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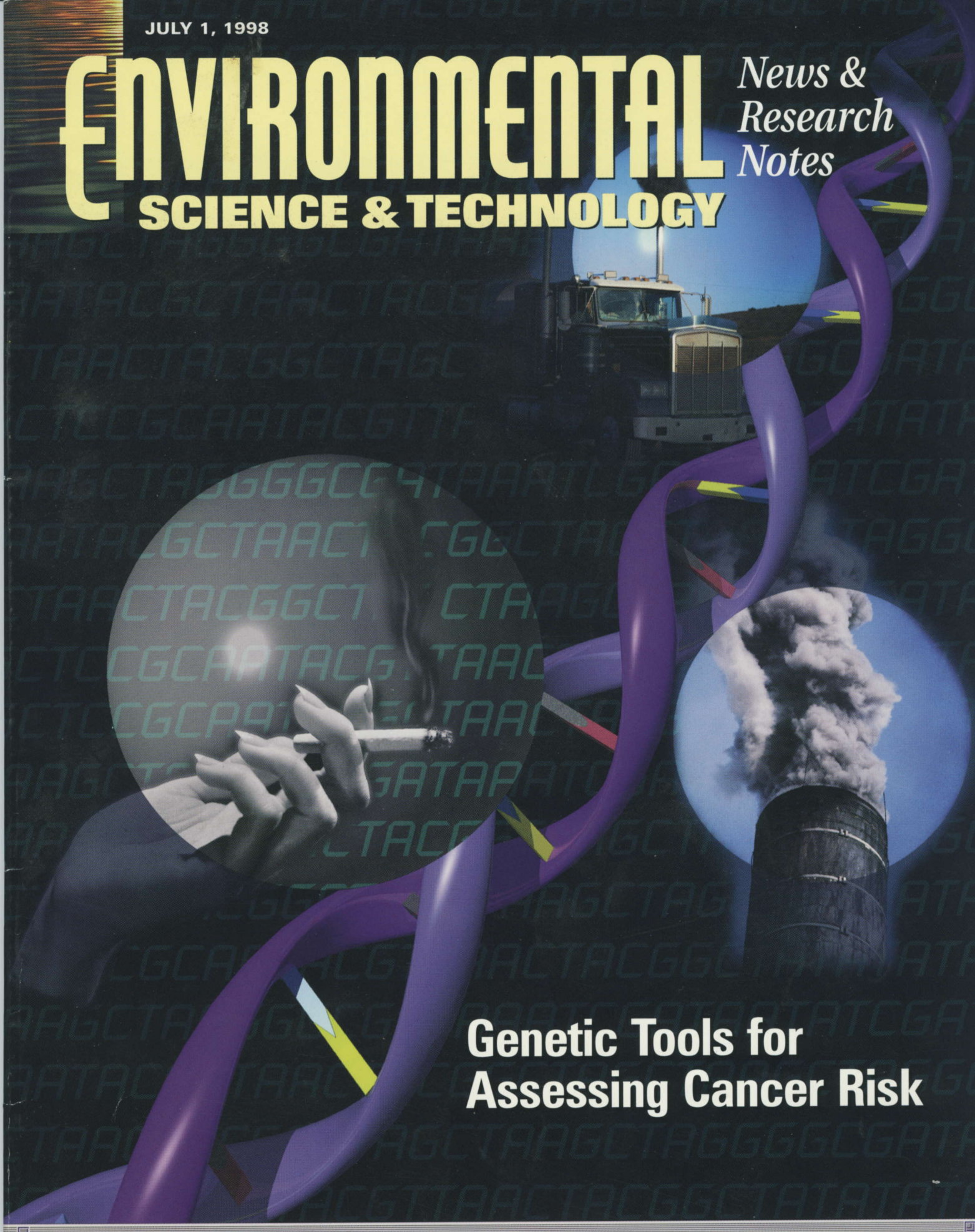

Genetic Tools for Assessing Cancer Risk

FEATURE

Unraveling Cancer Risk With Cellular and Molecular Tools

Innovative techniques and mechanistic insights are reducing uncertainties in risk assessments.

