrogen hypothesis during a presentation of his work at a meeting in Atlanta; among the attendees was L. Earl Gray Jr., a reproductive toxicologist with EPA.

Unknown to Guillette, Gray and William R. Kelce, also from EPA's Research Triangle Park laboratory, had already come to much the same conclusion. "The effects that Lou was seeing in the male alligators looked almost identical to the developmental effects we were seeing with vinclozolin in our rats," Kelce explains.

He and Gray were part of a research team that had just identified this widely used fungicide as the first known androgen blocker in the environment (SN: 7/2/94, p.15). By binding to androgen receptors in rodents exposed before birth, vinclozolin prevents sufficient testosterone from reaching androgen receptors.

The legacy of this early exposure in males included testes that failed to descend, infertility, a partially unfused phallus, and the development of a "vaginal pouch" characteristic of the female reproductive system. In other words, the males had suffered not only a demasculinization, but also a feminization.

Since DDE was the only major pollutant isolated from the eggs and tissues of Apopka alligators, Kelce and Gray set out to investigate its ability to bind to androgen receptors.

When Guillette stopped by their laboratory a month or so after his Atlanta talk, he found Gray and Kelce "looking like they'd swallowed canaries." The reason for their satisfied smiles, he quickly learned, was that their work had just confirmed that DDE readily binds to the androgen receptor.

Kelce, Gray, and their coworkers shared these and follow-up data with the rest of the world in the June 15 *NATURE*. Not only does DDE fail to unlock this receptor's normal sex steroid activities, but by tying up the receptor it also prevents true androgens from triggering their intended effects.

The roughly 60 parts per billion (ppb) concentration of DDE needed to block androgen receptors falls well below that seen in some DDT-contaminated communities. Eggs of some feminized male Apopka alligators contained up to 5,800 ppb. And blood from South Americans whose homes were treated with DDT to control malaria carries as much as 140 ppb.

Moreover, this DDT metabolite can cross the placenta. Tissues from stillborn infants in Atlanta during the mid-1960s—prior to the 1972 phaseout of DDT in the United States—contained DDE concentrations as high as 650 ppb in the brain, 850 ppb in the lung, 2,740 ppb in the heart, and 3,570 ppb in the kidney, Kelce's team notes.

Though most industrialized Western countries banned DDT use 2 decades ago, the toxicant still turns up in foods and soil throughout those nations. Why? In part, because other countries continue to use the compound. Indeed, points out Richard M. Sharpe of the Medical Research Council's Reproductive Biology Unit in Edinburgh in the same issue of *NATURE*, "Present use of DDT in developing countries (mainly for malarial control) probably exceeds the level of its use historically. Mexico and Brazil each used nearly 1,000 tons of DDT in 1992."

Kelce's team concludes that "the reported increased incidence of developmental male repro-

ductive system abnormalities in wildlife and humans may reflect antiandrogenic activity of the persistent DDT metabolite DDE.”

Three months earlier, the same EPA researchers reported new data showing that prenatal exposure to TCDD, the most toxic dioxin, permanently alters reproductive function in male rats and hamsters. The doses used were relatively small, they point out in the *March Toxicology and Applied Pharmacology*—well below those causing observable changes in the mothers.

Unlike vinclozolin, TCDD did not feminize exposed animals. Instead, males of both species suffered a slight emasculation of their reproductive system and sexual behavior. Though subtle, these changes did translate into “fairly large effects on the ejaculated sperm counts—even though the testes were only minimally affected,” Gray told *Science News*. He suspects the early exposures altered the development of the males’ epididymis—a gonadal structure that stores sperm and plays a role in ejaculation—and possibly that of related sex accessory glands.

Exposed male hamsters also developed mild epididymal inflammation, which Gray says “might imply a slight structural change.” Though hamsters are “typically insensitive to the lethal effects of dioxin,” he notes, “we’ve now shown the hamster fetus is not insensitive.”

This suggests that adult and fetal toxicology “do not correlate,” he says. “So if humans are insensitive to the lethal effects of dioxin, that might not mean the fetus will be also.”

“We still believe that dioxin is acting as a potent environmental hormone,” observes Linda Birnbaum, director of environmental toxicology at EPA’s Research Triangle Park facility. TCDD exerts its gender-bending effects by binding to its own distinct (aryl hydrocarbon) receptor. Once that binding occurs, the dioxin receptor complex “sends out mixed signals that alter the expression of a multitude of different hormones or hormone receptors,” she says.

