New Questions in Strobel Case

The microorganism Gary Strobel released in a controversial field test of Dutch elm disease may not be recombinant after all, which bears directly on which regulations he may have violated

MAJOR questions have arisen concerning whether the microorganism Gary Strobel injected into 14 American elm trees on the Montana State University (MSU) campus in June is in fact a recombinant DNA product. It has been widely reported in the press, including *Science*, that it is. The answer to this question bears directly on which federal and university guidelines Strobel may have violated in this controversial field test. On 27 August the Environmental Protection Agency (EPA) imposed mild sanctions on Strobel, a professor of plant pathology, for testing this organism without prior approval. Both the university and the National Institutes of Health (NIH) are investigating the matter.

As more facts emerge, however, it is becoming increasingly unclear whether this organism is properly considered a recombinant product. Strobel maintains that it is not—that it is a product of conventional mating, not recombinant DNA technology. The molecular biologists consulted by *Science* either support his view or concede that the question is ambiguous.

What this means in a practical sense, however, is not entirely clear. In a recent report, "Introduction of Recombinant-DNA Engineered Organisms into the Environment," the National Academy of Sciences said that the question should not matter—that living products such as microbial pesticides should be regulated on the basis of the risk posed by the organism itself, not the process by which it was made. But under the existing regulatory apparatus, or at least part of it, process still figures strongly. The NIH guidelines for federally funded university research apply only to recombinant DNA products. Thus, if Strobel's modified strain is not a recombinant product, he did not need approval of the NIH Recombinant DNA Advisory Committee (RAC) or of the university's institutional biosafety committee established under the RAC. For EPA, on the other hand, how the organism was created is not as important. Strobel clearly violated EPA regulations that require prior approval for a field test of any genetically *altered* microbial pesticide, however it was constructed.

The controversy hinges around a strain of *Pseudomonas syringae* that Strobel has modified in an attempt to protect trees from Dutch elm disease. The wild-type bacterium, a native of Montana, produces an antifungal agent that inhibits the fungus that causes Dutch elm disease. Strobel constructed a mutant strain of this bacterium through a conventional mating technique, known as conjugation, which allows a plasmid to move from one bacterial cell to another, in this case, from *Escherichia coli* to *P. syringae*. The *E. coli* plasmid he used carries a transposon, a moveable piece of DNA, that is sometimes taken up by the DNA of the host cell. The plasmid itself disappears, and what is left is a *P. syringae* containing the transposon, known as Tn*903*.

Plasmid conjugation is a conventional technique that has been used for centuries. Questions arise in this particular experiment, however, because Strobel used a recombinant plasmid, pRK2013, which was originally derived from *E. coli*. If this recombinant plasmid remains in the new host cell, then the mutant strain might be considered a recombinant product. But, according to Strobel, the plasmid is unstable in *Pseudomonas* and cannot survive; when these mutants are analyzed with Southern blots, no plasmid DNA is detected in the cell. The transposon that does remain, Tn*903*, is well characterized and unmodified.

One of the mutant strains produced by this process makes slightly more of the desired antifungal agent than the wild-type bacterium. (When the transposon inserts itself into the genome, it apparently causes a mutation that affects the level of antifungal agent production.) Tn*903* also carries resistance to kanamycin, an antibiotic, and thus can serve as a molecular tag for positive identification of the bacterial strain. It was this strain, which in laboratory and greenhouse tests inhibits Dutch elm disease, that Strobel injected into young American elms on the MSU campus. (He describes construction of the mutants and the greenhouse experiments in an article to be published in the 2 September *Proceedings of the National Academy of Sciences*.) He then infected these trees and a control group with the Dutch elm fungus. The experiment seems to have been a success, Strobel says; the unprotected trees were dead within 6 weeks, while those injected with the strain appear to be healthy. However, the university biosafety committee has recommended that the experiment be terminated and the trees cut down. MSU president William Tietz has yet to rule on the matter.

Strobel maintains that, plasmid notwithstanding, his strain is not a recombinant product and thus does not fall under the NIH guidelines. Others agree, including Mary-Dell Chilton of Ciba-Geigy, a molecular biologist who was recently elected to the National Academy of Sciences for her seminal work with the Ti plasmid of the soil bacterium *Agrobacterium tumefaciens*. "The bacterial strain he used does *not* have the original plasmid," says Chilton, who is familiar with Stobel's work. "It is armed with the transposon through natural mating and transposition processes. The only aspect that could be considered recombinant DNA is the plasmid, but the plasmid is gone. It is not a subject for the NIH guidelines."

If this description of Strobel's work is accurate, agrees RAC member Susan Gottesman of the National Cancer Institute, then it would not constitute recombinant DNA research or be covered by NIH guidelines. She cautions that she cannot make a definitive statement, however, without reviewing his work. To William Gartland, executive secretary of the RAC, the key question is whether any plasmid DNA remains in the mutant. And without more information on how the mutant was constructed, he says, he cannot tell.

"The crux of the issue" is whether the organism is recombinant, says David Young of MSU, who chaired an all-day hearing at the university on 28 August. "Evidence was presented on both sides, but more weighted on the side that it is not recombinant," says Young, whose committee will report its recommendations to the university president on 31 August. If they conclude that the organism is not recombinant, he says, then Strobel violated no university regulations.

Whether Strobel's organism is recombinant is a moot point at EPA, where it falls under the broader classification of a genetically altered microbial pesticide. EPA does not distinguish between microorganisms

modified by recombinant DNA techniques and those modified by other genetic techniques. For such products, as opposed to living but unaltered microbial products, EPA requires that researchers submit data 90 days before they plan to conduct a field test of any size. (For unaltered microbial pesticides, this requirement comes into play only for large-scale field tests.) EPA then has 90 days to determine whether an experimental use permit is needed.

