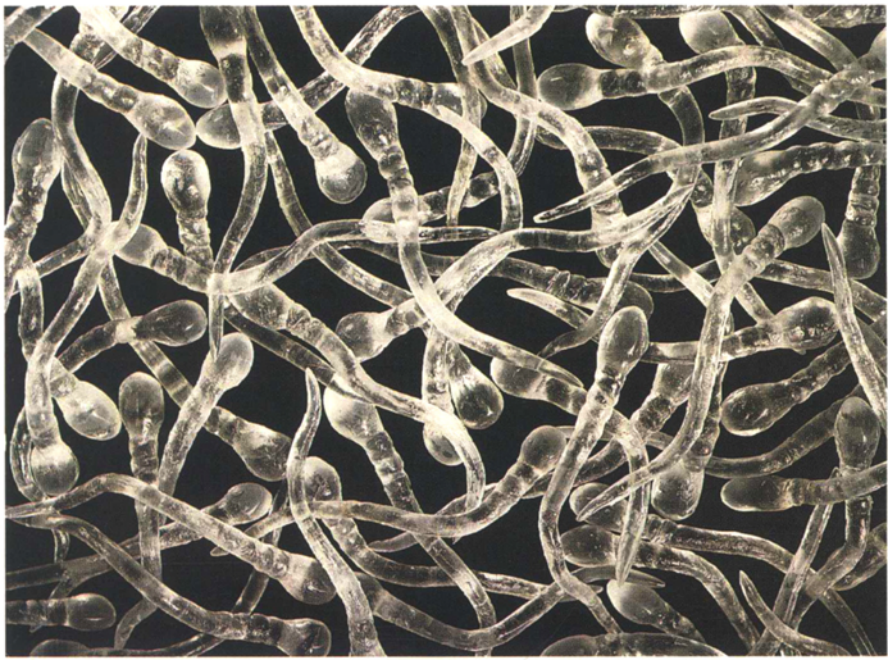
Anecdotal Evidence

DIANA LUTZ



Kiki Smith, Untitled (sperm), *1989–90*

No Conception

Masquerading as sex hormones, chemicals ubiquitous in the environment could threaten our children's ability to reproduce

As recently as 1960 infertility in couples was, to put the matter delicately, not a top priority for the medical establishment: it was a women's problem. Demographers routinely attributed the reproductive success of a couple to the woman if the fertility of the individuals was unknown. In other words, if a couple tried and failed to have children, the presumption was that the woman was barren, not that the man was sterile. In general, an infertile couple was regarded as exceptional.

These days infertility is not so casually dismissed. For one thing, the man falls under suspicion as well. The evidence of the past twenty years shows what, with hindsight, may always have been the case: that the male is a contributing factor in a couple's infertility 50 percent of the time—sexual equality with a vengeance.

But more, there is a substantial and developing body of evidence for disturbing trends in male reproductive health. For example, increases have been documented in the rate of testicular cancer and in the number of boys born with urethral abnormalities or undescended testicles. According to some investigators, though their assertions have been disputed, sperm counts have declined by about a third in the past twenty years—a rate of 2.1 percent a year—and the quality of sperm, measured by their morphology and motility, has declined as well. It is hard to resist the conclusion that fertility itself is on the wane. And whether or not fertility has declined, it is now estimated that one in six couples has trouble conceiving.

In some 25 to 40 percent of the cases of male infertility, the syndrome has no known cause. Recently the hypothesis has been put forward that the decline in male reproductive health, and the possible decline in fertility, may be related to the presence in the environment of chemical compounds that mimic or otherwise disrupt the estrogens (the female sex hormones) or the androgens (the male hormones).

One important argument in favor of that hypothesis is the case of the drug diethylstilbestrol (DES). DES is a synthetic estrogen, which was prescribed to more than five million women between the late 1940s and the early 1970s to prevent certain complications of pregnancy. The effects of the drug on the health of the daughters of those women have been widely publicized. Its effects on their sons are not so well known. In 1975 John A. McLachlan, now director of the Tulane/Xavier Center for Bioenvironmental Research in New Orleans, showed that exposure to DES in utero altered the sexual development of male mice. DES sons have since been shown to have an increased risk of reproductive disorders.

More recent research has demonstrated that certain other chemicals, now common in the environment, bind to and trigger or inhibit the activity of estrogen or androgen receptors in the test tube. To workers familiar with the effects of DES, it came as no surprise that the same chemicals also give rise to reproductive abnormalities, including low sperm counts, in animals exposed to them during fetal and neonatal development. Because of those effects, the chemicals have become known as endocrine disrupters, or, popularly, "gender benders."

