Chemical Carcinogens: The Scientific Basis for Regulation

The whole delicate framework of toxic substances regulation rests on the fragile premise that it is possible to identify which chemicals are hazardous and should be regulated and which are safe and can be ignored. That premise, in turn, now rests on the assumption that there is a close correlation between carcinogenic effects in animals and those in man. In the near future, it may rest on the even greater assumption that a similar correlation exists between results from short-term tests on bacteria and cultured cells and long-term tests in animals.

Unfortunately, while regulatory agencies are being pressed to make rapid decisions about the safety of chemicals, the validity of the assumptions have been the focus of sharp contention. Both assumptions seem basically sound, but there is enough doubt lingering around each one to cloud the issue when a proposed regulatory action threatens to affect the interests of a large number of people. In short, the scientific basis for regulation of toxic substances probably exists now, but regulations emanating from it are certain to be highly controversial.

The least controversial basis for regulation, when solid data exist, is epidemiology. By studying large numbers of individuals who have been exposed to specific environmental factors—chemicals, sunlight, tobacco smoke, radiation—it is often possible to show that there is an association between that exposure and the onset of cancer or some other disease. As long ago as 1775, Percivall Pott of London pointed out the association between the exposure of chimney sweeps to soot and their increased incidence of cancer of the scrotum. Asbestos, arsenic, and benzene are more recent examples of chemicals that have been classified as human carcinogens from epidemiological results. There is general agreement that positive results obtained from the best epidemiological studies are valid; these results provide the hard core of our knowledge about the effects of chemical carcinogens on humans.

Epidemiology, however, is of limited value for regulation of toxic substances because of the nature of chemical carcinogenesis. The crucial fact is that, for most chemical carcinogens, there is a latent period of 20 years or more between the first exposure to the chemical and the onset of cancer. By the time epidemiologists have confirmed that a chemical is a carcinogen, then, large numbers of people have already been exposed to it.

Epidemiologists are also limited by the difficulties of tracking down people who may have been exposed to any particular agent, since many have generally moved out of the area after exposure. Also, most of the people involved have generally been exposed to a number of other potentially hazardous agents. It is thus difficult to develop evidence of carcinogenicity from epidemiology alone; furthermore, epidemiological studies which show no effect from exposure are often virtually meaningless.

The best epidemiological results are thus obtained when there is a well-defined, relatively homogeneous group of people who have undergone exposure. For this reason, most chemicals that have so far been identified as human carcinogens are industrial chemicals, despite the fact that such chemicals probably account for less than 5 percent of all cancers. To date, according to David P. Rall, director of the National Institute of Environmental Health Sciences (NIEHS), approximately 26 chemicals (including those from five industrial processes in which the specific chemical agent has not yet been identified) have been firmly associated with cancer in man (Table 1). There are approximately 56 more, he says, for which there is less definitive epidemiological evidence.

Table 1. Chemicals known to be carcinogens in man. [Source: David P. Rall]

Aflatoxins
4-Aminobiphenyl
Arsenic compounds
Asbestos
Auramine (manufacture of)
Benzene
Benzidine
Bis(chloromethyl)ether
Cadmium oxide
Chloramphenicol
Chromium (chromate-producing industries)
Cyclophosphamide
Diethylstilbestrol
Haematite (mining)
Isopropyl oil
Melphalan
Mustard gas
2-Naphthylamine
Nickel (nickel refining)
N,*N*-Bis(2-chloroethyl)-2-naphthylamine
Oxymetholone
Phenacetin
Phenytoin
Soot, tars, and oils
Vinyl chloride

Clearly then, effective regulation of toxic substances requires identification of carcinogens before there is significant human exposure. The conventional way to achieve this is with long-term bioassays in animals. Practical considerations dictate that such testing be performed in small animals with short lifespans. Typically, these are rodents.

The current guidelines of the National Cancer Institute (NCI), which were published in 1976 after much study during the previous decade, suggest that each chemical should be tested in two strains of animals and in both sexes. Subgroups of 50 animals of one sex and one strain should be used for each experiment. The chemical should be administered by a route that closely approximates human exposure at a minimum of two doses, the maximum tolerated dose (the highest dose that can be predicted not to shorten the animals' life-span from effects other than carcinogenicity) and either one-half or one-quarter of the maximum tolerated dose. Treatment should be continued long enough to produce a maximum response (generally 24 months, their expected lifetime), after which the animals should be killed and autopsied. In practice, at least 500 animals are required, including controls. The total time required is at least 3.5 years or more and the cost is at least $250,000 per substance.

