The Gender Benders

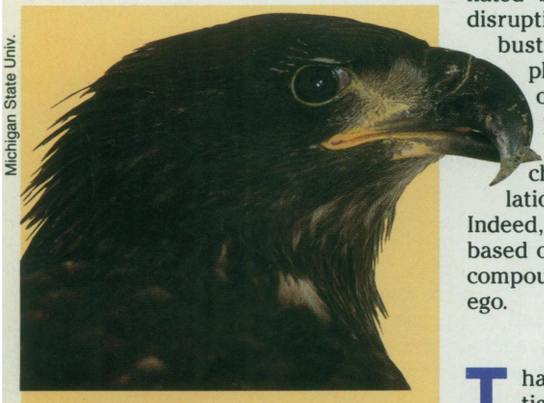
Are environmental "hormones" emasculating wildlife?

By JANET RALOFF

First in a two-part series

Mother Nature. The term conjures up images of a warm, nurturing, bountiful environment. But this sobriquet is proving increasingly apt for another reason — one that should offer anything but comfort.

New studies suggest that through pollution and other environmental factors, Mother Nature is exerting a feminizing hormonal influence on the animal kingdom.



Michigan State Univ.

Juvenile bald eagle collected in Michigan last year. Its life-threatening bill deformity may have been caused by exposure to estrogenic chemicals.

"We've been seeing many more deformities in recent years," reports David Best, a bald-eagle specialist with the U.S. Fish and Wildlife Service in East Lansing, Mich. "We've also seen some suggestion of deformities in the embryos [of eggs that didn't hatch]," he notes. Hatching rates within this population also fall below those seen in less polluted areas, such as inland Alaska. Reproduction in these birds starts to fall when PCBs in their bodies exceed 4 to 6 parts per million (ppm) or DDE exceeds 1 ppm. "We're finding very much higher levels than that around the Great Lakes," Best notes — such as eggs with PCB concentrations as high as 120 ppm.

Over the past 15 years, research has unmasked a number of "environmental hormones" — chemicals and pollutants that disrupt biological processes, often by mimicking the effects of naturally produced hormones such as the female hormone estrogen. On the ever-growing list of these agents are several restricted or banned pesticides — including DDT (and its even more toxic metabolite, DDE), kepone, heptachlor, dieldrin, mirex, and toxophene. Some polychlorinated biphenyls (PCBs) exhibit these disruptive properties, as do certain combustion pollutants, ingredients in plastics, and breakdown products of common detergents (SN: 7/3/93, p.10).

The hormonal activity of these chemicals usually bears little relationship to their intended function. Indeed, there is no way of predicting — based on structure or function — which compounds will exhibit a hormonal alter ego.

That fact troubles a number of scientists because such environmental hormones may be contributing to an increased risk of reproductive-system cancers in females. Moreover, prenatal exposure to hormone-like pollutants can derail the developmental processes that establish gender or ensure reproductive success.

While the health community has recently begun a host of studies to explore a possible link between estrogenic pollutants and cancers in women, few researchers have focused on the related reproductive risks such environmental hormones may pose for both sexes. That's unfortunate, says Theo Colborn, a zoologist with the World Wildlife Fund in Washington, D.C., because reproductive effects are likely to be "much more widespread."

Indeed, she notes, animal data are beginning to suggest that far smaller exposures are needed to trigger reproductive effects than to induce cancers. And because some of these reproductive changes may be subtle, they could evade detection for decades — even a lifetime — unless hunted for explicitly.

Colborn has convened a number of symposia in the past few years for researchers who study reproductively impaired wildlife populations or laboratory animals exposed to environmental hormones. Most of these scientists, she says, describe the links they're finding between impaired reproduction and "hormonal" pollutants as sobering — if not downright scary.

Indeed, she and many other environmental scientists worry that if hormone-like contaminants can feminize male animals, these ubiquitous pollutants may also underlie troubling reproductive-system trends being witnessed in men.

Some of the earliest data on unexpected reproductive risks posed by commercial chemicals came in the early 1950s. DDT, a potent and persistent organochlorine pesticide, was shown to cause the eggshells of many birds to thin. In fact, long after the compound was banned in 1972, DDT-thinned eggshells continued to put many embryonic birds — including bald eagles — at risk of being crushed to death.

