Animal Carcinogen Testing Challenged

Bruce Ames has stirred up the cancer research community by attacking one of the foundations of regulatory policy governing potential carcinogens

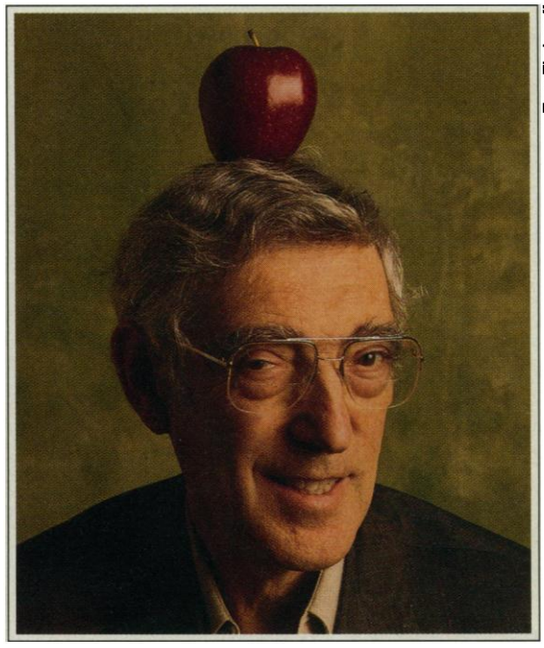
THE PAST 15 YEARS HAVE SEEN A LONG LIST of man-made chemicals indicted as potential human carcinogens. The sweetener saccharin and Alar, a ripening agent for apples, are among the most notorious, but scores of others have prompted widespread public concern as regulatory agencies have moved to restrict their use. Now, a prominent cancer researcher has touched off a heated debate in scientific circles by saying that the risks of those chemicals have been overestimated partly because of a serious design flaw in the animal tests used to assess their carcinogenicity.

The researcher, Bruce Ames of the University of California, Berkeley, is no stranger to controversy. Indeed, in recent years, he has become well known for his views that many of the news-making carcinogens pose little risk to the general human population. He has, for example, noted that the chemicals occurring naturally in foods may be a far more significant source of carcinogens than the low levels of synthetic pesticides, such as Alar, to which people may be exposed.

The criticisms that Ames has leveled at the animal testing methods are not new, nor is he alone in making them. Some other cancer researchers are raising similar questions about the animal tests, which are done in rats and mice. In fact, a committee on risk assessment methodology, recently established under the aegis of the National Academy of Sciences, has chosen the animal test issue as the first topic it will examine.

But Ames has gone farther out on a limb than most of the other researchers. He also argues that current policies for testing and regulating carcinogens, which emphasize synthetic chemicals, are misdirected. "I think we have to rethink the regulatory policy," he says. "When you exaggerate the risk you divert resources from other things [that might have a greater impact on reducing cancer rates]." Moreover, he has made his arguments very prominently, beginning with a Perspective coauthored with Lois Swirsky Gold of Lawrence Berkeley Laboratory, which appeared in the 31 August issue of *Science*, and following that up with three papers published in October in the *Proceedings of the National Academy of Sciences*.

Those challenges to established regulatory policy have helped draw fire to Ames. The regulators think that he has an oversimplified view of how they make the decisions about which chemicals are likely human carcinogens. David Hoel, who is currently the acting head of the National Institute of Environmental Health Sciences in Research Triangle Park, North Carolina, and William Farland, director of the Office of Health and Environmental Assessment at the Environmental Protection Agency, both point out that the results of the animal bioassays is only one of several factors they consider. "If one is careful to acknowledge that one can't make an estimate of cancer risk to humans solely on the basis of a rodent assay, we're okay [on current regulatory policy]," Farland says.



Tom Zimberoff

Tempting target. *Bruce Ames has drawn fire for claiming that chemicals like the apple-ripening agent Alar pose little risk.*

And many cancer researchers think that Ames' criticisms of the animal tests are wrong to start with. Even some of the investigators who share his concerns about them think he has oversimplified the issue. "Bruce has just gone too far," says one of them, James Swenberg of the University of North Carolina in Chapel Hill.

Whether or not Ames has gone "too far," his views have certainly come a long way in the past 10 years. During the 1970s, he was at the forefront of efforts to identify potential carcinogens and bring them to the public attention. Toward the end of the decade, for example, Ames was the one who pointed to the chemical Tris as a potential carcinogen, a suggestion that received a lot of attention because the chemical was widely used as a flame retardant in children's pajamas.