About 7000 chemicals have been reported as having been tested for carcinogenicity in animals, according to Umberto Saffiotti of NCI, and a little more than 1500 have been reported to be carcinogenic. A close examination, however, shows that at least half of those studies were completely inadequate for their purpose, he says, so the actual numbers are closer to 3500 and 750, respectively. The number of "known carcinogens" will vary considerably, he adds, depending on the degree of stringency adopted for accepting evidence of carcinogenicity. At present, between 100 and 300 new compounds are submitted to animal bioassays each year.

In those cases where it is possible to make comparisons, the evidence obtained in animal tests agrees well with experience in humans. Bernard Altschuler of New York University recently re-

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viewed animal bioassay data for the 82 chemicals for which there is some epidemiological evidence of human carcinogenicity. With one exception—arsenic, which is associated with skin cancer when ingested in drinking water and with lung cancer after occupational exposure—all of the 82 that have been tested in animals have been shown to be carcinogenic. The apparent discrepancy might be explained by recent results from Toby G. Rossman and Walter Troll of the New York University Medical Center; they found that arsenic inhibits DNA repair in unicellular organisms. It might thus potentiate the action of other carcinogens.

Virtually all investigators thus agree that chemicals which are carcinogenic in humans are also carcinogenic in animals. There is less agreement about whether chemicals that are carcinogenic in animals will be carcinogenic in humans. Here, unfortunately, there is less experimental verification. At least six chemicals—4-aminobiphenyl, diethylstilbestrol, mustard gas, vinyl chloride, aflatoxin, and bischloromethyl ether—were shown to be carcinogenic in animals before epidemiological evidence confirmed that they are carcinogenic in man. In most cases, though, epidemiological evidence is inadequate to confirm animal results, and it is impossible to conduct carcinogenicity studies in humans to confirm animal results. (Some investigators, such as Curtis Harris of NCI, are trying to confirm animal results by using cultured human tissues; these studies, however, are at a very early stage.)

Resistance to extrapolation of results from animal bioassays to effects in humans hinges on two key factors, namely, the use of the maximum tolerated dose and genetics—although nutrition, environment, and other host factors complicate the arguments. The controversy about dose levels has been accentuated in the media, particularly with regard to studies on saccharin. There is a misconception among the public that, at high enough levels, virtually any substance will be carcinogenic. Such an idea, says Rall, is completely fallacious; a large number of pesticides and industrial chemicals have been tested in rodents at the maximum tolerated dose and the majority have been given a clean bill of health. It should also be remembered that most chemicals that have been subjected to animal bioassays were already suspected of being carcinogens.

There is, however, legitimate concern that use of the maximum tolerated dose will overwhelm protective measures that might detoxify hazardous agents at lower doses (a concept closely related to that of the threshold dose, discussed in a second article next week) or introduce metabolic pathways that do not exist at doses to which humans might be exposed. A classic example of the latter effect is provided by results with nitrilotriacetic acid (NTA), a chemical that showed great promise for replacement of polluting phosphates in laundry detergents. Bioassays of NTA sponsored by NCI and NIEHS showed that it caused urinary, bladder, and kidney tumors at the maximum tolerated dose, and the chemical never found a place in the U.S. detergent market, although it is used in Canada and Sweden.

Special care must be taken to protect laboratory workers when potential carcinogens are tested in inhalation studies. [Source: National Cancer Institute]

Recently, however, Robert Kanerva and his associates at Procter & Gamble, Inc., have shown that high concentrations of NTA form chelates with magnesium, manganese, and zinc in the elimination system. Removal of these ions damages the system and leads finally to interference with DNA replication—but only at doses that are 100,000 to 1 million times higher than any potential human exposure. Use of the maximum tolerated dose for NTA or any other chemical, says Leon Golberg, president of the Chemical Industry Institute of Toxicology (CIIT), is "completely inappropriate." Instead, he and others argue, the complete spectrum of absorption, distribution, biotransformation, and excretion of the chemical should be considered before determining the maximum dose for bioassays. Rall says that he cannot disagree with this viewpoint, but notes that such studies would probably limit the number of chemicals that could be assayed to four per year.

For now, however, these concerns seem to be outweighed by the need to make animal studies manageable. Increased doses of a carcinogen produce tumors in a greater proportion of test animals; this, in turn, makes it possible to detect carcinogenicity with fewer animals. With ten animals, the lowest incidence of carcinogenicity that can be detected is 30 to 40 percent, since three or four animals must have tumors for statistical significance. Detection of an incidence of 1 percent would require approximately 4700 animals, which would be prohibitively expensive.