Strobel mailed his data to EPA on 15 June, only 3 days before he began inoculating trees on the MSU campus. The penalties EPA meted out last week are mild because he is a first offender. Under these rules, if Strobel plans to field test a genetically altered product during the next year, his application to EPA must be cosponsored by a responsible party, such as the university. And any application to EPA must first be reviewed by the university biosafety committee. These sanctions were imposed, EPA officials say, not because his experiment is unduly risky—indeed, it probably would have been approved without an experimental use permit—but because he failed to comply with known regulations. "He knew the rules," says EPA spokesman Al Heier, "he called us."

Strobel now admits his actions were wrong and says that his earlier remarks about defying regulations as an act of "civil disobedience" were spoken in anger. He says that until a colleague suggested that he check with the agency, he did not think his work was covered by EPA regulations. And after an EPA official assured him on the phone that his work would most likely not require a permit—but that he must wait 90 days while EPA reviewed his data—he went ahead anyway rather than delay the experiment until next season. "The problem is I acted in haste."

Although no one *Science* spoke with condones Strobel's behavior, several people said the episode does point out the need to clarify NIH and EPA policy. Mary-Dell Chilton, for one, believes there is legitimate room for confusion. She says she spent hours reading the *Federal Register* announcement of EPA's policy. "I could not make a judgment based on that document, I could not understand it." In her view, the problem with federal policy is not overregulation but simply a lack of clarity. "I don't want to put all the blame in the federal camp," agrees Young of MSU, "but there needs to be a consolidation of opinion" among the federal agencies.

Arthur Kelman, a plant pathologist who chaired the recent NAS panel, notes that when the "experts" are divided on a question as basic as whether Strobel's work constitutes recombinant DNA, it is not surprising if others are as well. One EPA official, who referred to the distinctions between the federal agencies on this question as "hairsplitting," says the recent episode makes a good case for regulating on the basis of the product alone.

Questions have also been raised about whether Strobel violated state and federal regulations—and, more important, created a hazard—by infecting trees with the Dutch elm fungus. Although the disease is in the state, it has not been detected in the Bozeman area. Strobel says he followed necessary precautions to prevent the disease from spreading, such as injecting the fungus into only young trees (beetles, which spread the disease, are not attracted to young trees), spraying the trees with insecticide, and burying diseased trees.

According to Terry Medley of the U.S. Department of Agriculture, if Strobel used fungus that originated in the state, then he did not need a federal permit. Data on that and other questions are incomplete, he says, but if standard procedures were followed, "the experiment sounds OK. Dr. Strobel is very knowledgeable in this area. It sounds as if he took extra measures, he followed good field procedures. But he failed to notify EPA or USDA."

And that, by all accounts, is the crucial step. Even if an experiment seems safe, says Martin Alexander, a Cornell University professor who serves on EPA's science advisory committee, "we want to have someone who is more sure than you are look at it, someone with nothing to gain or lose. That's what we have regulatory agencies for." ■

LESLIE ROBERTS

Researcher Accused of Plagiarism Resigns

Raymond J. Shamberger, a biochemist accused of plagiarizing a National Academy of Sciences report for a book on nutrition and cancer, resigned from his position at the Cleveland Clinic Foundation on 30 June.

Shamberger, head of the clinic's enzymology section, had been employed there since 1969. He could not be reached for comment, and a clinic spokesperson would give no details as to the circumstances of his resignation.

The resignation follows the recent withdrawal from the market of Shamberger's 1984 book, *Nutrition and Cancer*, by its publisher, Plenum Press. Plenum withdrew the book after Colin Campbell, professor of nutrition at Cornell University, called its attention to the fact that large portions of the book had been lifted from the academy's 1982 report, *Diet, Nutrition and Cancer*. Campbell was a member of the panel that produced the academy report.

The apparent plagiarism actually came to light in 1985 at a hearing held by the Federal Trade Commission. At that hearing, Shamberger appeared as expert witness on behalf of General Nutrition Inc., a company that manufactures nutrition supplements. The company had been accused of false and misleading advertising in its promotion of pills called "Healthy Greens," which it claimed could reduce the risk of cancer. According to Campbell, who appeared as a witness for the government, Shamberger cited his book to back up his contention that diet supplements may indeed reduce cancer risks. The academy report, however, while stating that dietary fat raises cancer risks, specifically states that there is no evidence that diet supplements reduce risks.

This was all aired at the hearing, which resulted in an order to General Nutrition to stop the offending advertising.

The publisher, however, took no action despite the fact that the apparent plagiarism became public when the *Journal of the American Medical Association* published a note from Campbell following the publication of a review of Shamberger's book. Campbell recently contacted Plenum directly.

Plenum people were not available for comment at the time of writing. But an editor told *Science and Government Report,* which published an article on 15 June, that no one there was aware of any problems with the book until Campbell called. They obtained a copy of the academy report and asked Shamberger for an explanation. Unsatisfied with Shamberger's response, they withdrew the book.

Shamberger is best known for his epidemiological work on the relation of soil selenium content and mortality. He originated the hypothesis, which is still controversial, that selenium may protect against heart disease and cancer. He is author of a 1983 book, *Biochemistry of Selenium*, also published by Plenum. Thressa C. Stadtman of the National Heart Lung and Blood Institute, who reviewed the book in *Nature*, wrote that the text is "full of glaring errors."

The Shamberger case has drawn little attention. The cancer book was not done in connection with any federally supported research. Campbell, however, calls it "the most serious case of plagiarism that I have ever heard about in all my years of research." ■ CONSTANCE HOLDEN