Toxicology Goes Molecular

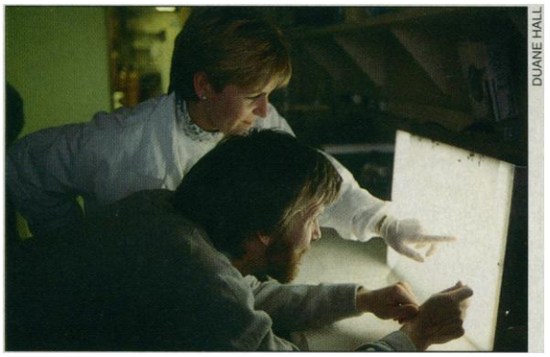
A new breed of toxicologists is using ultrasensitive probes to discern toxic effects at the cellular level, and working on preventive measures for those exposed

Toxicologists have long been interested in proliferative growth, and lately they've been witnessing one in their own backyard. Four years ago, the Society of Toxicology (SOT)—the oldest U.S. professional group in its field—launched a new specialty section on molecular biology. No sooner was the subfield created than it began to grow. Before long, it was infiltrating other sections—mechanisms, risk assessment, and carcinogenesis. It became obvious that it wasn't really a specialty at all but a fundamental part of the field—and so now its members are moving out into all the other sections of the society. That rapid growth and dispersion, and the overall rise in SOT membership, says SOT president John Emmerson, a scientist at Eli Lilly & Co., reflect profound changes in the field of toxicology itself. Since the late 1970s, Emmerson reports, there's been a sharp rise in SOT meeting attendance and a proliferation of submitted papers. These are a couple of signs that toxicology is on the upswing, and that molecular biology in particular is feeding the boom.

Other signs are out there, too. In 1991 a journal called *Pharmacogenetics* was born, devoted to the study of chemical-gene interactions, especially how genetic differences lead to variations in human responses to chemicals. This a hot research area, and SOT is making more room for it in its annual meeting. The trend to embrace molecular studies has been noticeable over the past 2 years, says biologist Cheryl Walker, an associate professor at the University of Texas M.D. Anderson Cancer Research Center in Austin. Walker chaired a session at the last SOT meeting on studies comparing responses to toxic compounds in animal and human cells. It held a standing room-only crowd for 2 hours. "Just about every symposium our specialty section [molecular biology] holds is packed," Walker notes.

Interviews with more than a dozen leaders in the field of toxicology revealed a widespread belief that molecular biology will have dramatic effects on their work in the future. One key to these developments comes in the form of molecular probes that enable toxicologists to detect exposure in samples of blood, urine, even exhaled breath—making it possible for them to understand events that precede a fullblown toxic response (see box on next page). As a result, toxicology as a whole, some argue, could well undergo a significant paradigm change, moving from a "high-dose discipline" to a "low-dose discipline." Instead of studying animals stressed to the maximum with near-lethal doses of toxic compounds, toxicologists may spend more time looking at biological processes much closer to normal ones in an effort to understand the biomechanisms by which damage is done.

Along with such changes, toxicologists say, could come a new reliance on engineered models derived from human cells and tissue—such as cultured liver tissue for testing cancer risks—in place of the high-dose animal models that have been the discipline's staple until now. The detailed information the new models provide may allow toxicology to become more potent scientifically, demonstrating cause and effect rather than merely pointing to associations. Already, new information is helping scientists move from levels of overall risk and zoom in on individuals susceptible to diseases caused by environmental toxins like aflatoxin B1. And that kind of knowledge is suggesting drugs to block toxic effects.



DUANE HALL

Lung cancer. Researchers at NIEHS pore over an electronic version of a human *p53* tumor suppressor gene.

Industry executives, of course, hope the new molecular toxicology will reduce public fears about chemical products. That may indeed come to pass, if molecular studies show that old risk models overstated the dangers. But some scientists warn that it's too early to predict what molecular-based research will show. And, in fact, a pair of case studies illustrating the impacts of molecular methods show that the new tools can cut both ways: Previously "safe" chemicals may turn out to be risky, while apparently lethal ones turn out to be relatively benign.