Under some circumstances, TCDD apparently produces antiestrogenic effects. At other times, this dioxin appears to need the presence of estrogen in order to unleash its toxicity. In still other cases, TCDD’s effects resemble those of estrogen.

“We know dioxin doesn’t bind to the estrogen receptor,” Birnbaum says. What it appears to do “is affect the expression of that receptor.”

Indeed, vom Saal maintains, “it’s virtually impossible to disturb any part of the endocrine system without seeing responses in other parts.” So adding hormone mimics or blockers to any part of the grand, interrelated community of hormones at work in the body may unexpectedly evoke changes elsewhere.

At no time is the body more susceptible than during fetal development.

Each individual begins life as a single fertilized cell that will divide again and again—eventually differentiating into tissues as diverse as muscle, bone, and brain. Yet each of these cells still carries the same genes. The difference, vom Saal explains, “is that during development, some genes are masked, others turned on. Hormones do that.”

By tweaking the system as tissues are differentiating, he says, “you may not damage the genes—just turn them off forever or turn them on at abnormal rates or times.”

What this means, Guillette explains, is that the hormones’ role in organizing fetal development does not stop at making sure organs end up in the right place. Hormones “even direct organization on the cellular and genetic level,” he says, sometimes modifying receptors or even changing the number present. This is why adult male rodents, exposed to DES as fetuses, respond to estrogen stimulation by producing reproductive proteins ordinarily seen only in females, he says. “Their cells were reorganized so that they now respond inappropriately.”

These features help explain why it’s so difficult to identify environmental hormones. “We now know that no one technique, assessment, or species will be able to tell us whether an ecosystem is polluted,” Guillette says.

In fact, vom Saal adds, “we have not even begun in science to identify the multitude of hor-

mones active during development and the receptor systems [that may be vulnerable]." In that sense, researchers don't yet know where to look for toxicity—beyond the classic, albeit simplistic, approach of identifying agents that bind to either estrogen or androgen receptors.

Moreover, vom Saal charges, these new data from studies on environmental hormones suggest that even the standard animal models for evaluating a chemical's safety—high doses over short periods—are inadequate. When it comes to hormones, he explains, "the body has a protective system. Whenever hormone levels get too high, the body basically shuts off its receptors [for those hormones]."

"So to find out how chemicals really impact development, you must know at what levels they occur in the environment and then go out and test animals at that level. Surprisingly," he says, "when we do that, we find effects not predicted by the very high dose studies."

In the future, vom Saal would like to see every licensed chemical—and every compound proposed for commercial introduction—tested in developing animals at concentrations likely to be encountered in the environment.

The concept is daunting. Where would toxicologists begin?

Research Triangle Park chemist Chris L. Waller hopes to offer some concrete suggestions soon. He leads a team working on computer models of the features that define estrogens, androgens, and other important hormones.

Waller's group hopes to assemble a family of tools that can be used to examine a molecule's three-dimensional structure and the electric charge associated with it; from these data, the team will predict the molecule's potential for binding with particular receptors. But because no one has yet identified the structure of those receptors, Waller's group is stuck with having to look for similarities between known members of each class of hormones (and their mimics). Among the compounds they've used in building the androgen model are about 20 unpublished antiandrogens—some as potent as DDE—that Kelce's group identified through more intuitive means.

"The androgen model I've put together is very predictive," Waller says. The group has refined the estrogen receptor model fairly well and has begun a model to screen for analogs of progesterone, another female sex hormone.

Kelce expects these models eventually to run through a library of thousands of structures in just a few hours. Compounds flagged as suspect could then be evaluated further in test-tube or animal toxicity assays.

In April, EPA convened a small international scientific conference to discuss the possible need for a U.S. research strategy to better understand environmental hormones. "The consensus of the workshop was that . . . [there] was sufficient concern to warrant a concerted research effort"—one that places especially high priority on identifying the risks of these compounds to the developing reproductive system, says organizer Robert Kavlock, director of developmental toxicology at the agency's Research Triangle Park facility.

Based in part on the conference, he says, EPA plans to beef up funding for research on environmental hormones, including their effects on wildlife and human health. Another by-product of the meeting, he says, will be the formation of a committee this month or next to coordinate new research efforts in this field by various government agencies, industry, and environmental groups. □