inflammation. In their study of 88 heart disease patients and 88 controls, *H. pylori* was found in 62% of people with heart disease and only 40% of those without the disease.

In 1998, the Penn State Harrisburg team, headed by microbiologist Katherine H. Baker, discovered that *H. pylori* contaminated surface water and shallow private wells in rural areas of Pennsylvania and Ohio. Of 42 surface water samples and 20 shallow private well samples collected, 40% and 65% of samples, respectively, tested positive for *H. pylori*. The findings demonstrate a major reservoir for the bacterium outside the human body and support the possibility of a waterborne transmission route of *H. pylori*.

A traditional indicator of microbial water quality involves testing for coliforms (microbes used as markers of fecal contamination) such as *Campylobacter* and *Salmonella*. Baker found that approximately 85% of the surface water samples contained coliforms. However, in four well water samples, *H. pylori* was detected in the absence of coliforms, suggesting that routine screening of water supplies for such bacteria may fail to protect consumers from exposure to *H. pylori*. These findings have been accepted for publication in the *Journal of Applied Microbiology*.

Baker and her team also analyzed water samples from the private wells of 10 people who were diagnosed with *H. pylori*-related illnesses or who had concerns about their drinking water source. Samples of tap water were collected and eight of the wells were found to be contaminated with *H. pylori*. The small sample size "is definitely a limitation of the study," says Baker, but the statistical agreement "is enough to raise a red flag." This first direct link between contaminated drinking water and stomach ulcers was presented 2 June 1999 at the American Society for Microbiology meeting held in Chicago, Illinois.

Baker suspects that *H. pylori* contamination of private well water could be caused by other household members infected with *H. pylori*; since the bacterium is believed to be transmitted through fecal–oral transmission, septic tanks could contribute to private well contamination. Although septic tanks should be located at least 100 feet from drinking water wells, sitting regulations often are not enforced in rural areas. In one case, Baker found a well just 15 feet from a septic field.

Unfortunately for private well owners, testing for *H. pylori* is not a routine laboratory task. Baker uses an expensive and time-consuming research method that involves a commercial monoclonal antibody specific for *H. pylori* and immunofluorescence, followed by direct microscopic examination. "The last thing I want is people paying a lot of money to have their water tested for *H. pylori*," says Baker. Instead, she recommends that people with well water have it checked for coliforms, an inexpensive test performed by local health departments. "The presence of coliforms is a good, but not absolute, indicator of the likelihood of *H. pylori*," Baker says. Levels of chlorine that kill coliforms also kill *H. pylori*. For this reason, municipal water supplies treated with chlorine will not be contaminated with *H. pylori*.

**Scientists Find MTBE Degradates Naturally**

The reformulated gasoline additive methyl tert-butyl ether (MTBE) is a mixed blessing: the oxygen it contains decreases the production of smog-producing carbon monoxide during combustion, but it can be a pollutant in its own right, and it is classified as a possible human carcinogen by the U.S. Environmental Protection Agency (EPA). But the scales may be tipping, thanks to a study published in the 1 June 1999 issue of *Environmental Science & Technology* in which scientists found that naturally occurring microbes can digest MTBE and convert it into less toxic by-products.

MTBE enters the environment by leaking from underground gasoline storage tanks and through gasoline that evaporates as vehicles are being fueled. It migrates much more quickly through the soil than most petroleum distillates and has been found in groundwater at numerous sites in the United States. Despite MTBE's utility in reducing carbon monoxide, in July the EPA suggested to Congress that the use of the chemical be "reduced substantially" (the phrase was not defined further) because of fears over water contamination. In California, where traces of MTBE have been measured in 10,000 wells, the state has ordered a phaseout of the additive by 2002.

In the June *Environmental Science & Technology* article, researchers from the U.S. Geological Survey in Columbia, South Carolina, reported on a study in which they extracted sediment from beneath two streambeds that received groundwater discharge from storage tanks that had each leaked about 1,000 gallons of gasoline containing MTBE. The study sites were "typical, garden-variety underground storage tank releases that were adjacent to sensitive receptors," says research hydrologist James Landmeyer, an author of the study. "We didn't want something so unique that the results would not be transferable to other locations." The sediments were taken from an aerobic zone of sand and gravel about 2 inches below the streambed.

In the laboratory, the researchers added radioactively labeled MTBE to the sediment samples, then measured how much radioactive carbon dioxide was produced as an indicator of how much MTBE was degraded. After 100 days, microorganisms from one site had degraded 30% of the MTBE. The comparable number for the other site was 73%. The reason for the difference is unclear. The degradation only occurred, however, in samples that were maintained in aerobic conditions. MTBE was not degraded when oxygen was not available or when the samples had been heated to kill microbes. After 80 days, 84% of tert-butyl alcohol, another component of reformulated gasoline, had also been degraded in samples from both sites.

