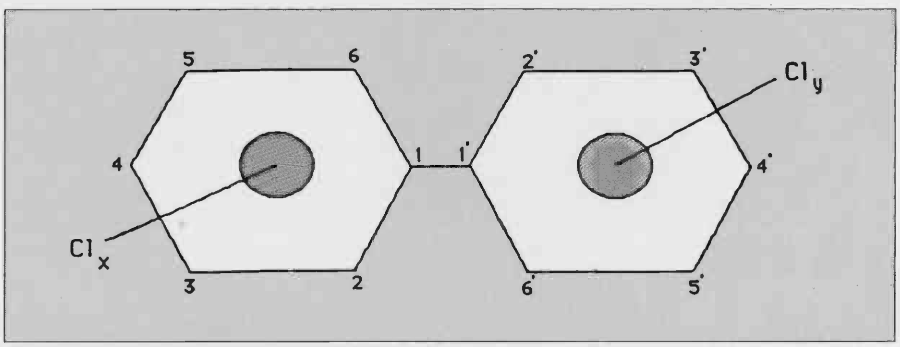
New Plants to Clean Up the Environment

By Jan McCoy

Michael Stoklos

Don P. Bourque monitors the growth of transformed plants.



The generic structure of PCBs. There are 209 possible structures with different numbers of chlorine (x and y) in each ring of the parent compound, biphenyl.

Genetically engineered plants may someday help clean up PCB-contaminated environments in a way humans cannot—by metabolizing the toxic compounds.

After polychlorinated biphenyls (PCBs) were introduced in the 1930s, the compounds found wide use in industry because of their ability to transfer heat. In time, PCBs were manufactured and used worldwide in electrical transformers and capacitators, paints, hydraulic systems and even in carbonless paper. But as the use of PCBs increased, so did the number of contamination incidents. Accidents and improper disposal methods caused the compounds to turn up in: cooking oil in Japan; New York's Hudson River; grain silos in the Midwest; packaged foods in Norway, Sweden and the United States; and fertilizers in Indiana. Although PCBs are no longer produced in most parts of the world, nearly a half century of use left behind a global problem.

PCBs are a class of chemicals characterized by two attached ring structures containing varying numbers of chlorine molecules. These compounds are stable and non-biodegradable, making them difficult to remove from the environment. PCBs accumulate in the fatty tissue of humans and other animals, most of which are unable to eliminate the compounds from their systems, causing a variety of toxicological problems including liver tumors, miscarriages, and skin and testicular problems.

Current environmental treatment methods are limited to containing the contaminants in order to prevent them from further spreading into soil and water. Although scientists continue to explore biological remediation techniques (such as using bacteria that metabolize PCBs), federal guidelines and public concerns restrict the release of such genetically altered organisms into the environment.

Don P. Bourque, a University of Arizona associate professor of biochemistry, says he's working on a possible answer to the PCB dilemma. Bourque and his research team have developed a method to genetically alter plants to metabolize PCBs.

"We also can engineer these plants to be male sterile," Bourque says. "They won't reproduce and spread all over the place. The impediment of restricting field-use of genetically engineered organisms based on the assumption they may not be containable doesn't exist in this case."

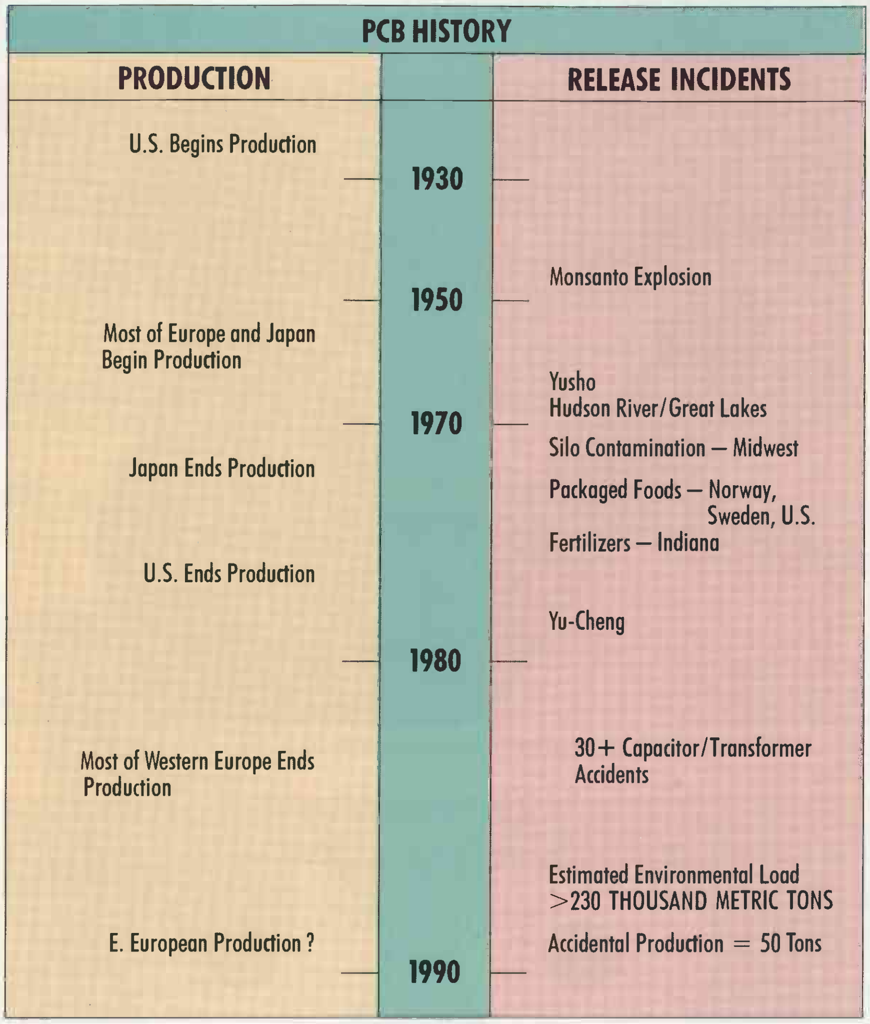
The project began more than four years ago as a collaboration with James Halpert, a UA associate professor of pharmacology and toxicology in the UA College of Pharmacy. Halpert studies how drugs are metabolized by a class of enzymes called cytochrome P450s. Enzymes are proteins capable of inducing chemical changes in other substances without being changed themselves. Cytochrome P450s are known to chemically modify drugs and chemical pollutants, including PCBs.