Consider two of the ingredients in the infamous herbicide Agent Orange. Those two ingredients have received converse treatments at the hands of the new molecular methods. The contaminant dioxin, a potent carcinogen for rodents, even at minute doses, has begun to look less threatening to humans as a cancer inducer (though it may have teratogenic effects). But the opposite is happening with Agent Orange's active ingredient 2,4-D, long considered safe. 2,4-D belongs to a class of compounds called peroxisome proliferators that are coming under close scrutiny now, as the National Institute of Environmental Health Sciences (NIEHS) grows more concerned about them. Indeed, the new toxicology has set the old view of these chemicals "on its ear," says NIEHS scientist Daniel Marsman.

The dioxin mystery

In many ways dioxin has become a test case —both for those who want to use molecular analysis to adjust risk standards based on new science, and for scientists trying to learn how manmade substances influence genes and cell growth. The dioxin field has exploded in scope and sophistication since the early 1970s when Alan Poland, now at the McArdle Laboratory at the University of Wisconsin in Madison, discovered that dioxin binds tightly to a protein (the Ah receptor) in mouse livers. Once bound, dioxin triggers a cascade of events like those set off by the hormone estrogen, revving up the synthesis of certain enzymes. So far, scientists haven't found a natural substance that binds to the Ah receptor as tightly as dioxin, nor are they sure why the receptor exists.

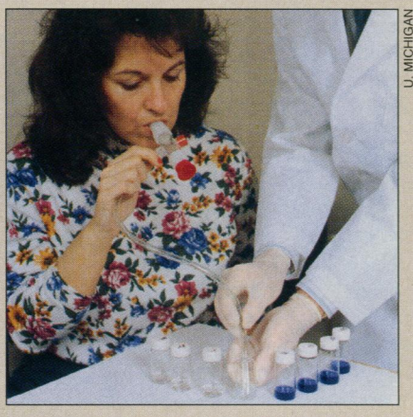
What is clear, though, is that dioxin has different effects on different species—and on specific tissues within each species. Rodents, for example, develop liver tumors. Yet data from a 1976 chemical factory explosion in Seveso, Italy, suggest that the response seen in humans may be limited mostly to a skin disorder called chloracne. Daniel Nebert, director of the Center for Environmental Genetics of the University of Cincinnati Medical Center, says rats and rabbits were "practically crawling out in the street and dying" after exposure to the Seveso dioxin cloud. But, so far, he says, even children with skin lesions have shown no significant increase in cancer.

Health standards in the United States,

A Breath Test for Cancer?

It isn't a simple job to untangle the causes of liver cancer in a typical clinic in Shanghai. In the areas around the clinic, people live crowded lives and, as a result, many are exposed to the same pathogens and toxins—including aflatoxin and hepatitis B, both known causes of liver cancer. How, then, can toxicologists determine the role of aflatoxin, which is a toxic byproduct of mold on peanuts and corn, in these cancers? The answer is: molecular methods to the rescue, says Gerald Wogan of the Massachusetts Institute of Technology, a leader in this research. And China isn't the only place where some remarkable, sometimes offbeat high-tech methods are being used to detect risks posed by toxic agents.

Armed with some of these new tools, John Groopman and Thomas Kensler—Wogan's former students and collaborators at Johns Hopkins University—are proposing to try a new form of therapy in a Chinese clinic. First, they plan to use two molecular-based bioassays to find vulnerable individuals: a urine test that looks for a "point" mutation (a single base pair change) in DNA, along with a blood test that looks for a change in hemoglobin. Surveys using these methods, together with blood tests for hepatitis B, have already yielded startling results. Near Shanghai, the chances of getting liver cancer are doubled for people exposed only to aflatoxin, according to Groopman, and they are increased fivefold for those exposed to hepatitis B. But if you're exposed to both, the risks jump to an incredible 60 times the risk for nonexposed people—the equivalent, says Groopman, of a death sentence.



U. MICHIGAN

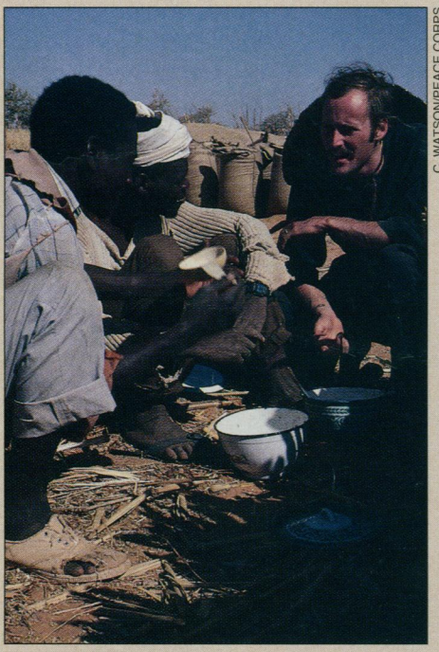
Telltale breath. Subject in Watkins' lab exhales after an erythromycin shot, revealing P450 enzyme level.