MARGIE PATLAK

The hazy science behind environmental risk assessments currently relies much more on general principles and experience than on a detailed understanding of how specific chemicals cause cancer or other adverse effects. Without those mechanistic details, risk assessments suffer from being sketchy and uncertain—a blurred picture that is open to interpretation. But in the not-too-distant future, risk assessors may be able to provide a sharper picture of health risks posed by environmental contaminants, thanks to advances in genetics and molecular biology that are filling in some of the details. Not only do these advances show promise for improving the certainty of risk assessments, they also are dramatically changing how these assessments are done.

Right now, to determine if a substance might cause cancer in people, for example, researchers give large doses of test compounds to laboratory rodents for two years and observe them to see if they develop significantly more tumors than control animals not exposed to the same compound. These studies are expensive and take a long time to perform. "What is worse, when you are all done, it is hard to know how to extrapolate your findings to humans at reasonable doses," said Dick Albertini, a physician and geneticist at the University of Vermont. Future cancer risk assessments, in contrast, may not require showing whether a chemical causes cancer in rodents, but rather rely on more short-term tests that show whether the chemical causes the genetic changes that lead to tumors. "It will take time for people to accept that," said senior geneticist Vicki Dellarco of EPA, "but as we understand carcinogens better and how they operate, we will continue to move in that direction."

"There is a danger, because cancer is a very complicated picture, that once you start looking at the pieces you may miss the issue," she said. Laura Rosato, senior director of public health policy, speaking for the Chemical Manufacturers Association, stressed the importance of validating new tests and models. "I am concerned about these new tools being used to regulate a material when not enough is understood about them," she said.

To meet that concern, the Interagency Coordinating Committee on the Validation of Alternative Methods, which had its first meeting in May 1997 and comprises representatives from 14 government and research organizations, will help developers of new tests or animal models acquire resources to conduct validation studies. These studies will indicate the usefulness and accuracy of the new models under various conditions.

In the meantime, EPA is willing to consider data

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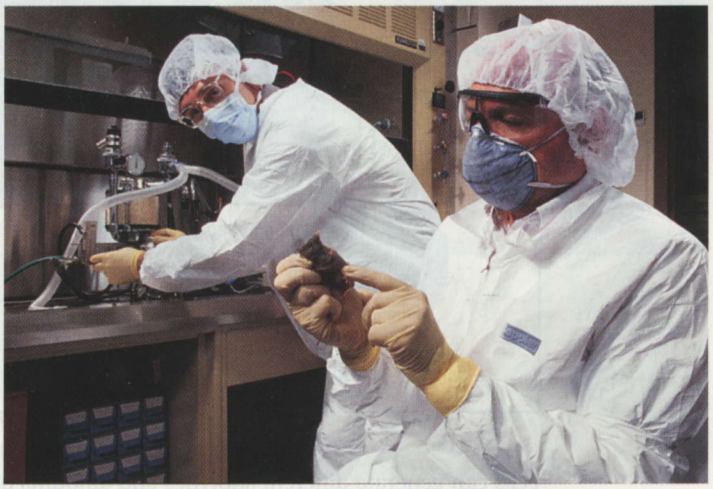
generated by alternative tests and animal models. Dellarco stated that the Food Quality Protection Act and the Safe Drinking Water Act Amendments of 1996 specify that the agency consider health risks for particularly susceptible populations such as children and the elderly. "We are obligated to look at all the relevant findings and bring them into the risk assessment whether they come from standard accepted assays or not," she said.

Assessing health risks

Environmental risk assessment, which EPA formally adopted in 1986, is a method regulators follow to determine if a substance in the environment is likely to pose significant health risks to people. New chemicals, such as pesticides, must pass such risk assessments before they can go to market. Environmental risk assessments of pollutants also determine what measures various industries must take to meet clean air and water standards. The key steps involved in a risk assessment are hazard identification, dose–response assessment, exposure assessment, and risk characterization.