But Ames' ideas began to change, he says, as a result of information that he and Gold accumulated in the Carcinogenic Potency Database. Originally set up in the early 1980s, the database now contains the results of some 4000 experiments assessing the carcinogenicity of about 1000 different chemicals in rats and mice. Slightly more than half of those chemicals were found to cause cancer in at least one species—and Ames thinks that that's just too many carcinogens. "You wouldn't predict that so many would be positive," he says.

Ames thinks that the large number of positives is essentially an artifact of the way the animal tests are done. The chemicals are usually administered in the maximum tolerated doses (MTDs), which are the highest doses that can be given without causing severe weight loss or other life-threatening signs of toxicity. Even though these levels are much higher than the doses to which people are likely to be exposed, MTDs are used to cut down on the number of animals—and thus the cost—required to obtain statistically significant results.

Although the MTDs don't cause overt signs of toxicity, they can still have more subtle toxic effects, however, and that is what Ames thinks accounts for the large number of compounds that test positive for carcinogenicity. He proposes a chain of events in which a chemical given at the MTD damages an organ, killing some of the cells and thereby triggering a compensatory

increase in cell proliferation to repair the injury.

The increased cell proliferation in turn increases the opportunities for mutations that can cause cells to become cancerous. "The dominant thing is cell proliferation," Ames says. "It's much easier to get mutagenesis when cells are dividing." Below the toxic dose, carcinogenesis would not be a problem, he maintains, because there would be no increased cell proliferation.

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>
> —JOHN BAILAR

By making this argument, Ames has raised a specter that has long haunted carcinogenesis testing—that of the "threshold." The current models for extrapolating the cancer-causing effects of a chemical from the high doses at which the animal assays are performed to the low doses at which human exposures occur assume that there is no threshold; in other words, the number of cancer cases will decrease linearly as the doses fall all the way to zero. This assumption is considered conservative from a regulatory point of view. With a threshold, there would be a dose below which no cancer cases occur, and therefore no need to ban a chemical provided exposures remained below that threshold.

Carcinogenesis experts have argued for years about whether thresholds exist. "The fact is that I don't know what goes on at those low doses. Dr. Ames doesn't. Nobody does," says biostatician John Bailar, a former official of the National Cancer Institute who is now at McGill University in Montreal and also serves as science adviser for the Office of Disease Prevention and Health Promotion at the U.S. Department of Health and Human Services.

But Ames is not alone in arguing that there are thresholds. "The current assumptions [of the animal assays] are skewed to what we knew 30 to 40 years ago," says Samuel Cohen of the Eppley Institute in Cancer Research in Omaha, Nebraska. He and Leon Ellwein of the University of Nebraska Medical Center in Omaha have developed a computer model that they have used to analyze the mechanisms by which two well-studied chemicals, 2-AAF (2-acetylaminofluorene) and sodium saccharin, induce cancers. Their conclusion: cell proliferation induced by toxicity can play a major role in carcinogenesis, and there is a threshold at which the effect kicks in. In fact, they say, saccharin's ability to induce bladder tumors in male rats is solely due to the proliferative effects that high doses have on the bladder lining.

Swenberg has found something similar for D-limonene, a chemical found in orange juice that also causes kidney tumors in male rats. The chemical binds to a specific protein found in the rats (and not in humans) and the complex induces kidney toxicity. "It appears," Swenberg says, "that cell proliferation is the cause of the cancers and that D-limonene is not a risk for man," which is good news for orange juice lovers.

Apparent supporting evidence for Ames' case also comes from Daniel Krewski, a statistician at Canada's Environmental Health Directorate in Ottawa, who has performed an analysis of the published information on chemical toxicity and carcinogenicity for the academy's committee on risk assessment methodology. He finds, he says, a "fairly strong" correlation between toxicity and carcinogenicity, an association that would seem to buttress arguments that toxicity-induced cell proliferation underlies the large number of positive results seen in the animal bioassays. But, Krewski adds, "The interpretation of that correlation is uncertain."

To Ames' critics, however, these observations simply don't add up to a compelling case. Bailar says that many chemicals cause just as many tumors in animals when given at half the MTD as they do at the MTD. "There may still be toxicity there," he says, "but it shouldn't be as high as at the maximum dose. It seems to me to be directly contrary to his hypothesis."