To break this lethal synergy, the Johns Hopkins team is focusing on aflatoxin and would like to give high-risk individuals a protective drug called oltipraz—formerly used as an anti-schistosomiasis medication. It stimulates metabolic enzymes that interact with aflatoxin compounds and divert them from the liver, where they damage DNA. Already, by giving oltipraz to aflatoxin-exposed rats, Groopman says, his colleagues have shown that it blocks carcinogenesis.

This attack on aflatoxin could be used in other areas, including, for example, South Africa and the Gambia, where close living makes it difficult to sort out the effects of aflatoxin and hepatitis. But intriguing as it is, it's only one of several projects using molecular toxicology to identify—and assist—people at high risk.

Among other molecular strategies:

• A caffeine breath test developed by George Lambert, a neonatologist at Loyola University in Chicago who is interested in preventing birth defects due to toxic substances. Though not a toxicologist by training, Lambert has developed a biomarker to spot people who have been exposed to manmade chemical toxicants and show signs of a metabolic response. His method may be the simplest of all: He gives a two-cup-of-java dose of caffeine, waits a few minutes, then collects a breath sample. Lambert analyzes the collected breath to see how rapidly the person metabolizes caffeine. The metabolic rate correlates with enzyme activity induced by exposure to substances such as dioxin, PBBs, and PCBs. Lambert has traveled around the world, from Cana-da to Italy to Taiwan, collecting samples of breath from chemically exposed people.



C. WATSON/PEACE CORPS

Food hazard. In high aflatoxin areas, new tests may help identify exposed individuals.

• George Lucier and Douglas Bell of the National Institute of Environmental Health Sciences are working on a blood test that would check for dioxin exposure. The technique uses the polymerase chain reaction (PCR) to measure a combination of effects, including the activation of receptors that elevate enzyme activity and cell growth factors. Lucier thinks that, using PCR, it will be possible to identify a high-risk group of individuals who may be at risk for cancer as a result of dioxin exposure. After 2 years of development, they're just about ready to take their test on the road.

• Frederick Kadlubar, scientific director of the National Center for Toxicological Research, recently published data on a caffeine-in-urine test designed to focus on people at risk of getting colon cancer after exposure to heterocyclic amines in food. (A common source of heterocyclic amines is charred red meat.) The new tests identify people who may be genetically programmed to produce high levels of two specific enzymes—N acetyl transferase and cytochrome P450 1a2. Kadlubar and his colleagues have looked at eight populations around the world and identified subgroups with fast and slow enzyme activity rates. The National Cancer Institute has set up a clinical trial to see whether high-risk individuals can reduce their risk with a change in diet.

• Paul Watkins, director of the general clinical research center at the University of Michigan, Ann Arbor, has developed another breath test that looks at the rate at which people use a member of the cytochrome P450 enzyme family to metabolize erythromycin. In this test, a carbon-14 labeled form of erythromycin is injected, and the patient is asked to blow bubbles into a liquid that captures the exhaled carbon dioxide. Fast metabolizers, indicated by the ratio of labeled carbon atoms, are at increased risk for developing aflatoxin-related cancer.

Aflatoxin isn't a big problem in Michigan, but Watkins and his colleagues have been using the technique to classify patients undergoing heart and kidney transplants according to their P450 levels. Those with lots of P450 require larger amounts of the immunosuppressant drug cyclosporin to assist the transplant. Using the breath test, Watkins can now predict how much cyclosporin a patient will require.

–E.M.

however, use the rodent model to estimate risks for humans, extrapolating from observed high-dose effects to predict hazards at low doses, where none has been observed. This cautious approach (not used in Europe) assumes that the mechanisms of toxicity are similar in both species and that altering the dose-rate makes no crucial difference in effect. Michael Gallo of the Robert Wood Johnson Medical School is one of a group of toxicologists who are now arguing strongly that such assumptions ignore biology. He feels that it would be scientifically more sound to develop a model that incorporates up-to-date molecular data, reflecting the real differences between rodents and humans.