Hazard identification uses the results of human and animal studies to determine if a particular compound, such as formaldehyde, is associated with a toxic effect, such as cancer. If there is enough evidence to make the compound suspect, researchers then use additional studies and mathematical models to more precisely define the connection between dose and toxic effect. If findings from these studies warrant concern, researchers then determine exposure pathways and try to measure or estimate amounts of the suspect compound to which people are exposed. Emissions of a pollutant from an industrial smokestack, for example, may be measured, and then air flow dynamics and other information are used to estimate how much of that pollutant is reaching people in a nearby residential community. Risk characterization uses the information generated by the prior three steps in the risk assessment process to estimate the likelihood of a health effect given specific conditions of exposure.

In recent years, shortcomings of the environmental risk assessment process have become increasingly apparent (*1*). Highly criticized by toxicologists are the mathematical leaps of faith the process often takes to project risk from animal studies to risk in humans, low-dose effects from high-dose effects, and effects in a small number of people to effects in the population at large. Some of those projections made from animals to humans are likely to be faulty because the path taken by a toxin to cause damage in rodents is not necessarily the same as that followed by the chemical in humans. For example, studies conducted over the past decade or so reveal that in order for a number of chemicals to cause kidney cancer in male rats they must first bind to a protein found in the animals' urine. Since human urine lacks this protein, chemicals that prompt kidney cancer in male rats may not induce such cancers in people (*2*).



Genetically engineered knockout mice are used by investigators to determine the role of selected genes in mediating the potentially adverse effects of chemical exposure. (Courtesy Russell Cattley, Chemical Industry Institute of Toxicology, Research Triangle Park, N.C.)

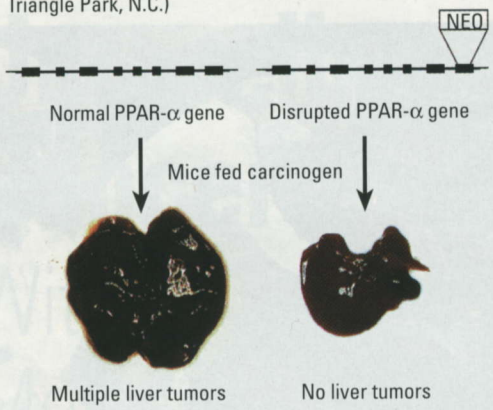
Although changes are underfoot with EPA's proposed revisions to cancer risk assessment, traditionally, the agency has ignored possible mechanistic differences between animals and humans, just as it has ignored differences in susceptibility to cancer within human populations. But a growing understanding of these differences is opening informative avenues for risk assessors to follow so that risk assessments can be placed on sounder footing.

Hazard identification research

The bulk of the new genetic and molecular biologic developments making their way into risk assessments focuses on ways to strengthen assessments of the carcincogenicity of compounds. Current high-dose, long-term laboratory studies of rodents exposed to chemical compounds may provide uncertain results. The animals can have unique molecular pathways to cancer that people lack and vice versa. Some of these pathways start in the liver, which is a staging ground for toxins in the body. This organ has an array of enzymes that breaks down or adds compounds on to toxins. Some of these enzymes, known as cytochrome p450s, can change innocuous toxins into cancer-causing agents, while others detoxify some carcinogens. Human liver

Unraveling the mechanisms of carcinogenesis

When receptor knockout mice were fed a toxin known to prompt liver cancer in rodents via the PPAR-α gene, these mice did not develop liver tumors, unlike their normal cousins. The receptor was inactivated by placement of a bacterial gene for neomycin resistance (NEO) in the coding region of the mouse receptor gene. (Courtesy Russell Cattley, Chemical Industry Institute of Toxicology, Research Triangle Park, N.C.)



cells can generate different subtypes and amounts of these cytochrome p450 enzymes than can rodent liver cells. Cells in culture, even liver cells, do not independently generate p450 enzymes at all (*3*).

