

## How paper mill wastes may imperil fish

For years, biologists have observed that some fish downstream of pulp and paper mills reach sexual maturity much later than normal. For the white sucker, a distant relative of the salmon, that delay can be up to 2 years, "effectively reducing its lifetime fecundity by almost 50 percent," notes Glen J. Van Der Kraak of the University of Guelph in Ontario.

For lake whitefish, another commercially important species, "we have no indication of natural reproduction occurring," he says.

Other symptoms—such as exceptionally small reproductive organs and abnormally low concentrations of sex hormones circulating in the blood—suggested that something had greatly perturbed the fish's hormone systems.

Veterinary pathologists had traced similar symptoms in sheep and pigs to estrogenlike compounds in the plants they ate. So Van Der Kraak and Deborah L. MacLachy, now at the University of New Brunswick in Saint John, looked into a tree-derived estrogen that researchers with Environment Canada had identified in paper mill effluent.

They injected goldfish—the aquatic equivalent of laboratory rats—with this beta-sitosterol, then assayed each animal's blood a few days later. In the just-issued October *TOXICOLOGY AND APPLIED PHARMACOLOGY*, they report that the pollutant significantly depressed concentrations of estrogen, the primary female sex hormone, and testosterone, the primary male reproductive hormone, in both male and female fish.

The new study showed that sitosterol affects "predominantly. . . the gonads—the ovary and testis," explains Van Der Kraak. "And this pattern tends to parallel what we see in wild fish populations." His team has begun experiments to evaluate whether sitosterol can also affect gonad size.

"I don't think beta-sitosterol is going to explain the entire picture," Van Der Kraak cautions. Fish downstream of pulp and paper operations frequently exhibit detoxifying liver enzymes. "And we're not seeing that with beta-sitosterol," he points out. "So there must be other chemicals the fish are exposed to—such as some chlorinated organics—[activating] those enzymes."

Certain chlorinated organic compounds—especially dioxins—not only trigger these detoxifying enzymes but also act as hormones, impairing the reproductive development of mammals (SN: 7/15/95, p.44). Moreover, chlorine bleaching of wood pulp generates dioxins (SN: 5/12/90, p.303).

Van Der Kraak doubts, however, that such agents pose the primary endocrine risk from paper-making operations. "We've sampled fish before and after

normal mill shutdowns—such as for annual holiday maintenance—and we see a recovery [of normal hormone concentrations] in wild fish. This was one clue that dioxins weren't the causative agents." These long-lived chemicals would not have disappeared during the mill shutdowns.

"That plants would develop compounds that are reproductively active in animals is perfectly understandable" as a strategy for keeping predators in check, maintains Louis J. Guillette Jr. of the University of Florida in Gainesville. Though most predators would develop a tolerance to these hormone mimics,

## X chromosome again linked to homosexuality

In a reprise of the most controversial study of 1993, researchers surveying the genetic landscape of the X chromosome have discovered further evidence that it contains a gene or genes that may steer some men to homosexuality.

"Once again, we've found that more than half of gay brothers share the same chromosome region. It gives us additional confidence that there is something in this region involved in sexual orientation," says Dean H. Hamer of the National Cancer Institute in Bethesda, Md.

Two years ago, Hamer reported that men might inherit a predisposition to homosexuality through the X chromosome, whose genes are passed on by mothers (SN: 7/17/93, p.37). Some scientists harshly criticized the study, as well as the general effort to probe the genetics of behavior. This summer, the Chicago Tribune reported that a former member of Hamer's group had raised questions about how Hamer conducted the 1993 study, prompting a still-ongoing inquiry by the Department of Health and Human Services.

In the new study, as in the original, Hamer and his coworkers examined the X chromosomes of families that have two homosexual brothers. Since a son can inherit parts of his X chromosome from either of the two versions his mother has, the investigators looked for genetic markers, brief sequences of DNA, that differ between the two maternal X chromosomes.

Two-thirds of the 32 pairs of homosexual brothers included in the final analysis share the same markers for one portion of their X chromosome and thus inherited the same span, Hamer and his colleagues at the University of Colorado in Boulder and the Whitehead Institute for Biomedical Research in Cambridge, Mass., report in the November *NATURE GENETICS*.

Ordinarily, if that region played no role in sexual orientation, says Hamer, only about half the brothers would share it.

Guillette says, it would take generations. Thus, when animals such as these fish encounter what for them is a novel phytoestrogen, they may suffer dramatically—at least initially.

Indeed, says Peter Thomas of the University of Texas Marine Science Institute in Port Aransas, "the importance of the [Guelph study] is that it's showing that we should be concerned about other components of effluent, not just dioxins."

The study may also point to a solution to the fish's problems, he says. "Because most of these phytoestrogens are on the outside of plants, where insects can get to them, [industry] might solve this problem by debarking the wood before they ground it up." —J. Raloff

In another test, which Hamer had been chastised for not doing in the 1993 study, investigators examined the X chromosomes of heterosexual brothers of the homosexual men. At most, one-fourth of the 12 heterosexual brothers have the chromosome X region that their homosexual brothers share—less than the expected one-half, says Hamer.

Investigators also inspected the X chromosomes of 36 pairs of lesbian sisters but found no evidence that the chromosomes contain genes affecting the women's sexual orientation. "To me, it's not a surprise, because the pathways leading to female and male sexual orientation are probably quite different," says Hamer.

The researchers stress that the so-called gay gene on chromosome X would be neither sufficient nor necessary to make a man homosexual. Some heterosexual brothers possess the suspect region, while some homosexual men do not. "This gene is not acting like some automatic switch that makes you one way or the other," says Hamer.

The new study is not likely to settle whether the X chromosome holds a gene that sways sexual orientation. "It certainly falls short of a replication, considering it doesn't come from an independent team," says George C. Ebers of the University of Western Ontario in London.

Ebers' own, not-yet-published genetic analysis of homosexual brothers does not show any evidence of a gay gene on the X chromosome. "I don't have any explanation for why we differ," he says. Similar studies by other investigators are already under way.

"This issue won't be resolved by picking apart Hamer's studies. It will be resolved by doing a larger, definitive study," comments J. Michael Bailey of Northwestern University in Evanston, Ill., whose studies of twins have suggested that genetics does influence an individual's sexual orientation. —J. Travis