Research has shown that some animals are able to metabolize PCBs and eliminate them from their bodies. At the time Bourque and Halpert began working together, Halpert had just identified the cytochrome P450 responsible for PCB metabolism, which he named PBD-2. Bourque offered to work with Halpert to isolate PBD-2's genetic information.

"Our ultimate goal was to learn how the protein (PBD-2) works to metabolize PCBs and to understand how the gene coding for the protein might be turned on in the presence of PCBs," Bourque says.

The DNA that codes for the protein was isolated with the help of postdoctoral fellow Penny Graves, and a paper on the work was published in 1990. Halfway through the isolation process, Bourque, a plant molecular biologist, began to wonder how the work could be applied to plants.

"The idea I came up with fairly quickly was, 'what if plants had this



A brief history of PCB production and the major incidents of PCB releases into the environment.

Michael Stokios

Transformed plants containing the PBD-2 gene represent different ages and isolates.

gene and could metabolize PCBs?'" Bourque says. "Perhaps we could improve the environment by developing genetically engineered plants that break down toxic compounds into nontoxic materials? I think this is a novel and viable concept."

Bourque and graduate student Dan Wall collaborated with UA plant scientist David Galbraith to use *Agrobacterium tumefaciens*, a soil bacteria, to transfer the PBD-2 gene into tobacco plants. In nature, *Agrobacterium* transfers a segment of its DNA into plant cells, causing the plant to grow a tumor. By eliminating its tumor-causing properties, scientists created a versatile vector able to transfer foreign genes into many types of plants.

The work went quickly. Preliminary results indicated the probability was high that the gene could be put in plants and that the plant would produce the protein.

"This is only the tip of the iceberg."

"We went ahead, easily obtaining undifferentiated masses of transformed plant cells. We regenerated them into new plants and found that the leaves of these new plants also produced the protein. We proved the gene was there and that it functioned in the transformed plants."

One more step was needed for the team to show the potential for practical application of the technology. In November 1991, Bourque got the data he needed. Using an assay that uses a steroid in place of PCBs, Bourque and Wall proved the protein is active in plants and able to metabolize the steroid. Typically, cytochrome P450s react with steroids, so steroids can be used as a diagnostic substrate for the PBD-2 protein.

"PCBs are nasty and difficult chemicals to work with," Bourque explains. "The steroid substrate was our first choice because it was simple to use. We're now in the process of working with PCBs to see whether the protein metabolizes PCBs as well as the steroid substrate."

Bourque also is trying to transfer the PBD-2 gene into Dunaliella algae in hope of engineering marine organisms able to metabolize water pollutants. Dunaliella was chosen because of its many species and tolerance to salt and polluted water.

Using well-chosen cytochrome P450s to metabolize specific chemical pollutants holds a multitude of commercial possibilities, Bourque says. He's now hoping to begin working with two different cytochrome P450s known to metabolize herbicides.

"Consider this," he says, "what if you could plant a crop containing this kind of gene, use herbicides at the beginning of the season and know the enzyme would detoxify the field and even the crop by harvest?"

"This is only the tip of the iceberg. We've only chosen a couple of compounds to work with, but we have reason to believe this approach will be applicable to many kinds of pollutants that need to be dealt with in our environment."

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