Charles Crespi at Gentest in Woburn, Mass., however, reported in 1991 that he had genetically engineered cultured human blood cells so that they produced five different types of human cytochrome p450 enzymes simultaneously (*4*). He also created human cell lines that each produce one of the 12 types of these enzymes. Crespi's cell lines are useful cancer screening tools because they are likely to more authentically mimic the human liver's ability to change toxins into cancer-causing agents than rodents or rodent cells. Tests done on these cells indicated that the drug tamoxifen, which is used to treat breast cancer, caused genetic damage (a precursor to cancer) even though the drug caused no such effects on rodent cells cultured with rodent liver enzymes. Researchers at the Massachusetts Institute of Technology have been using the Crespi cell lines since 1994 to help identify pollutants in air and soil samples that might cause cancer in people (*5*).

An alternative to using genetically engineered cultured cell lines for assessment of potential human health risks is to perform studies using genetically engineered knockout mice. Russell Cattley and Chris Corton at the Chemical Industry Institute of Toxicology have been evaluating a genetically engineered mouse created by Frank Gonzalez at the National Cancer Institute. This knockout mouse lacks a gene, called peroxisome proliferator-activated receptor-α (PPAR-α), whose protein product in the liver responds to certain chemicals by triggering a cascade of events that leads to the development of liver cancer in rodents.

In a collaborative study between the National Cancer Institute and the Chemical Industry Institute of Toxicology (*6*), treatment of the knockout mouse with a highly potent model carcinogen failed to cause any tumors, clearly establishing the critical role of PPAR-α for cancer in mice (see figure on the left). According to Corton, several studies suggest that the human counterpart of the level of the PPAR-α gene product found in people is one-tenth of that found in mice. This lower level of PPAR-α expression, as well as other observations, indicates that people are much less likely to get liver cancer from exposure to the chemicals that act via this mechanism in mice. If a compound causes liver cancer in the standard two-year tests in mice and rats, Corton suggested, researchers could evaluate the mechanism of action using the PPAR-α knockout mice. If these knockout mice did not develop cancer, then the compound may also be less likely to cause cancer in people.

The researchers are currently conducting studies aimed at verifying the limited role this gene has in triggering cancers in people and expect to have results in a few years, which would bolster the usefulness of the knockout mouse model in cancer risk assessments. By tampering with some of the key genes that play a role in cancer, researchers have also genetically engineered mice to be particularly susceptible to cancer (*7*, *8*). Preliminary findings by researchers at the National Institute of Environmental Health Sciences suggest that these mouse models can indicate in just six months whether a substance is carcinogenic. More extensive testing is needed to assess the overall usefulness of these models.