"Our results indicate that [certain] microorganisms are able to degrade MTBE to nontoxic by-products if oxygen is present in the microbial environment," says lead author Paul Bradley. The decay microbes have not been identified yet, but the researchers suspect that a community of microbes is responsible.

Although the samples were taken from active spill sites, there is evidence that the decay process is more effective in nature than in the laboratory. The maximum groundwater concentration of MTBE at
About micrograms seeing any sites, charging means the MTBE, “costs organic improved the additive he Landmeyer MTBE Administration and the Forum Green 30 other Program corrosivity. A ICCVM-sponsored the Validation material of established institution Occupational Safety regulatory agencies says. “We years.”

William Stokes, the ICCVM cochair and director of the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods, says, “This is the first in vitro test to be reviewed by an ICCVM scientific panel and recommended for consideration by regulatory agencies. The review of this method showed that the test may be useful even when it does not completely replace the current animal test.” Corrosivity tests are used to determine whether a chemical will cause irreversible damage to human skin or eye tissue. They are also used to ascertain the type of packaging necessary for shipping a particular chemical in order to comply with Department of Transportation regulations.

Corrositex was developed by In Vitro International of Irvine, California. The test method apparatus is a glass vial filled with a chemical detection system consisting of water and pH indicator dyes, and overlaid with a collagen matrix biobARRIER membrane. If a sample is able to penetrate the biobARRIER either by diffusion or destruction, the fluid will change color. The tester records the time it takes (usually between 3 minutes and 4 hours) for the sample to break through the membrane. ICCVM’s evaluation considered Corrositex data (either provided by In Vitro International or obtained from peer-reviewed sources) from tests of 163 different materials for which there were corresponding in vivo rabbit corrosivity data. At a public meeting held 21 January 1999 to formulate a final recommendation on Corrositex, the ICCVM Peer Review Panel determined that the test is useful as a stand-alone assay for acids, bases, and acid derivatives, and as part of a tiered assessment strategy for testing other chemical and product classes. When used as a stand-alone assay in some testing situations, Corrositex replaces the use of animals for corrosivity testing; when used as part of a tiered approach, the test reduces and refines the use of animals in testing by providing a basis for decisions on which, if any, further in vivo tests need to be conducted.

Robert Scala, a toxicology consultant who served as the panel chair, says, “The ICCVM report states very carefully that a negative test may suggest that the investigator may want to pursue further testing using alternative methods such as knowledge of chemistry of the material or a limited animal test.” Follow-up tests using in vivo methods could employ fewer animals and less potent test doses to minimize possible pain in any individual animal. In addition to its animal welfare advantages, Corrositex is less expensive than the traditional rabbit test, displays results more quickly, and requires no special equipment, facilities, or training.

Before testing with Corrositex, all test chemicals are prescreened by directly applying a small amount of the test material to the detection fluid. If a chemical is unable to shift the pH of the fluid to less than 4.5 or greater than 8.5, it does not qualify for testing with Corrositex and must be tested using another method. Some nonqualifying chemicals may actually be corrosive, and in fact, the primary limitation noted in Corrositex is the proportion of test chemicals that do not qualify for use with the test, which, in the case of the chemicals culled from different databases for the ICCVM assay, came to about 18%. However, of the 75 nonqualifying test chemicals evaluated, 85% were not corrosive according to available in vivo test results, indicating that nonqualifying test materials are most often not corrosive.

The panel recommended several changes to the current test method protocol that will address issues of tester instruction and variability in testing conditions. Overall, says Scala, “For those categories of materials for which there is evidence that the test worked well, this report is a strong endorsement.

Green Light for Alternative to Rabbit Test

On 22 June 1999, the National Toxicology Program and the NIEHS, along with 13 other federal agencies that support the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), announced the results of an ICCVM-sponsored independent peer review of Corrositex, an in vitro test for corrosivity. The test provides an alternative to the traditional assay in which the sample material is applied directly to the skin of a rabbit. This review provides the basis for regulatory agencies such as the Occupational Safety and Health Administration and the U.S. Department of Transportation to determine whether and how the Corrositex test may be used to assess dermal corrosivity, proper chemical packaging and labeling, and safe transportation and storage methods. ICCVM was established in 1997 to coordinate the development, validation, acceptance, and harmonization of new toxicological test methods throughout the federal government, including alternative tests that reduce, refine, or replace animal use.

Color proof. In the Corrositex assay, a test chemical is applied to a biobARRIER membrane suspended over a chemical detection fluid contained in the test vial. Corrosive agents will penetrate the membrane and cause the detection fluid to change from yellow (left) to a shade of orange/red (right). The time it takes the fluid to change color helps determine the potency of the test chemical.