Use of high doses also compensates for unknown synergistic and cumulative effects, argues Edward J. Baier of the National Institute for Occupational Safety and Health (NIOSH); it also compensates for the greater metabolic rate and shorter life-span of rodents. Current guidelines thus maximize the possibility of detecting carcinogenicity, even though there is a slight risk of obtaining positive results with noncarcinogens.

A more difficult argument involves genetics. The rodents used for bioassays are highly inbred strains that have very uniform genetic characteristics selected for sensitivity to the effects of carcinogens. One objection to these animals, says James H. Jandl of the Harvard Medical School, is that many strains have a high incidence of spontaneous tumors. Many critics seem to think that this will lead to a higher incidence of false positive results. But if there is a high spontaneous incidence, argues Saffiotti, a greater proportional increase in the number of animals bearing tumors must be observed for statistical significance. In fact, then, only the most potent carcinogens will give positive results.

The genetics of humans, in contrast to those of the test animals, are highly heterogeneous. It is thus not clear, says Frederick de Serres of NIEHS, to what proportion of the population animal stud-

Who Chooses Chemicals for Testing?

With an estimated 63,000 chemicals in common use in this country (*Science*, 13 January 1978, p. 162), the problem of selecting the 100 chemicals that the National Cancer Institute (NCI) has funds to submit to animal bioassays each year is obviously complex. Until recently, the majority of the chemicals to be tested were selected rather informally by a small group of NCI investigators, who relied on a combination of experience and intuition to choose chemicals that seemed likely to be carcinogens. Their intuition was fairly good, and more than half of the chemicals tested to date have been found to be carcinogens—a high percentage that has, unfortunately, somewhat distorted the public perception of the incidence of carcinogens among all chemicals. Many of the chemicals tested, however, are produced in limited quantities, and there is no significant exposure to them of industrial workers or the population at large. To correct this deficiency, a more formalized procedure was established about 2 years ago.

The central committee for test selection is the NCI's Chemical Selection Working Group (CSWG), which is composed of representatives from NCI and other regulatory agencies and chaired by Herman F. Kraybill of NCI. Chemicals that may be candidates for testing are referred to CSWG by regulatory agencies, industry, academicians, and other individuals. CSWG also employs a contractor to examine the chemical literature on a systematic basis to identify other candidates. Now, for example, NCI has directed the contractor to examine structural classes such as aldehydes, metals, and ethers, and use classes, such as chemicals for rubber processing, pulp and paper processing, and water treatment, to identify chemicals from each class that seem likely to be the biggest risk. NCI and the contractor have already reviewed 49 structural classes and 47 use classes. Another source of candidates is monographs of the International Agency for Research on Cancer; chemicals cited in the monographs as having received inadequate testing are prime candidates for bioassay. Candidates for testing are selected for many reasons, including exposure and production volume, and are not necessarily suspect carcinogens.

In the course of a year, CSWG will review about 5000 candidates and reduce the list to about 150 to 200 of the most promising. For each of these, a summary sheet is prepared listing all relevant information, including production volume, uses, exposures, and results of prior tests. CSWG meets monthly to review these summaries and to assign each chemical a priority of high, medium, or low. The lists are then referred to NCI's public advisory group, known as the Chemical Selection Subgroup of the Clearinghouse on Environmental Carcinogens.

The subgroup, chaired by David B. Clayson of the Eppley Institute for Cancer Research, is composed primarily of representatives from academic, industry, consumer public interest, and labor groups. The subgroup, says Kraybill, gives testing decisions the benefit of opinions of nongovernment groups. It reviews and assigns each chemical a numerical priority ranging from 1.0 to 10.0. With its present members, Kraybill says, the subgroup places great emphasis on chemicals to which there is a great amount of exposure, particularly food additives, pharmaceuticals, and pesticides. CSWG, for example, assigned a low priority to phenolphthalein, a widely used ingredient of laxatives. Because of that wide use, however, the subgroup gave it a priority of 7.7. Similarly, the subgroup assigned a priority of 7.0 to the aspirin substitute acetaminophen, which had also been assigned a low priority by CSWG. Because there is now almost no backlog of chemicals awaiting testing, any chemical with a rating higher than 3.0 will be tested very quickly.

