A Molecular Approach to Cancer Risk

In a major change in how toxic substances are tested and regulated, federal agencies will soon require molecular data on how chemicals cause cancer

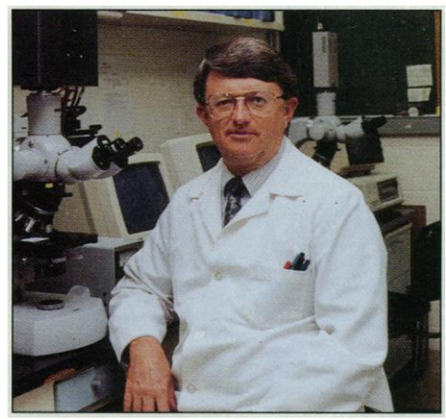
Readers of Victorian murder mysteries know that massive doses of chloroform can kill silently and swiftly. And toxicologists know that the chemical can also kill in a more insidious and painful way: Give large quantities of chloroform to a colony of mice, and many of the animals will die of cancer. Based on data from such studies, the Environmental Protection Agency (EPA) has suggested that drinking water in the United States should contain no more than an infinitesimally small amount—0.004 parts per million (ppm)—of chloroform, a byproduct of water chlorination and other industrial processes. Since its inception 25 years ago, EPA has applied the same logic to hundreds of other substances, extrapolating from high levels in animal studies to arrive at acceptable levels for humans.

But that approach, say scientists both inside and outside the federal government, may no longer be the best way to safeguard public health. In what is being hailed as a major shift in the regulation of toxic chemicals, EPA plans this summer to unveil guidelines for assessing cancer risk that will require regulators to incorporate into risk assessments such factors as how a chemical's structural features might affect its toxicity and how potential poisons are absorbed, metabolized, and distributed in the body (see table on p. 357). "It's a big step," says William Farland, director of EPA's office of health and environmental assessment. "The idea is to incorporate better science into cancer risk assessments."

It is, indeed, a big step. In many cases, such as chloroform, EPA has based cancer risk assessments on the carcinogenic carnage wrought by high doses of chemicals. That's because measuring the risk from small doses of a carcinogen would require testing thousands of rodents to produce statistically meaningful results. But there's the rub: Toxic effects of high doses often do not occur at low doses. EPA's new guidelines, a draft of which has been obtained by *Science*, take a new tack. They will require regulators to assess data on how chemicals affect the workings of cells and to use those data to judge whether high-dose extrapolations are likely to provide a realistic indication of low-dose risks. The guidelines also urge the development and use of ways to directly measure the effect of suspect carcinogens on thousands of organisms, such as fish, in single, low-dose tests.

This philosophy is taking hold at other agencies, too. The National Toxicology Program (NTP)—an interagency effort to assess the hazards of industrial chemicals, food contaminants, and drugs—is adding a low-dose test to high-dose rodent assays for carcinogenicity. The lower dose reflects levels more likely to be found in human tissues, says Kenneth Olden, director of the National Institute of Environmental Health Sciences (NIEHS), which runs the NTP.

The changes are expected to affect existing standards as well as rules for new substances. "Some risks will go up, and others will go down," predicts Lynn Goldman, EPA's top risk-assessment official. Indeed, some chemicals for which EPA now provides a thick cushion of safety may receive a cleaner bill of health. And that should reduce the costs of regulation—a big plus in today's anti-regulatory climate. "The last thing we want to do," Goldman says, "is to put our limited resources into protecting people from things that are harmless."



Back to reality. Toxicologist Byron Butterworth says EPA needs to make "real-world decisions."

Outside scientists give EPA points for moving in the right direction, but they worry that the quest for greater scientific rigor could hold up needed regulations. They point, for example, to the controversy surrounding efforts to regulate dioxin on the basis of molecular changes wrought by the chemical. "If EPA delays regulatory action while conducting thorough analyses of competing mechanistic hypotheses," says University of California, Berkeley, toxicologist William Pease, "it will address fewer toxic chemicals and may fail to prevent exposures to significant hazards." Adds University of Maryland toxicologist Ellen Silbergeld, who works with the Environmental Defense Fund: "Risk assessment is like clinical medicine—at some point you have to do surgery."

Still, Silbergeld and other EPA watchdogs say the agency is moving in the right direction. "If EPA doesn't take mechanistic data into account, someone should blow the whistle," Silbergeld says.

Low-level reassessment

EPA's new emphasis on molecular data is based on a growing body of evidence that extrapolations from megadoses can provide a misleading picture of the effects of low-level exposure. Chloroform is a good example. EPA's current strict standards were derived from a study in which mice developed liver tumors after exposure to massive daily doses of chloroform pumped into their stomachs over several months. However, those findings may not be relevant to human exposures, according to a paper picked by the Society of Toxicology as the best published last year in its journal (*Fundamental and Applied Toxicology*, vol. 22, p. 90). A team led by Chemical Industry Institute of Toxicology toxicologist Byron Butterworth found no cancer or liver toxicity in mice exposed to chloroform concentrations in water as high as 1800 ppm. Considering that municipal drinking water supplies are required to maintain levels of chloroform several orders of magnitude lower, says Butterworth, "our studies thus far indicate no increased risk of cancer from the levels of chloroform found in drinking water."