The list of chemical suspects is incomplete, but it includes phthalates, which are added to plastics to give them flexibility and are also ingredients in paints, inks and adhesives; alkyl phenols, which are products of the microbial breakdown of certain

ingredients of detergents, paints, herbicides and cosmetics; and organochlorine pesticides, including DDT, aldrin and dieldrin.

Those substances are everywhere. Phthalates, for instance, occur in plumbing pipes and in the plastic, paper and cardboard common in food packaging. As the endocrinologist John Sumpter of Brunel University in Uxbridge, England, notes, "You can't have a Western life of any sort at all without being exposed to phthalates." Alkyl phenols find their way into surface waters and aquatic sediments, and then accumulate in living organisms, wherever detergents or cosmetics are washed into the environment or paints or herbicides leach into soil and groundwater. And even though many organochlorine pesticides were banned in the West in the 1960s, they are still very much with us. The half-life of DDT in the environment varies, but it can exceed fifty years. The current use of DDT in developing countries probably exceeds past levels of its use in the developed world.

The evidence that such environmental chemicals are agents of human infertility is still largely circumstantial. There are still many missing links in the causal chain that would connect receptor binding to changes in reproductive health to human infertility. No one is more aware of those missing links than the investigators working to forge them. But the general feeling is that the evidence gained so far is too plausible to ignore. Last summer an ecologist and a climatologist made a $15,000 bet with an economist who maintains that environmentalists are painting a misleadingly dark picture of the human condition. The wager was that fifteen benchmarks of the quality of life will grow worse in the next ten years. One of those benchmarks was the average count of human sperm.

THE RECENT BURST OF RESEARCH ON endocrine disrupters was triggered by the observations of their effects in wildlife or laboratory animals. In 1980 a small chemical-mixing company spilled massive amounts of sulfuric acid and DDT-laced dicofol, a mitacide that is a close chemical relative of DDT, into Lake Apopka, the fourth-largest body of freshwater in Florida. The volume of the spill is still not known for certain, but two weeks after the event investigators from the U.S. Environmental Protection Agency (EPA), who had been alerted by local residents, found that water near the lake's spring had a *p*H of 1.7—roughly the *p*H of stomach acid.

Florida is renowned for alligator ranching. The ranchers, ever mindful of the need to protect a multimillion-dollar industry, wanted to know how many wild alligator eggs could be harvested without damage to future populations. In 1986 the Florida Game and Freshwater Fish Commission asked Louis J. Guillette Jr., a professor of zoology at the University of Florida in Gainesville, to find out what makes a good alligator egg.

Guillette and his colleagues began to take an inventory of the hatching rates at nest sites on the shores of seven Florida lakes, including Apopka. The reproductive conditions of the alligators was in general poorer than the investigators had expected, but Lake Apopka was in a class by itself. Nests along the lakeshore contained an unusually large number of unhatched alligator eggs. Guillette and his team followed the situation and in 1988, the worst year, found that only 4 percent of the eggs hatched.

THE PENISES *of juvenile alligators exposed to "gender-bender" pollutants were notably reduced in size.*

Moreover, those alligators that did hatch had problems that were likely to impair their own ability to reproduce. The testes of the males and the ovaries of the females were abnormal. Measurements of the levels of sex hormones in the hatchlings showed that in both sexes the estrogens had come to dominate. In females the ratio of estradiol, the primary female hormone, to testosterone, the male hormone, was twice its normal value. In males the ratio was even more abnormal; levels of circulating testosterone were typical of females. As the hatchlings grew into juveniles, the penises of the males were notably reduced in size. Guillette concluded that the chemical contamination had somehow interfered with hormonal instructions in the developing alligators.

MEANWHILE OTHER INVESTIGATORS were stumbling onto similar evidence, linking certain chemical pollutants to estrogenic or androgenic effects that interfere with sexual differentiation and reproduction. William R. Kelce of the EPA's National Health and Environmental Effects Research Laboratory in Research Triangle Park, North Carolina, says his research on endocrine disrupters began when his group received data on the chemical vinclozolin from its manufacturer. The compound was being submitted to EPA for testing in order to be registered as a fungicide for use on grapes. "We noticed that there were problems with the reproductive systems of the male [rat] pups born in a multigenerational study," Kelce recalls. "We repeated the animal studies, and we found that the development of the male reproductive system was totally female-like. When the male pups were born, they had a very small anal–genital distance, which is an androgen-dependent measure, and the external genitalia of older animals had female characteristics. We had male rats with vaginal pouches and many abnormalities of the penis."