Understanding dose–response

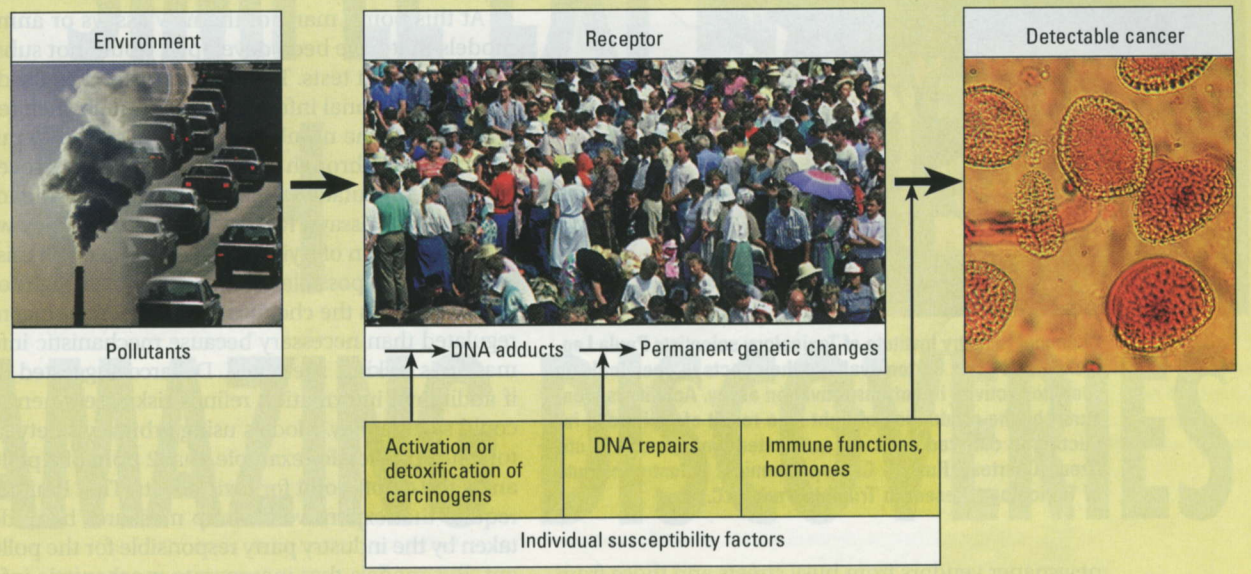
Not everyone responds equally to a chemical exposure, but toxicologists are just beginning to tackle such human variability. Their efforts are being aided by increased understanding of the genes known to govern susceptibility to adverse effects from various chemicals. These genes include those for enzymes that break down or detoxify toxins, those that govern DNA repair or other cellular events involved in cancer, and those that influence immune responses (see figure on page 315A).

Research over the past decade reveals that minor changes in the gene that produces an enzyme that breaks down various foreign chemicals in the body, for example, cause some people to be what are termed "slow acetylators" (*9*). If these people work in textile factories where they are exposed to dyes, they have a much higher risk for bladder cancer than people who are rapid acetylators. The wide range in frequencies of this genetic trait among ethnic groups is striking. Whereas only 5% of Canadian Eskimos are slow acetylators, 90% of North Africans harbor this trait. Consequently, people of North African descent working at textile mills are likely to be particularly susceptible to the toxic effects of the dyes to which they are exposed.

Although dose–response assessments currently do not usually consider particularly susceptible populations when calculating which doses are adverse to human health, that situation may change in the near future as the National Institute of Environmental Health Sciences' Environmental Genome Project progresses. It will characterize the range of variation in many of the

The complexity of cancer development

The path an environmental pollutant takes to cause cancer in people is complex and is influenced by several factors. Recent advances in genetics and molecular biology have fostered the development of new tests or animal models that can be used to help assess how far along the path to cancer a pollutant is likely to go, and which individuals are most susceptible to the potential carcinogenic effects of the pollutant.



200 known genes that govern susceptibility to adverse health effects from environmental substances in hundreds of Americans from five ethnic groups. Another objective is to relate the various gene variations with susceptibility to specific health problems resulting from exposure to environmental chemicals. The enormous database that this project is creating should greatly enhance the strength of dose–response assessments in the future. But Dellarco noted that using such information in a risk assessment will not be a simple task. "There are going to be a lot of genes involved and a lot of interactions. At this point we do not have a framework for dealing with this information," she said.

Adducts suggest exposure

Another area of research that has taken great strides in recent years and should help improve the power of both dose–response and exposure assessments entails measuring chemical-cell component links (adducts) in exposed individuals. These highly sensitive measurements can detect and characterize low-level exposures that people have to environmental chemicals, a fundamental requisite for a more thorough determination of cancer risks.

Using more conventional means, scientists can determine the chemical concentrations in a particular environment, such as amounts of a chemical emitted from an industrial smokestack, but these evaluations fail to accurately reflect what quantity actually reaches the human body in a form that can cause damage. Such environmental concentration measurements do not account for human bodily absorption, breakdown, or detoxification of a chemical to create what is known as an internal dose. Traditionally, risk assessors combine environmental concentration data with complex mathematical models to predict the internal dose of environmental chemicals. A lack of information, however, forces risk assessors to make many assumptions that may or may not be true.