DDT even wreaked havoc among birds resistant to eggshell thinning, such as sea gulls. Recognition of the extent of these problems, however, didn't emerge until decades after the initial reports of eggshell thinning.

Though heavily contaminated gull embryos managed to hatch, reproduction in gull colonies exposed to large amounts of DDT began to decline precipitously in the late 1960s. Biologists observed not only that many female gulls in these communities were sharing nests with other females — the so-called lesbian gulls — but also that the young within these communities bore grossly feminized reproductive tracts. Female gulls, which should have developed mature reproductive organs only on the left side, also carried

vestigial oviducts on the right side. Many males also bore feminine characteristics, such as oviducts, recalls avian toxicologist D. Michael Fry of the University of California, Davis. Moreover, he notes, the males' left gonad "had tissues that were both ovarian and testicular — so it was an intersex, or hybrid, gonad."

To connect these effects with estrogenic pollutants, Fry and his colleagues conducted a number of experiments during the 1980s. In one, they injected eggs of contaminant-free gulls with estradiol or with an estrogenic pesticide such as DDT. When the hatchlings emerged, they exhibited the same array of feminized sex organs as DDT-contaminated Western gulls on Santa Barbara Island, off the coast of California.

In effect, DDT "chemically castrated" the males, Fry says. He suspects the males' likely lack of interest in mating explains not only why female gulls dominated Santa Barbara Island's breeding colony in the late 1960s and early 1970s, but also why the females cohabited.

More recently, Fry has turned his attention to the effects of other estrogenic pesticides and PCBs. This summer he began studying common terns, a relative of the gull. Fry studied male embryos from nests along New Bedford Harbor, Mass., located near a toxic waste site contaminated with PCBs. Only four of the 15 males that he analyzed appeared normal. The rest exhibited varying degrees of feminized sex organs.

Dunbar/FG&FWFC

Florida panthers: Researchers are investigating whether environmental hormones might help explain their testicular problems, puzzling sex-hormone concentrations, and falling fertility.

"I never set out to do any toxicology," maintains Louis J. Guillette Jr., a reproductive endocrinologist at the University of Florida in Gainesville. But the team he heads has recently distinguished itself as one of the foremost in environmental-hormone toxicology. It all began six years ago, when the state of Florida asked him to find out what makes a good alligator egg.

Alligator ranching has become a multimillion-dollar industry in Florida, and ranchers wanted to know how many eggs they could harvest from the wild without jeopardizing the survival of this once-endangered species. So Guillette's team began surveying the hatching rate of eggs on various lakes: in all, more than 1,200 nests accounting for more than 50,000 alligator eggs.

It didn't take long, Guillette says, "before we realized there was something fundamentally different about one lake." It was Apopka, Florida's fourth largest freshwater body.

Whereas 70 to 80 percent of the eggs in most alligator nests hatched, between 80 and 95 percent of those from Apopka *failed* to hatch. Moreover, of the alligators that did hatch at Apopka, roughly half died within two weeks — a mortality rate at least 10 times that expected for such neonates.

As one measure of the health of these animals, Guillette's team began two years ago to examine the fluid that leaks out of eggs at the time of hatching and to analyze it for estrogen and testosterone. In females, estrogen should predominate, whereas males should have more testosterone. Eggs from Lake Woodruff — with normal hatching rates — displayed those classic patterns.

Apopka eggs didn't. One group showed what at first appeared to be the normal female pattern. Another group appeared to be "superfemales," with ratios of estrogen to testosterone twice as high as normal. "We didn't have any group that looked like males," Guillette recalls.

It turns out that there were indeed males — the gators emerging from eggs exhibiting the standard female ratio of hormones. But the concentrations of the hormones contributing to that ratio were not normal. "These animals were making almost no testosterone and almost no estrogen," Guillette explains.

Six months later, the researchers returned to Lakes Woodruff and Apopka to measure hormones in the young. "We found exactly the same condition that we had seen in the eggs," he says — "females with about twice the estrogen typical of a female and almost no testosterone in the males."

Apopka's animals also possessed feminized internal reproductive organs. The males bore what looked like ovaries, for example, while follicles in the females possessed not only abnormal eggs, but also far too many eggs.