EPA scientists acknowledge that the agency has inadvertently exaggerated chloroform's risks. "Butterworth's work has gone a long way toward showing us that chloroform is not the worry it once was," says Rex Pegram, an EPA toxicologist who is studying the health effects of disinfection byproducts. And that reassessment may have a big economic impact: In an effort to meet current standards for chloroform and other chlorinated chemicals, EPA drafted a costly proposal to switch from chlorination of drinking water—a process that generates chloroform—to a process using ozone. But EPA regulators are having second thoughts in light of Butterworth's findings, as well as recent studies indicating that ozonation byproducts may be more hazardous than chlorination

byproducts. Butterworth says that's as it should be: "EPA has to make real-world decisions. ... We can't let incorrect hypothetical risks drive our expenditures and waste money."

Chloroform isn't the only substance with an improved reputation at low doses. Recent findings suggest that tiny amounts of thioacetamide—once used as a fungicide but shelved after studies showed that high doses destroy the liver—may counteract some of the harmful effects the chemical causes at high doses. A team led by Northeast Louisiana University toxicologist Harihara Mehendale injected rats with a range of thioacetamide doses and found that the rat liver cells exposed to lower doses were furiously synthesizing DNA and proliferating, thereby replenishing the damaged liver with fresh cells. This process, the researchers found, did not occur at higher doses.

"We are finding that almost all toxic chemicals are able to stimulate cell proliferation," says Mehendale, whose results appeared last month (*Environmental Health Perspectives*, vol. 103, p. 260). "If we only look at mechanisms of how chemicals inflict injury to predict the risk of exposure, we're obviously going to be wrong," says Mehendale.

ILLUSTRATIONS BY K. SUTLIFF

Dioxin: Molecular uncertainties

And then there's dioxin. Applying molecular data to assess the risk of exposure to low levels of dioxin is difficult, EPA has discovered. With some compounds, the exercise may even generate pressure to tighten restrictions.

In 1991 EPA began reviewing evidence of a threshold for dioxin's effects that suggested dioxin was less hazardous than previously thought. But last fall, based mainly on molecular data, the agency circulated a draft risk assessment concluding that low levels do pose a threat to human health. In particular, the draft pointed to certain biochemical changes induced by low dioxin doses—such as production of an enzyme implicated in carcinogenesis—that could lead to cancer and other illnesses such as endometriosis (*Science*, 16 September 1994, p. 1650).

However, the idea that some of the changes are inherently harmful was attacked last winter by a panel of top scientists reviewing the draft. The panel, chaired by former EPA official John Moore, now director of the Institute for Evaluating Health Risks, and toxicologist Gilbert Omenn, dean of the school of public health at the University of Washington, said the EPA report "does not maintain a clear distinction between induced biochemical changes and toxic effects, nor does it explicitly consider the significance of the dose-response relationship between the two." The panel argued that homeostatic mechanisms in the cell would overwhelm perturbations induced by low levels of dioxin.

Linda Birnbaum, EPA's top dioxin toxicologist and an author of the risk characterization, agrees that biomarkers indicate only the potential for toxic effects and that the agency needs to be more careful about how it uses the term "adverse." However, she says, the effect of subtle biochemical changes over the entire U.S. population must also be weighed. For some susceptible fraction of the population, she says, subtle changes in sperm count—an effect seen in some animal studies but unconfirmed in humans—may affect fertility.

The shift from rodent assays

In spite of such uncertainties, EPA is already moving away from its traditional reliance on high-dose studies. The trend began with a 1991 report from EPA's research office that told agency regulators to ignore a particular kind of tumor in certain strains of male rats that cropped up after exposure to high doses of chemicals such as dichlorobenzene (an insecticide) and limonene (an industrial solvent and constituent of orange juice). Researchers linked the tumors to a buildup in the kidneys of the protein alpha 2u-globulin. However, similar high-dose studies on female rats and other species yielded neither tumors nor excess alpha 2u-globulin, prompting EPA to conclude—for the first time—that a mechanism harmful to rats was irrelevant in assessing the risk to humans.

The agency has applied similar logic in an ongoing reassessment of the toxicity of atrazine, a popular herbicide. At high doses, atrazine triggers mammary tumors in a mouse line prone to such tumors. "The question is whether that model is valid for humans," says Goldman. In the past, EPA risk assessors would have automatically banned atrazine based on the mouse tumors. Now they will probe the mechanism behind atrazine's harmful effects before passing judgment.

EPA's draft guidelines essentially elevate such thinking to official policy. They also put a premium on using species capable of directly showing the effects of low dosages at a reasonable cost. For example, scientists at the University of Southern Mississippi's Gulf Coast Research Laboratory are using 36,000 medaka fish—the largest carcinogenicity test ever conducted—in an EPA-supported study to determine the shape of the dose-response curve for low doses of diethylnitrosamine, which at high levels causes liver cancer in rats.

For the NTP, a shift toward mechanistic data is intended to shed light on which chemicals should receive primary attention and further studies. The standard test of carcinogenicity—the 2-year rodent study—costs as much as $4 million and takes at least 5 years. With an $83 million annual budget, the NTP can launch only about 10 rodent bioassays each year, says George Lucier, director of NIEHS's environmental toxicology program. Although NTP won't select 2-year rodent studies solely on the results of mechanistic studies, says Lucier, the studies should help to identify candidate chemicals for the long-term, expensive bioassays.

Once implemented, the changes should challenge risk managers "to develop a much deeper understanding of the science," says EPA's Goldman. The same goes for NTP, says Olden. "That's something we should have done a long time ago," he says.

–Richard Stone