ENDOCRINE DISRUPTERS

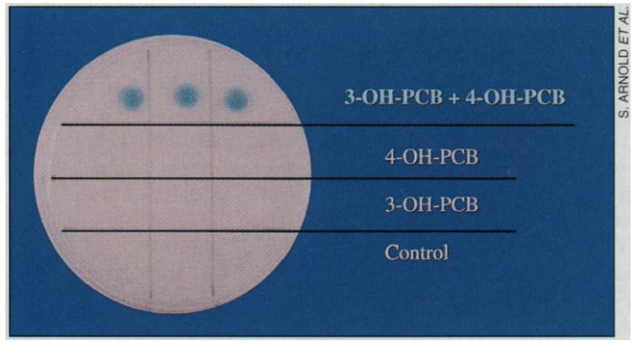
Synergy Paper Questioned at Toxicology Meeting

CINCINNATI—For a modest set of test-tube experiments, a study published in *Science* last summer made quite a splash. It was part of a rush of work looking into the controversial hypothesis that hormonelike chemicals in the environment could be contributing to cancer and reproductive problems in humans. The study found that in cultures of yeast cells specially fitted with receptors for the hormone estrogen, pairs of certain pesticides appeared to be up to 1600 times more potent at triggering an estrogenlike response than was either chemical alone.

This stunning synergy—reported by Steven Arnold and others in the laboratory of endocrinologist John McLachlan at Tulane University in New Orleans (*Science*, 7 June 1996, p. 1489)—didn't just intrigue basic scientists studying steroid hormones. It sent a chill through the Environmental Protection Agency (EPA) and other regulatory agencies, which now faced the possibility that all their safety tests of single chemicals might be suspect. And some observers say it helped give a final nudge to provisions in federal pesticide and drinking-water laws passed last August, requiring that EPA screen chemicals for estrogenic effects. "I never saw a paper have such impact," says one federal scientist close to the issue.

In the months since, however, the findings have been getting attention for a different reason: Two studies involving five laboratories have tested the same chemicals for synergy in yeast and mammalian cells; they have come up empty-handed. The ensuing debate has deepened rifts among scientists already sharply divided over the risks of endocrine disrupters, as was evident this month at a meeting here of the Society of Toxicology, where new studies failing to find synergy were presented. Although some scientists agree that the results still merit further study, others doubt that the findings will hold up or have already written them off as unlikely to be of relevance to animals or people.

The paper caused a hubbub because it seemed to address a central criticism of the endocrine disrupter hypothesis: that the suspected chemicals appear to be far less biologically active than natural estrogens in the body. The synergy results suggested that, in combination, endocrine disrupters might not be so weak after all. The McLachlan lab used an ingenious piece of genetic engineering: They manipulated yeast cells to express human estrogen receptors and some related genes—so that when estrogenic substances docked onto the receptors, the cell made a protein that turned the cell blue. They then tested combinations of the pesticides dieldrin, endosulfan, toxaphene, and chlordane (all but endosulfan have been banned but persist in the environment). Singly, these chemicals bound weakly with the estrogen receptors. But when two pesticides were tested together, their estrogenic activity shot up 160- to 1600-fold. The team also found fivefold synergy with polychlorinated biphenyls (PCBs), using transgenic human endometrial cells. The results, says John Sumpter of Brunel University in the United Kingdom, appear to be "immensely important. It has ramifications for not just endocrine disrupters but the whole spectrum of how you test and assess chemicals."



True blue? In a controversial study, three combinations of two chemicals—here, PCBs—were far more potent at triggering an estrogenlike response in yeast cells than was either chemical alone.

But last fall, the synergy theory began to fray at the edges, as other groups tried to repeat or extend the findings. At least five teams have now looked for synergy, using the same chemicals in 10 standard endocrine test systems. These range from transgenic yeast cells to breast cancer cells (which proliferate when treated with estrogen) to uterine assays—in which young female rats are injected with a suspected estrogen to see if it causes the animal's uterus to grow. The effects of the chemical mixtures, reported as Technical Comments in *Science* (17 January, p. 405) and in *Nature* (6 February, p. 494), were merely additive in every case, according to the investigators.

McLachlan maintains that a key difference may be that his custom-made yeast cells had very low levels of receptors—only 500 per cell. Because the cells of a developing fetus also have few receptors, he says, the finding still could be relevant to humans. McLachlan says he and collaborators are now looking for synergistic effects in newborn mice. But two other groups—William Kelce's laboratory at the EPA in Research Triangle Park, North Carolina, and Steve Safe's group at Texas A&M University in College Station—reported at the toxicology meeting that they have tried the experiments in yeast and mammalian cells with low levels of receptors, and they have found no synergism. McLachlan wasn't at the meeting, but after viewing portions of the session posters, he said, "I can't explain the difference."

Some scientists, such as Sumpter, say that even if synergy happens only in one cell system under specific conditions, the explanation could be "potentially a very interesting one." Postdoc Tom Wiese, in Kelce's lab, says he thinks it's important to design future experiments so that synergy could be detected. For others, the possibility may not be worth pursuing. "If you can't reproduce it [the Tulane group's results], you can't ask questions or extend it any further," says Kenneth Korach of the National Institute of Environmental Health Sciences, a co-author on the January Technical Comment in *Science*.

At the meeting, there was speculation that contaminated reagents might have given a false result. Recently, McLachlan told *Science* that in his lab's latest experiments, synergy "[has] not always been at the same magnitude." He declined, however, to say what level of synergy the group is now seeing, because "we're doing the additional studies right now." McLachlan, who some researchers say has been slow to share his materials, has recently given samples of his yeast cells to three other groups, and they hope to have results within a few weeks.

"I think it's incredibly important that we resolve the issue," says Duke University pharmacologist Donald McDonnell, also an author of the Technical Comment. "This is not meant to be a witch hunt, but this issue got so much public press." Some researchers even say that if, in this new round of studies, the Tulane group's finding of 1000-fold or more synergy turns out to be much lower, the team should issue a formal retraction.

To some of McLachlan's colleagues, the furious debate going on among toxicologists is a healthy sign. "If they truly got these results, ... in a way you have to admire them for going with it," says John Gierthy of the Wadsworth Center at the New York State Department of Health in Albany. "I just see this as how science works. We're trying to find the truth through debate."

–Jocelyn Kaiser