Last summer, Guillette's team collected more than 100 juvenile alligators — animals 2 to 8 years old — from each of five lakes. Apopka's gators again distinguished themselves. The phallus on males was one-half to one-third the normal size, and the females' ovaries "looked burned out," Guillette says. Moreover, estrogen and testosterone production in all Apopka gators was minimal — as if, Guillette says, the ovaries and testes were indeed burned out.

What accounts for Apopka's feminized alligators? The culprit is estrogenic pesticides, Guillette testified at an Oct. 21 hearing before the House Subcommittee on Health and the Environment. Tower Chemical Co. for years made the pesticide dicofol — a molecule that he says looks like DDT with an extra oxygen atom. Production methods at the plant, situated on the shore of Lake Apopka, weren't always ideal, Guillette says. Spills occurred and much of the dicofol was laced with up to 15 percent DDT or DDE. Tower's defunct plant is now a toxic waste site.

While high concentrations of DDT have been measured in Apopka gators, Guillette cautions that this doesn't prove DDT is responsible for the observed feminization. To test that link, his team this summer painted gator eggs from Lake Woodruff with concentrations of DDE and dicofol to produce tissue contamination typical of hatchlings from Lake Apopka.

Though not all their tests have been completed yet, Guillette told SCIENCE NEWS that "we're finding hormone levels in these hatchlings that are almost identical to those in Apopka hatchlings." He adds, "That's about the closest thing to proof science is ever likely to give."

In the meantime, Apopka's gators continue to suffer. Since a catastrophic

dicofol spill in 1980, there has been a 90-percent reduction in the number of juvenile alligators at the lake. And in a population of animals that can live to be 60 years old, that's not healthy, he says.

Another reluctant toxicologist, Brent Palmer of Ohio University in Athens, has begun studying a substance in the blood of egg-laying vertebrates that he suspects will one day prove a sensitive biomarker of exposure to estrogenic pollutants, at least in males. It's vitellogenin, the egg-yolk protein.

When stimulated by estrogen, the liver produces this protein, then dumps it into the blood. From there it circulates to the ovaries, where it is deposited in an egg. Though males can produce vitellogenin, usually only females possess sufficient estrogen to do so.

That's good, Guillette points out, because "if you have enough estrogen in a male to turn on vitellogenin, then you probably have enough to shut off the normal functioning of the testes."

Working with the red-eared slider, America's most common turtle, Palmer has demonstrated that DDT can turn on vitellogenin production in males. But DDT doesn't elicit the same broad suite of changes that estrogen does. For instance, it fails to trigger the liver's production of two other proteins and it turns on the production of some other substances that estrogen doesn't. "So even though DDT is mimicking estrogen in some ways," Palmer points out, "it's not *exactly* the same."

"Certainly, if we can find vitellogenin in males in the wild, that's a sign they've been exposed to an environmental estrogen," he says. However, Palmer is not yet sure whether the converse also holds: that the lack of vitellogenin proves no estrogen was encountered. He says his new data "make me wonder if there might not still be an environmental estrogen present, just one that's having some other effect." Indeed, he says, interpreting the lack of vitellogenin "could prove a very sticky problem."

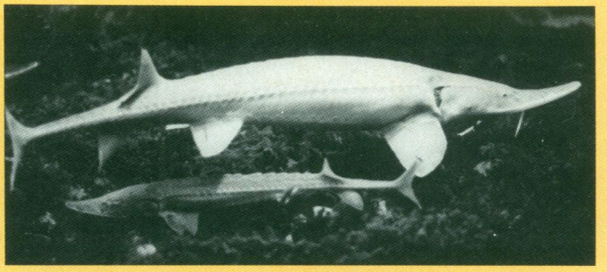
It's not a problem John Sumpter has had to cope with. The rainbow trout and carp that he and his colleagues have studied throughout the waterways of England and Wales have displayed plenty of vitellogenin — even the males.

Sumpter and Charles R. Tyler, biologists at Brunel University in Uxbridge, England, collaborated with scientists from Britain's Ministry of Agriculture, Fisheries, and Food to measure vitellogenin concentrations in fish that were caged and suspended for three weeks in the river outfalls of 30 different sewage treatment plants.

In the January CHEMISTRY AND ECOLOGY, these researchers describe finding widely varied production of vitellogenin by the fish. However, "in all cases," they say, "exposure of trout to effluent resulted in a very pronounced increase (500- to 100,000-fold, depending on the site) in the [blood] plasma vitellogenin concentration." In some cases, male trout exhibited vitellogenin concentrations in their bloodstreams typical of mature females during egg production. Carp showed similar, though far smaller, increases.