Several of the critics also point to a study done about 2 years ago by a group at the National Institute of Environmental Health Sciences in Research Triangle Park, North Carolina, as refuting Ames' hypothesis. The group, led by Hoel, looked for signs of toxicity in tissues taken from rats and mice used in carcinogenesis studies performed as part of the National Toxicology Program, which includes the U.S. government's largest carcinogen-testing operation. They did not find a good correlation between their toxicity indicators and tumor formation. Cancers occurred in organs that did not show apparent damage and, conversely, organs with damage could be tumor-free. For only 7 of the 53 chemicals that tested positive in the animals did there appear to be a clear link between organ toxicity and cancer formation.

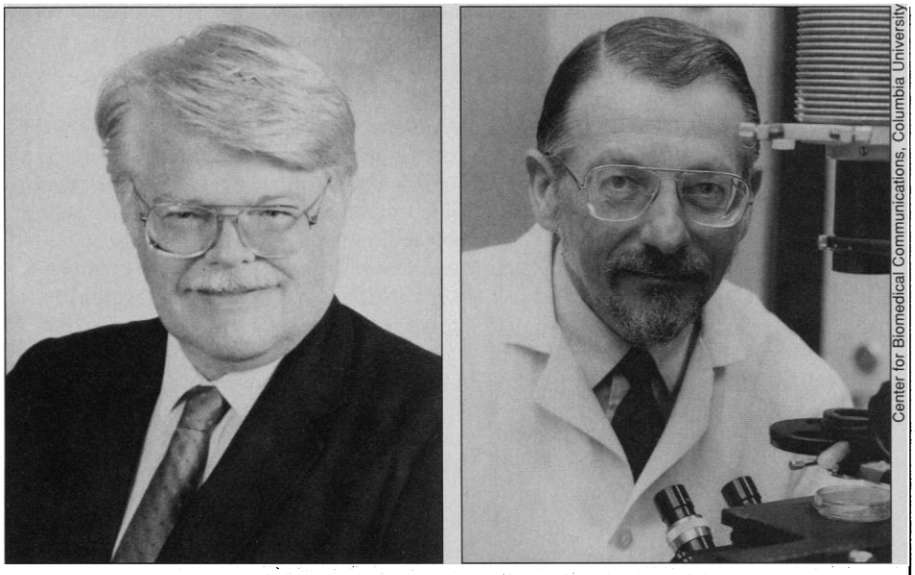
According to Swenberg, however, the NIEHS evidence is not airtight. The researchers looked for such signs of toxicity as dead or damaged cells. But they did not directly measure cell proliferation, which is supposed to be the critical element in carcinogenesis, according to Ames. "They're implying that the paper refutes the cell proliferation idea, and it doesn't," Swenberg says.

Despite all the furor over Ames' proposals, no one disputes that increased cell proliferation plays *some* role in the formation of cancers. That's been known since the work of Peyton Rous in the early 1900s, notes veteran carcinogenesis researcher I. Bernard Weinstein of Columbia University's College of Physicians and Surgeons. "This is an old idea that he [Ames] has resuscitated in a half-baked way," Weinstein says. "Cell proliferation is just not going to be the only mechanism for all chemicals," Swenberg concurs. The general consensus now is that while increased cell proliferation may contribute, carcinogenesis is a complex process, taking place in several stages and generally involving mutations in several genes.

SOME NATURAL CARCINOGENS IN RODENTS

CARCINOGEN	SOURCE	CONC., PPM
5-/8-Methoxypsoralen	Parsley Parsnips	14 32
Sinigrin (converted to allyl isothiocyanate)	Cabbage Cauliflower Brussels sprouts	35–590 12–66 110–1560
D-Limonene	Orange juice	31
Caffeic acid	Apples, carrots, celery, cherries, eggplant, grapes, lettuce, pears, plums, potatoes Coffee	50–200 1800
Neochlorogenic acid (converted to caffeic acid)	Apples, apricots, broccoli, brussels sprouts, cabbage, cherries, kale, peaches, pears, plums	50–500

Taken from B. N. Ames, M. Profet, and L. S. Gold, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 7777 (1990).