Those results suggested that vinclozolin was inhibiting the action of the androgens. For the male reproductive tract to develop, a number of proteins have to be synthesized, and that synthesis depends on androgens secreted by the testes during development. The androgens circulate in the blood and freely enter cells. In the cells of target organs, they bind to special proteins that act as their receptors. By binding, they change the shape of the receptor in such a way that it can, in turn, bind to DNA. That coupling initiates a sequence of events that results in the manufacture of the proteins encoded by the genes.

After eliminating several other possibilities Kelce's team demonstrated that vinclozolin interferes with androgens by binding to the androgen receptor and preventing the transcription of DNA. Whereas the androgens normally act like keys that open doors to reproductive development, vinclozolin acts like a key that jams in a lock.

The EPA team was just finishing its studies of vinclozolin when the team members attended a symposium given by Guillette. Guillette was still trying to figure out exactly why young male alligators in Lake Apopka had suffered "severe demasculinization." Kelce and his colleagues were struck by the resemblance between the alligators that had been exposed to DDT and their own rat pups that had been exposed to vinclozolin.

Back in their laboratory they repeated the vinclozolin studies with DDT metabolites. In a letter to the journal *Nature* last summer they reported that p,p'-DDE, the major persistent metabolite of DDT, has little or no ability to bind to the estrogen receptor. Instead it inhibits androgen binding and thereby prevents the genes ordinarily activated by the androgen receptor from being expressed. Animal studies showed that it gives rise to abnormalities in male reproductive development similar to the ones generated by vinclozolin.

WHEN KELCE DISCOVERED THE EFFECTS of vinclozolin in his rat pups, John Sumpter had already been working on environmental estrogens for some time. Sumpter originally set out to study vitellogenesis, or the production of egg yolk, in fishes. In female fishes the ovary makes estradiol, which stimulates the liver to make vitellogenin, or yolk protein. The vitellogenin is carried by the blood from the liver back to the ovary, where it is taken up and stored in the eggs.

Sumpter was studying egg making not because he expected to find anything amiss, but because he was trying to understand why animals vary in the number and size of

the eggs they produce. "But in the early 1980s," Sumpter says, "we found that in one or two places male fish, which we were using as controls, had quite substantial concentrations of vitellogenin in their blood. We didn't expect this, because vitellogenin production is stimulated by estrogen, and males don't have any estrogen. The site where the males had the highest levels was half a mile downstream from a sewage-treatment works, and one day, one of us said, 'I wonder whether it's the contraceptive pill in the sewage effluent.'"

Sumpter's group approached the British government with its findings and suggested further study might be warranted. "To our amazement," he recalls, "the British government got back to us. They said, in effect, that they knew all about it." There had been reports of hermaphroditic fishes in one or two rivers, and government investigators had been studying them since the late 1970s. But no one had been aware of the work because it was classified.

IN THE END THE GOVERNMENT COMMISsioned Sumpter's group to study the feminization of the fishes. To determine how widespread the problem was, the group placed male fishes in cages near sewage outflows and studied the blood level of vitellogenin in the exposed fishes. According to Sumpter, "There's no doubt whatsoever. Every single effluent across the whole of the U.K. that we've tested, and we've tested quite a few, is estrogenic." He adds that he knows of similar data from France and similar unpublished data from Germany.

One goal of Sumpter's group is to identify estrogenic chemicals present in sewage effluent. To save time the group has tested some chemicals known to be prevalent in effluent for estrogenic activity. Sumpter's student Susan Jobling reported last June that nine of the twenty common sewage contaminants she assayed bind to estrogen receptors, and three of the nine trigger the receptor's normal activity. Two of the three are phthalates and the third is BHA, a common food preservative.

For the past two years Sumpter has also been feeding information to Richard M. Sharpe, a mammalian physiologist with the Medical Research Council Reproductive Biology Unit in Edinburgh, who is considered Great Britain's leading expert on sperm production. As *The Sciences* went to press, Sharpe was about to publish a study showing lowered sperm counts in rat pups exposed in utero to estrogenic compounds. Sumpter, a coauthor of the paper, agreed to outline the main findings. He says the study shows that when pregnant rats are exposed to what most people would consider quite low levels of estrogenic chemicals (including two phthalates), the male offspring have significantly smaller testes than do control rats. "It's not a gross effect," Sumpter says, "but it's real, and it leads to reduced sperm production in [the exposed] rats."

