two sites upgradient from the stream was 64 milligrams per liter at one site and 138 micrograms per liter at the other. Yet no MTBE was detected in the stream water at either site, indicating that microbes in the streams were indeed cleaning the groundwater. "The fact that we're not seeing any MTBE in the stream water means the groundwater is probably discharging slowly to the stream at both sites, [giving the microbes time to digest the MTBE]," says Landmeyer.

Learning what happens to MTBE in the environment is a critical question as scientists and regulators continue debating the costs and benefits of oxygenated fuels. About 3 billion gallons of MTBE were produced in 1997, according to John Zogorski of the Rapid City, South Dakota, office of the U.S. Geological Survey. Zagorski says the additive is one of the most widely used organic compounds. And, despite the improved regulation of underground storage tanks, leaks continue to be a problem: Landmeyer estimates that South Carolina alone has 3,000 past or present tank leaks, although not all feed directly into surface waters. "I don't think the problem of MTBE in groundwater is going away by any means, even if it is banned tomorrow," he says. "We will have this legacy for 10 to 30 years."

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 ICCVAM-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 used to assess dermal corrosivity, proper chemical packaging and labeling, and safe transportation and storage methods. ICCVAM 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.

William Stokes, the ICCVAM 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 ICCVAM 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.

NICEATM

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.

Corrositex was developed by InVitro 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.

ICCVAM's evaluation considered Corrositex data (either provided by InVitro 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 ICCVAM 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 ICCVAM 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 ICCVAM 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.