Sharon Hayes and David Yang, researchers at Columbia University's School of Public Health, perform experiments using advanced diagnostic techniques to investigate adduct formation in exposed individuals. (Courtesy Regina Santella, Columbia University, N.Y.)

However, recent advances are providing some of that missing information. By measuring certain adducts, which form after the main processes that confound internal exposure assessment (absorption, breakdown, and detoxification) have occurred, researchers can determine the internal dose of specific chemicals. Such determinations must be cautiously evaluated. A study conducted in Poland in 1992 found that DNA adduct levels were closely tied to air pollution levels: In the summertime, when the air is cleaner, DNA adduct levels in people's blood cells dropped accordingly. However, an Italian study found no significant differences in DNA adduct levels between

Chemical Industry Institute of Toxicology scientists Paula Lapinskas and Chris Corton evaluate the effects of chemicals on receptor activity in cotransactivation assay. Activity is measured by the production of light as a result of luciferase induction in cultured cells. An automated luminometer is utilized. (Courtesy, Russell Cattley, Chemical Industry Institute of Toxicology, Research Triangle Park, N.C.)

newspaper vendors from busy streets and those from the outskirts of Milan (*10*). "Adduct data may have a role," said Dellarco, "but they need to be interpreted carefully. You may be misled because you may be looking at the wrong adduct or the wrong target tissue."

Next steps

Each of these recently developed assays or animal models has its own limitations and uncertainties, some of which are already known, while others will become more apparent as they are used more frequently. Rosato would like to know if the new tests and models are as accurate as or more accurate than standard assays—a task now being supported by the Interagency Coordinating Committee on the Validation of Alternative Methods. The organization and its sister center at the National Institute of Environmental Health Sciences will also coordinate peer review of validation studies and ultimately make recommendations to agencies regarding the scientific usefulness of test methods as well as how they can be applied. "The Interagency Coordinating Committee on the Validation of Alternative Methods' opinions would be taken very seriously by the EPA," said Dellarco. The committee is still in its fledgling stages and has not yet begun reviewing specific tests.

Tumor data no longer take precedence in cancer risk assessments, Dellarco said. Instead, a weight of evidence approach is taken in which information from in vitro studies may be on equal footing with the standard two-year animal tests.

Dellarco noted that EPA completed risk assessments this spring on chloroform and formaldehyde in which data from DNA adduct studies or other nonstandard methods or models were used. The International Life Sciences Institute, which is affiliated with the Risk Sciences Institute based in Washington, D.C., is also conducting an extensive study that is exploring how nontumor data can be used to predict which doses of pollutants, such as butadiene and benzene, are likely to be carcinogenic to people. Results from this study are expected to be published this year. "How we are going to use data from new assays is a legitimate question," said Dellarco, "and what is going to help us figure that out are these case studies."

At this point, many of the new assays or animal models that have been developed would not substitute for current tests. They would instead be used to provide additional information. Although such tests would boost the number of resources used to push a compound through the risk assessment process, chemical manufacturers are not balking at the idea of using such assays, Rosato commented. "They welcome the notion of trying to get as much mechanistic information as possible," she said. There are high costs associated with the chemicals being more stringently regulated than necessary because mechanistic information is lacking, she noted. Dellarco suggested that if additional information refines risk assessments, it could save money. Models using arbitrary safety factors may predict, for example, that 2 ppm of a pollutant is the cutoff point for toxic effects. That limit may require that expensive cleanup measures be undertaken by the industry party responsible for the pollutant. But models that incorporate mechanistic information currently under development may indicate that 4 ppm is a more realistic limit, in which case little to no cleanup effort may be required of the responsible party. "If you are setting up a cleanup standard for site remediation," said Dellarco, "a twofold difference can make quite a difference in cost."

The ultimate advantage of new information spinning off the molecular biological and genetic revolution in toxicology is that the soundness of risk assessments may be improved. "Every piece of information that decreases the uncertainty in risk assessment is good. Even if that information indicates that a chemical needs to be regulated more stringently, if that protects public health, then it needs to happen," said Rosato.

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