ONE CRITIC OF THE ENDOCRINE-disrupter hypothesis is Stephen Safe, a toxicologist at Texas A&M University in College Station. "As far as we know," Safe says, "we take in two or three micrograms a day of these so-called estrogenic pesticides, but we take in about a million micrograms a day of potentially estrogenic bioflavonoids." (The bioflavonoids are a class of compounds that occur in many fruits and are essential for the absorption and metabolism of vitamin C.) "Both classes of compounds are weakly estrogenic," Safe says, "so I'd like someone to tell me what the difference is."

Sumpter responds, "I don't have much problem with anything that Safe's saying, but he misses some things." One is that the health effects investigators are worried about occur not in the adult animal but in the developing animal. It could be that during that sensitive period, when androgen levels are quite low, doses of estrogen mimics or of anti-androgens that leave adults apparently unruffled can do substantial harm.

ONE INVESTIGATOR *told Congress, "Every man in this room is half the man his grandfather was."*

Why should it matter whether a developing animal is exposed to synthetic or to plant-derived hormones? First, the mechanisms protecting people against natural or plant-derived hormones may be ineffective against synthetic ones. For example, special sex-hormone-binding proteins bind to natural estrogen in the blood, but not to the synthetic estrogen DES (which has caused the reproductive abnormalities I mentioned earlier). The plant-derived estrogens may stimulate the synthesis of binding proteins. Since bound estrogen is not physiologically active, the plant-derived estrogens might thereby decrease the concentration of available estrogen.

A second difference is that many of the synthetic hormones are resistant to biodegradation, and so they accumulate in the body. In contrast, natural or plant-derived hormones are quickly metabolized and excreted. The phthalates, the precursors to the alkyl phenols, and DDT, for instance, accumulate in body fat. The levels of organochlorine compounds in women today are ten times higher than their levels of estrogen. The worry is that if the fat is broken down during pregnancy or lactation, the fetus or suckling child might be exposed to high concentrations of the chemicals at a time of great sensitivity.

Kelce's EPA colleague L. Earl Gray Jr. says that "one problem with Safe's argument is that if everything he says is true, we never would have had any problems with estrogens in wildlife and domestic animals, and we do. Most of Safe's evidence for his arguments comes from in vitro single-cell assays that do not always correspond to what's going on in the whole animal."

THE HYPOTHESIS THAT SPERM QUALITY is diminishing is not new. According to the *British Medical Journal,* it attracted some attention in the 1970s, but the debate was "temporarily silenced" by an article that compared large-scale studies and records from a New York laboratory. The same criteria of patient selection and the same analytical techniques had been used at the laboratory for thirty years. No evidence of a decline in sperm quality was detected.

Then, in 1992, a Danish team from the National University Hospital in Copenhagen published in the *British Medical Journal* a meta-analysis of sixty-one studies, published between 1938 and 1991, of semen quality in men without a history of infertility. They noted that the average sperm count had declined by more than 40 percent, to 66 million per milliliter in 1990 from 113 million per milliliter in 1940. As Guillette told a congressional committee during testimony about the endocrine disrupters, "Every man in this room is half the man his grandfather was."

But partly because the Danish study came to the attention of the popular press, it was soon mired in controversy. As Stephen Farrow of Middlesex University in Enfield, England, put it in an editorial in the *British Medical Journal,* "By the nature of their work epidemiologists erect hypotheses and invite others to test them to destruction. They run a constant occupational risk, that of being mistaken." When the dust had settled, two of the study's authors concluded that although the data for the period from 1970 until 1990 were compatible with several interpretations, a real decline in semen quality had taken place between 1938 and 1991.

Controversy over the Danish study also drew heightened attention to geographic patterns in male reproductive health, and particularly to the discrepancy between Denmark and Finland. Danish men have a fivefold increase in testicular cancer, compared with their Finnish counterparts, and their sperm counts are half those of the Finns. Niels E. Skakkebæk of the National University Hospital in Copenhagen, one of the authors of the Danish study, is organizing a multinational study of male reproductive health that will include teams in Denmark, Finland, France and the United Kingdom.

Meanwhile another study was published

that left less room for argument. In February 1995 the *New England Journal of Medicine* published a study covering twenty years of donations at a sperm bank in Paris. The French team found that the mean sperm concentration had decreased, as I noted earlier, by 2.1 percent a year, from 89 million per milliliter in 1973 to 60 million per milliliter in 1992. That study was harder than the Danish one to dispute because the way men were recruited for donations and the method of semen analysis had remained the same throughout the period.