Gallo has been a leader of this campaign, which was triggered partly by toxicologists' realization that nearly all the dioxin effects they'd seen were triggered by the activated Ah receptor (*Science*, 8 February 1991, p. 624). Since it takes a certain number of molecules to fill the available receptors and shift toxic processes into high gear, it seemed logical that there would be a threshold of exposure below which dioxin does not disrupt normal processes. That logic, if accepted by the Environmental Protection Agency (EPA), would be good news for defendants in toxic tort cases—since levels of human exposure are usually very low. It also suggests that in time researchers might learn precisely how the Ah receptor triggers its deadly effects.

Accordingly, research on the Ah receptor has expanded rapidly. Gallo says, "It just took off after about 1978, and we are still in a straight-up line." He estimates that 8000 papers or abstracts have been published on it. They have been spurred by more than just a desire to answer regulatory questions, says Gallo: The fundamental biology is fascinating. "Whole groups are now spinning off and looking at other binding proteins," Gallo adds. "We are now looking at the work on the Ah receptor as a tool to understand molecular biology"—probing genetic regulation of cell growth and differentiation.

For example, these studies have opened a window on the way cells produce an important family of enzymes, the cytochrome P450's, which metabolize foreign substances. According to William Greenlee of Purdue University, one particular cytochrome P450 (P4501a1) is so well understood now that its presence can be monitored by several assays and used as an indicator of past dioxin exposure. Meanwhile, Greenlee and his colleagues have zeroed in on a target that may be more relevant—human skin cell genes that are turned on by the dioxin receptor. Dioxin increases the output signal for these genes, which encode for proteins that may influence skin cell growth and inflammation, among other things.

This gusher of information is now seeping into the debate over regulating dioxin risk, because EPA has been enthusiastic about revising its models to reflect the new molecular data. Indeed, the initial hopeful news—that the existence of the Ah receptor implied a threshold for toxicity—encouraged EPA to launch a major toxicology review in 1991. The aim, according to EPA's chief of carcinogenesis review, William Farland, was to create a new model for the future and "incorporate more science into risk decisions"—also, presumably, to modify risk estimates. Scientists from outside, including Gallo and George Lucier of NIEHS, have been invited to contribute.

But molecular findings have not by any means persuaded everyone to reduce their estimates of the risk from dioxin. Indeed, the debate that once focused on rodent livers and whole animal counts has now moved to the molecular realm while retaining much of its previous heat. The people who perceived dioxin as risky before—when standards were based primarily on its ability to kill rodents—see no basis for reducing risk estimates today. Lucier, for example, points out that no one knows just how many Ah receptors must be activated before they do their dirty work. Nor is it clear yet which of the many responses triggered by the Ah receptor is the most threatening.

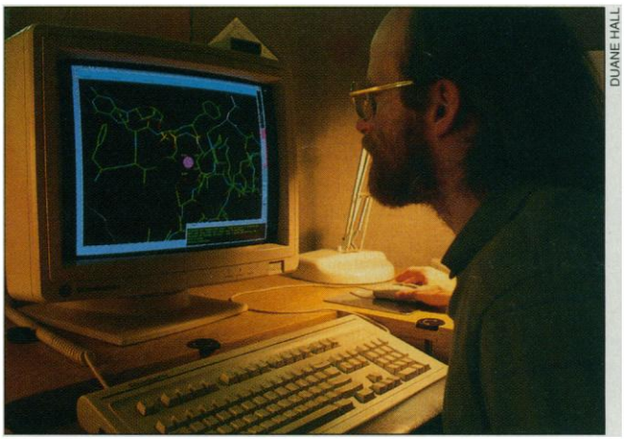
Lucier says that his personal view is that the dioxin risk lies "in between" the ultraconservative standards of the past and the low-risk estimates that some have been promoting in the 1990s. Others, like Gallo, are convinced the risk is still greatly overstated. But if the debate among the toxicologists is not yet resolved, it has moved to a new realm—that of molecules—and EPA has had to move with it. Farland says the agency plans to deliver a public draft this summer. Even if it leads to no immediate change in standards, Farland says, the work "will allow us to put our traditional views in perspective."