Attempts to isolate the agent responsible for these increases proved fruitless. However, at least one of the researchers strongly suspected that ethynylestradiol (EE) — the main estrogenic compound in birth-control pills — was responsible for much of the vitellogenin effect they observed. He reasoned that women on the pill excreted the EE in their urine and that some share of this chemical may have passed through the water-treatment plants.

To test the theory, the researchers incubated fish in aquariums containing dilute concentrations of either estradiol — the animal kingdom's primary estrogen — or EE. Concentrations of EE as low as 0.1 nanogram per liter of water caused a significant spike in the animals' production of vitellogenin — proving EE "very



Kent Keenlyne, FWS

Pallid sturgeon, an endangered fish native to the Missouri and Mississippi Rivers. Though most U.S. sturgeons aren't faring well, "there hasn't been any record of reproduction in the pallid sturgeon for 10 years," notes Richard Ruelle of the U.S. Fish and Wildlife Service in Pierre, S.D. Indeed, he says, any pallid sturgeon seen these days are usually 30 to 40 years old.

Altering the river — chiefly, damming and straightening its path — has reduced the fish's habitat. But the high concentrations of PCBs and DDT that have been found in some pallid sturgeon have led Ruelle to suspect that environmental estrogens might also be jeopardizing its reproductive health. Indeed, he notes, for 15 years researchers have reported that sturgeon gonads "aren't distinctly male or female anymore." Currently, Ruelle is awaiting lab results on vitellogenin in the gonadal tissues saved from sturgeon that were confiscated from anglers who caught the fish illegally.

EMFs — another environmental feminizer?

If electromagnetic fields (EMFs) can affect the amount of estrogen in animals — and their susceptibility to breast cancer (SN: 7/3/93, p.10) — might they also alter the fetal development of a male? Two studies investigating the topic suggest the answer may be a qualified yes.

Robert F. McGivern of Harbor-UCLA Medical Center in Torrance, Calif., and his co-workers exposed pregnant rats to low-level, pulsed magnetic fields twice daily for six days during the period when the fetal brain is undergoing sexual differentiation. In the January 1990 TERATOLOGY, the team reported finding that these low-frequency (15 hertz) prenatal exposures demasculinized the scent-marking behavior of mature males.

The study was notable for another reason, asserts McGivern, now at San Diego State University. Prenatal exposures led to "really huge" testes and prostate glands in the adult animal. The researchers have no explanation for the unexpected effect.

In 1972, researchers at the University of Manitoba in Winnipeg conducted a similar experiment. "We exposed male rats chronically to magnetic fields — either pre- or postnatally," notes study leader Klaus-Peter Ossenkopp, now at the University of Western Ontario in London. His team found that "if the rats were prenatally exposed, they developed heavier testicles. If you exposed them as adults, testicle size actually decreased."

"The reproductive system of the rat is built like a Sherman tank," McGivern says. As a result, he maintains, "any disruption in the rat becomes interesting because the human is usually much more susceptible to the same things."

And because EMFs appear capable of altering susceptibility to estrogen-mediated tumors in female laboratory animals, Ossenkopp asserts, these findings may represent EMFs' male reproductive corollary.

Neither group has followed up on the work nor knows of others investigating the reproductive effects of EMFs.

much more potent" than estradiol, Sumpter's team says. Indeed, they conclude, EE represents one of "the most potent of biologically active molecules."

If present in potable waters, however, EE must occur in concentrations below the limits of detection, the British team found. In fact, Sumpter notes, it was only after their research was completed that his team learned of another possible candidate: nonylphenols (SN: 7/3/93, p.12).

These are breakdown products of alkylphenol polyethoxylates (APEs), a class of surfactants first marketed in the 1940s. Today, APEs are used in detergents (including many U.S. dishwashing liquids), pesticides, herbicides, toiletries, and products that need to wet surfaces. Though the parent APEs are not estrogenic, Sumpter describes the nonylphenols as "directly estrogenic" – which means that they can bind to and activate the body's estrogen receptor.