Ames' challengers. *John Bailar (left) and I. Bernard Weinstein are among many researchers who think that Ames is off base in his criticisms of animal carcinogen tests.*

Ames' critics also take issue with his contention that far too large a fraction of chemicals tested in animals show up as carcinogens. That's not surprising, says Richard Griesemer, who, as head of the Division of Toxicology Research and Testing at NIEHS, is in charge of carcinogen testing for the National Toxicology Program. "Most were selected for study because of some suspicion that they cause cancer; it's a biased sample," he explains.

The Berkeley researcher counters, however, that while that may have been true in the early years of carcinogen testing, more recently, compounds have been chosen because they are widely used, not because they are suspected carcinogens.

To Ames, the high proportion of chemicals that produce cancers in the animal assays means that the way the tests are done leads to an exaggeration of cancer risks. Not so, says Weinstein. "His bottom line is that rodent bioassays are misleading, but as a matter of fact the rodent bioassays have been extremely useful," Weinstein says. Not only have almost all the known human carcinogens tested positive in the animals, but he says there have also been cases in which compounds that caused cancer in rats or mice later turned out to be human carcinogens. He cites vinyl chloride as an example. It caused angiosarcomas, an unusual form of liver cancer, in rats and mice, and several years later was found to cause the same kind of cancer, and at the same doses, in humans. "In that case the rodent assay was right on," Weinstein says.

Then there is Ames' suggestion that people consume so many natural carcinogens in the diet that their exposures to synthetic carcinogens are trivial by comparison, at least for members of the general population, although possibly not for workers who are exposed to higher occupational doses. Ames has, for example, taken the provocative step of entitling one of the papers in the *Proceedings of the National Academy of Sciences* "Dietary pesticides (99.99% all natural)."

In that paper, Ames, Gold, and their Berkeley colleague Margie Profet point out that plants make numerous toxic chemicals as part of their defenses against insects and other predators. The limited testing of the natural products done so far has revealed that some of those chemicals, which have been found in a wide variety of fruits and vegetables, are carcinogens in the rodent assays. And yet, Ames says, epidemiological studies have indicated that eating a diet rich in fruits and vegetables protects against cancer. His conclusion: that humans and other animals have evolved defenses to protect them against the natural toxins and that those defenses are general enough to take care of synthetic chemicals, too.

Ames' critics also find that theory a little far-fetched. Weinstein points out that some types of synthetic compounds, including halogenated hydrocarbons such as PCB, are not found in nature. "Our defense mechanisms may not be prepared to handle them, while we may be very well equipped to handle the natural pesticides," he says.

And says Frederica Perera, an epidemiologist at the Columbia University School of Public Health in New York City, "The point is we are producing and releasing a lot of toxic chemicals," in excess of 20 billion pounds per year according to estimates by the Environmental Protection Agency. Perera has evidence that environmental exposures to some of those toxic chemicals, the polycyclic aromatic hydrocarbons, can cause changes in the genetic material that might lead to cancer. She and her colleagues found that people living in Silesia, a heavily industrialized area of Poland, showed more of the DNA changes characteristically induced by the chemicals than did people in a rural area.

And finally, several of the people contacted by *Science* were unhappy that Ames is suggesting rethinking the testing policy on carcinogens without suggesting what might replace it. "What are the alternatives?" asks Eugene McConnell, formerly at NIEHS and now a consultant in Raleigh, North Carolina. "It's not the ideal, but what have we got that's better?" He notes that the rodent assays are now the only way to identify chemicals that cause tumors.

Previous efforts to use cultured cells did not work. In fact, a test using bacterial cells, which was developed by Ames in the early 1970s, was one of those that failed to pan out. "We spent millions and millions of the taxpayers' money to show that the Ames test is not an adequate predictor of carcinogenicity," McConnell says.

Ames concedes that he has not "thought through" just what carcinogen testing and regulatory policy ought to be. He suggests, however, that rodents might be put to better use for studying the role of diet in causing or preventing cancer and also for pinning down the mechanisms of carcinogenesis.

So, are there any points of agreement in all this? Surprisingly enough, the answer is yes. Several researchers agreed with Ames that more research is needed on naturally occurring carcinogens. "I think that Bruce has performed a service in emphasizing the occurrence of naturally occurring carcinogens and toxins," Weinstein says.

And there was unanimous agreement that more research is needed to understand how different chemicals contribute to cancer development, so that realistic estimates of their risks can be obtained. Meanwhile, Ames is standing firm in his views. "I wouldn't go out on a limb if I didn't think I would be right," he says. ■ JEAN MARX

ADDITIONAL READING

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