But there are those who doubt even that study. In an editorial in the same journal, Richard J. Sherins of the Genetics and IVF Institute in Fairfax, Virginia, objected that the men were selected from groups unrepresentative of the general population and "differences in age, abstinence before semen analysis, ejaculatory frequency, and the number of samples analyzed per person were not controlled for."

SKAKKEBÆK IS IMPATIENT with the debate and particularly with its narrow focus on sperm counts. Such a focus, he maintains, is not biologically sound; the sperm counts are only one of the many related indicators of male reproductive health that investigators are monitoring. For example, the incidence of testicular cancer in the United States has doubled among white people and tripled among black people since 1950. The point, says Skakkebæk, is that the testicular abnormalities appear to be linked. The risk of testicular cancer, for instance, is higher in men with a history of undescended testicles.

Writing in *The Lancet,* Sharpe and Skakkebæk have argued that because testicular cancer, undescended testicles and urethral abnormalities all are errors that probably arise during fetal development, those medical conditions and reduced sperm counts may have a common cause. Sharpe and Skakkebæk suggest that altered exposure to estrogens during pregnancy is a likely candidate, in part because of the similar abnormalities observed in the sons of women exposed to DES.

One final complication: Suppose sperm counts are indeed lower. Does that make men less fertile? After all, it takes only one sperm to fertilize an egg. Skakkebæk and his colleagues say that sperm concentration has been shown to correlate with male fertility. But Sherin's editorial asserts that data collected by the National Center for Health Statistics and the Princeton National Fertility Study indicate that rates of infertility have remained constant for three decades.

According to Douglas T. Carrell of the University of Utah School of Medicine in Salt Lake City, who works with infertile couples, it is hard to tell whether infertility has increased. Moreover, there are no good historical data, because in the past women discussed such matters privately with their gynecologists.

IN SPITE OF THE WAVE OF PUBLICITY about the endocrine disrupters in the past year, most men are still unaware that their reproductive health is under scrutiny. Among those who have heard casual reports about the endocrine disrupters, a common response is that a decline in fertility is not a bad thing. As a friend of mine put it, "the only command God has ever given that we have adequately fulfilled is be fruitful and multiply. We should give it a rest."

Kiki Smith, Uro-Genital System (male), *1986*

Why worry? Frederick S. vom Saal, a biologist at the University of Missouri–Columbia, gives two reasons. One is the consequences of endocrine disruption. "Look at congenital hypothyroidism," he says. "The consequences of abnormally low levels of thyroid hormones include mental retardation and motor abnormalities, so you're talking about lifelong debilitation." There are chemicals in the environment that resemble thyroid hormones, he continues, such as PCBs and dioxin. Although no one yet knows whether those chemicals are disrupting thyroid levels in humans, the possibility "is nothing to laugh at."

A second worry is that the methods worked out for assessing risk from carcinogens are inappropriate when it comes to endocrine disrupters. "The whole field of toxicology," vom Saal says, "grew out of industry concern over high-dose exposure of its workers. The goal was to protect against cancers that occur at extremely low frequency. The method people came up with was to run one or two studies using exceedingly high doses, plot the responses and then draw a straight line down to some zero value. The assumption was that this gave you some idea of the risk at lower doses.

"This method is criticized even within the cancer field," vom Saal continues, "but in the field of endocrine disrupters, there is no argument about it whatsoever. It simply doesn't work. That's because there is no linear response in endocrinology. The response is shaped like an upside-down U. At high doses you don't get a response. The first therapy for breast cancer was a high dose of estrogen, because a high dose of a hormone does exactly the opposite of what a low dose does. It inhibits the system. That means you can't extrapolate from a few high-dose studies, and that, in turn, means no chemical in the environment today has been tested in a relevant fashion for its endocrine-disrupting ability."

VOM SAAL CONCEDES THAT risk assessment must take into account political, economic and moral considerations and so extends beyond science. But for that very reason, he argues, we all are stakeholders in the debate; we all have to eat. "Most people assume that if a chemical is in food, it's got to be safe," he says. But there are many chemicals in food that have never been tested—indeed, many are nowhere even listed as present. One example is BPA (bis-phenol A), which occurs in many plastic products, including the epoxy lining of food cans. Only a few studies have looked at the compound, vom Saal says, and those have investigated its effects only at levels higher than the ones in a normal diet. But vom Saal himself has recently done an animal study that indicates it is a very potent DES-like compound. "Chemicals that are going to end up in our food need to be examined for health effects," he says, "not after but before they are placed in our food. We all have a stake in that." ●

***DIANA LUTZ** is a freelance journalist based in Ames, Iowa, and an editorial associate of* THE SCIENCES.