Though nonylphenols occur in concentrations of more than 1 milligram per liter of water in poor-quality English rivers – especially downstream of textile mills – concentrations of 1 to 50 micrograms per liter (μg/l) are more typical of waters in England and Europe, Sumpter says. U.S. concentrations, by contrast, tend to fall below 1 μg/l.

"Because of their ubiquitous presence in the aquatic environment and the 'high' concentrations," Sumpter told SCIENCE NEWS, "we consider them a good candidate to account for the estrogenic effects [found in the study with trout and carp]." Though only perhaps 1/10,000 as potent as EE, nonylphenols "are pretty resistant to degradation and [they] bioaccumulate, which will increase the likelihood of them producing physiological effects," he argues.

But nonylphenols are not the only products formed by the breakdown of APEs. And because many of those others are not monitored, Sumpter says, "the total concentration of all the closely related degradation products remains unknown."

Environmental estrogens are also suspected of playing a role in reproductive problems plaguing the Florida panther, a species whose surviving members total only 30 to 50 animals.

Between 1985 and 1990, 67 percent of male Florida panthers were born with one or more undescended testes, a condition known as cryptorchidism. Just 10 years earlier, only 14 percent of males were cryptorchid, observes Charles Facemire, an ecological geneticist with the U.S. Fish and Wildlife Service in Atlanta. In addition, he notes, at least one noncryptorchid male is sterile, and even some of the apparently normal males produce abnormal or deformed sperm.

Initially, these problems were assumed to trace to a loss of genetic diversity in the heavily inbred species (SN: 9/25/93, p.200), Facemire says. But a few months ago, he and Mike Dunbar, a veterinarian with the Florida Game and Fresh Water Fish Commission in Gainesville, decided to investigate whether estrogenic contaminants might also be contributing to these reproductive problems.

Their initial blood sampling program turned up males with unusual steroid hormone ratios. For instance, one male had nearly twice as much estrogen as testosterone. (This animal should have had two to three times as much testosterone as estrogen.) At least two other males had similarly skewed ratios; both of them were also cryptorchid. Equally perplexing, at least one female had more testosterone than estrogen.

"We don't know enough about the species to know if these hormone levels might be normal under certain circumstances. But we don't think they are," Facemire says. Though genetic problems cannot be ruled out, he acknowledges, "I suspect we're going to find that the problems are due more to estrogenic chemicals in the environment."

Working under that assumption, Facemire's office has just issued a prohibition on the use of estrogenic chemicals – principally pesticides – in the 100 or so federally managed wildlife refuges in the southeastern United States. At the same time, Facemire's office has initiated four other investigations into possible effects of environmental hormones on wildlife – including one involving the prothonotary warbler in Alabama and another involving sea turtles in Georgia.

Nor are these the only animal studies linking reproductive changes with exposures to hormone-mimicking contaminants. Laboratory studies on fish at the University of Guelph in Ontario, for instance, have shown that white suckers exposed to papermill effluent – often rich in dioxins and related compounds – took longer to mature, developed smaller gonads, experienced reduced fertility, and had lower than normal concentrations of steroid hormones in their blood. Moreover, Glen Van Der Kraak and his coworkers reported at an international meeting on the topic in September 1990, male fish exposed to papermill wastes developed reduced secondary sex characteristics.

Other researchers have begun linking reproductive problems in salmon to relatively high concentrations of hormone-like contaminants. And at a conference sponsored by the U.S. and Canadian governments three years ago, PCBs in such fish were linked to dramatic declines in the reproduction of minks and otters around the Great Lakes.

Finally, University of Wisconsin scientists demonstrated two years ago that low prenatal exposures to dioxin feminized the behavior of male rats during adulthood – and sharply reduced their production of sperm. Indeed, the researchers concluded, the developing male reproductive system appears to be more sensitive to the effects of this hormone-like toxicant that any other organ or organ-system studied (SN: 5/30/92, p.359).

"Because we're only just getting to the basics in this field," Palmer says, even simple questions about the reproductive effects of environmental hormones for most species must go unanswered. But he suspects that biologists are going to have to move fast in finding those answers if some contaminated populations are to survive.

Toxic-pollutant concentrations in the environment have dropped to where they can seldom kill most adult animals outright, he says. However, in some species, he fears, "We may have gotten to a point where the adults look healthy but are so reproductively impaired that that population may already be extinct – and we're just waiting for the last remaining adults to die [of old age]." □

Jan. 22: Environmental hormones and men