The other side of the coin

Understanding the details of how dioxin works in the human body may some day allow EPA to relax its public exposure limits. Meanwhile, for other chemicals, the insight provided by molecular analysis may drive the odds in the opposite direction, shifting them from low-risk to high-risk status.

One group that's beginning to look as though it might fit in this category are the peroxisome proliferators (PPs). Peroxisomes are structures in the cell that enclose enzymes, often associated with the breakdown of fatty acids. The PP group got its name because of the effects on lab animals: Among other things, these chemicals cause peroxisomes to proliferate. They also induce cell proliferation, liver tumors, and testicular damage in rodents.

The family to which the PPs belong is large, including such products as clofibrate, a drug given to patients to reduce cholesterol levels; 2,4-D, a defoliant used in Agent Orange and in garden herbicides such as Weed-B-Gone; and phthalates, oily additives that make hard plastics flexible. Plastic soda bottles, for example, contain phthalates, as do plastic medical containers. Marsman of NIEHS notes that "everyone" has the chemical in their body these days.


DUANE HALL

Virtual proteins. Computers let scientists "see" target substances, like this oncogene product.

Although PPs induce liver tumors in rodents, they have not been associated with human liver cancer. For many years, toxicologists have assumed that these chemicals were more threatening to rodents than humans because they appeared to induce cancer through "oxidative stress" conveyed by the peroxisomes. After exposure to PPs, animal cells generate peroxisomes far more abundantly than human cells, so it seemed reasonable to conclude that humans were not in danger. Now, suddenly, the assumptions about mechanism are changing, says Marsman, and the reason is that molecular biology has found credible evidence that carcinogenesis could occur by another route. It may be a receptor-based route, like the dioxin pattern.

The stir began about 2 years ago, says James Popp, a vice president at the Chemical Industry Institute of Toxicology (CIIT), with the announcement of a discovery by Stephen Green and Isabelle Issemann of Imperial Chemical Industries. They found a new receptor in the mouse that's activated by chemicals in the PP class. Like dioxin's Ah receptor, it seems to alter gene transcription, controlling basic enzyme functions in the cell. This process—perhaps in addition to oxidative stress—may be what's promoting cancer in test animals. Toxicologists are now hunting for a human PP receptor. If it turns out

that humans and rodents are similar in the way they process PP chemicals, that could make health officials more concerned.

There's no evidence at present of any similarity in human and animal responses. But the discovery that PPs are involved in a poorly understood receptor system is reason for concern in its own right. Any chemical that triggers cellular events like those dioxin triggers is going to raise eyebrows—particularly if the chemical is spread as widely through the environment as PPs are.

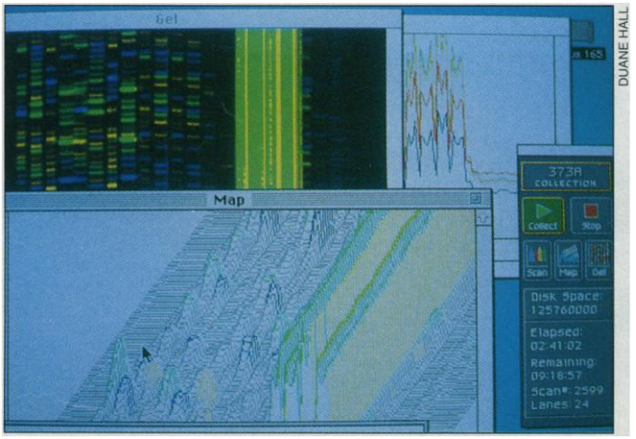
Marsman reports that last year, EPA started the ball rolling on a new research program in this area. EPA headquarters asked NIEHS to investigate one of the phthalates as a hazardous air pollutant. This means that the entire class will come under scrutiny, and we can expect to hear a lot more in the future about peroxisome proliferators.

What is the bottom line?

The movement toward molecular methods within toxicology isn't being accomplished without stress. Some traditionalists feel the new toxicology is "reductionist," as Gallo says, in its fine-grained focus on events within the cell. John Doull, emeritus professor of toxicology and pharmacology at the University of Kansas Medical Center and co-editor of one of the essential texts in the field, says the new biology fascinates him—but he worries about its applicability. "We get a lot of groups doing molecular toxicology for its own sake," but sometimes people "fail to relate it to the whole animal," says Doull. And if it lacks context, he worries, it may lose touch with toxicology's ultimate goal—protecting public health. Gallo concedes that the focus often seems to have narrowed, but he argues that "we are actually broadening our thought patterns at the other end" by describing fundamental biological mechanisms that underlie many toxic effects.

Most leaders in the field of toxicology believe, as Roger McClellan, president of CIIT says, that molecular biology is enabling officials to "get smarter in using animal data" in assessing the risks of exposure to chemicals. And he points to a small victory last year that may prove to be a blessing for gasoline makers. EPA accepted on its scientific merits the argument that a particular type of animal tumor—one produced in male rat kidneys by chemicals binding to a protein called alpha 2 micro globulin—should not be viewed as evidence of carcinogenic risk for humans. This message—the first of its kind, NIEHS scientists say—has now gone out from headquarters to all EPA toxicologists. That may be good news for sellers of unleaded gasoline, which induces male rat kidney tumors.

The benefits of molecular toxicology are spreading to other areas—for example, to drug companies' labs, says Jeffrey Theiss of the Warner-Lambert Co. Instead of methodically running a series of slightly similar compounds through the same battery of animal tests, says Theiss, "now the emphasis is more on understanding the molecular biology of a particular disease...and we can rationally select targets... [and] develop agents that can disrupt the process." For example, he mentions that the lab has developed a special culture of adrenal cells to precheck compounds before testing them in vivo.



Toxic wizardry. NIEHS's automated DNA sequencer projects a lung tumor gene on the screen.

At the M.D. Anderson Center, Walker has developed a series of molecular tests to yield an index of toxicity without resorting to expensive animal studies. Consider a company, Walker says, that wants to develop a new, safe substitute for asbestos. Before making a big investment, the technical staff would consult a group like her own to learn how to target the toxicology studies to get the best information on potential lung effects. She might even help them set up the assays to run in parallel with product research.

Small improvements like these mean a lot to enthusiasts—like Gallo—who insist that toxicology stands at the beginning of a new era. In the future, they say, big decisions will be based more on an understanding of toxic mechanisms and less on rigid mathematical models based on dose response curves derived from animal models. Gallo says the shift will "shake a lot of trees," and "people will get nervous." But he argues that changes brought about by the molecular revolution will be good for everyone—if for no other reason than that they will bring more fundamental science into the debate.

–Eliot Marshall

INSTITUTIONAL PROFILE

Ken Olden Heals NIEHS's 'Split Brain'

When cancer researcher Kenneth Olden left his job as director of Howard University's Cancer Center to become director of the National Institute of Environmental Health Sciences (NIEHS) in June 1991, he inherited a 25-year-old organization with a split in it that resembled the one between the hemispheres of a human brain. The creative, synthetic "right brain" was the division of intramural research, which carried out basic research into epidemiology and toxicological mechanisms. The analytical, linear "left brain" was the National Toxicology Program (NTP), which carried out routine animal tests of potentially toxic substances. Every brain needs these faculties working in tandem, but, like a brain in which the corpus callosum doesn't function well, the halves of NIEHS communicated poorly over the years, says an institute official, and "a deep gulf developed between the people" in each half—leaving the nation's top toxicology center with a dissociated sense of its own mission.

Olden knew a key to success in his new role was getting the halves of his agency to think in sync. And whether he succeeded or failed would have significant consequences for the field of toxicology, because NIEHS (a part of the National Institutes of Health (NIH) located in Research Triangle Park, North Carolina) is the government's main toxicology center, spending $150 million on research every year, more than any other federal entity. But when Olden came on board, the agency, created as a division of NIH in 1966 and elevated to institute status in 1969, was perceived to be slipping in its leadership role, coming under criticism from Congress and environmental groups for testing fewer toxic chemicals each year. Under those circumstances, it was tough to persuade Congress to add to the institute's research budget—something researchers there wanted badly.

Confronted with a divided institute hard up for cash, it didn't take long for Olden to realize he needed to make major changes in the shape of his institution. Although the 54-year-old cell biologist from Parrottsville, Tennessee, had little experience in environmental research, he didn't hesitate to order up a reorganization. "I want to get toxicologists talking to and collaborating with molecular biologists, epidemiologists, and pharmacologists," Olden told *Science* in a recent interview. To do that, the new director broke down the rigid institutional barriers between