The subgroup has been meeting regularly since last October and has thus far rated 61 chemicals—although the pace should quicken now that members are more familiar with the process. Some of the chemicals that have received the highest ratings are the pesticide dichlorvos and the drugs furosemide, phenylephrine, tetracycline hydrochloride, isoproterenol hydrochloride, and hydrochlorothiazide. These have already been submitted to testing or will be soon.—T.H.M.

ies are relevant. It may be that 90 percent of the population shares the genes necessary for any given carcinogen to induce cancer, or it may be only 1 percent. (It is also possible, says Saffiotti, that some percentage of the population may have genes, not shared by the inbred strains, that can activate carcinogens.) Unless such a determination can be made, he says, it may not be possible to make a definitive assessment of the risks associated with a chemical.

Despite these reservations, there is clear historical evidence, says Rall, that a chemical which is carcinogenic in appropriate laboratory animal test systems must be treated as if it were carcinogenic in man. That conclusion was affirmed by a National Academy of Sciences panel chaired by Matthew Meselson of Harvard University. Despite the uncertainties, the panel concluded, enough is known to indicate what dependencies on dose and time may operate and to provide rough predictions of induced cancer rates in human populations. In most cases, in short, animal results are the only tool available to identify risks.

The problem is in obtaining those results. There are simply not enough toxicologists, pathologists, animal suppliers, and laboratory facilities to test all chemicals. Saffiotti estimates that, with current resources, no more than 500 chemicals could be started on bioassays each year, while current estimates indicate that as many as 1000 new chemicals will be introduced in the same period; even if all 1000 could be tested, that would still ignore all those already in use. There has thus been a great deal of interest in the development of short-term, relatively inexpensive assays that could be used to identify those chemicals that are potentially most hazardous. The controversy about animal bioassays, however, pales in comparison to that surrounding short-term tests.

One of the first short-term tests—and certainly the most thoroughly studied—was developed about 12 years ago by Bruce Ames of the University of California at Berkeley. He used mutant strains of the bacterium *Salmonella typhimurium* that are unable to synthesize the essential amino acid histidine. Exposure of the mutant strains to mutagens (chemicals that alter genetic information by interaction with DNA) corrects this defect and allows the bacteria to grow in a histidine-free medium. Since interaction of a chemical with DNA is believed to be the first important step in carcinogenesis, Ames says, most mutagens are potential carcinogens.

The test shows a strong correlation be-

tween carcinogenicity and mutagenicity. Ames and other investigators have tested several hundred chemicals in the *Salmonella* system, and nearly 90 percent of those chemicals which are known carcinogens tested positive, Ames asserts. (About 13 percent of compounds which are not known to be carcinogens also test positive; many of these, argues Ames, are close relatives of carcinogens or are chemicals that have not received adequate bioassays.) In its current form, the Ames test—which costs between $300 and $1000 per chemical tested—is used in as many as 1000 laboratories, and it is used routinely by a number of companies, such as DuPont and American Cyanamid, for premarket testing of new products. The Ames test provided the first suggestion that hair dyes and Tris, the flame retardant used on children's pajamas, are potential hazards.

The major deficiency of the Ames test and others in which microorganisms or cultured cells are used is that the test species lack the enzymes, found in the liver and other parts of the mammalian body, that activate carcinogens. Many carcinogens, perhaps even the majority, are not carcinogenic in the form in which they are inhaled or ingested. Instead, they are converted by mammalian enzymes into more reactive forms that can attack cellular macromolecules.

This problem can be overcome in part, and even made to work for the investigator, by adding to the test system a homogenate of liver cells containing enzymes that metabolize the test chemical. Cells from a variety of species, including humans, can thus be used to demonstrate species sensitivity. Results obtained in such systems, however, are highly variable from laboratory to laboratory, says Marvin Legator of the University of Texas Medical Branch at Galveston, because of differences in the way the systems are prepared. What is needed, argues Joyce C. McCann, a colleague of Ames's at Berkeley, is a greater use of internal standards to make results reproducible from laboratory to laboratory.

The initial successes of the Ames test have stimulated a great deal of interest in short-term tests, according to McCann. There are now at least 80 different tests in various stages of study, she notes. They can be broken down into at least four broad classes. The examples cited represent some of the most thoroughly studied tests.

▶ Tests with microorganisms. The Ames test is the best example of this category. Another test with bacteria is the Pol-A test developed by Herbert Rosenkranz of New York Medical College. It is based on mutant strains of *Escherichia coli* that lack a particular DNA repair system; chemicals that interfere with DNA kill the mutants. The yeast mitotic recombination test refined by Vincent F. Simmon of SRI International looks for exchange of material between homologous chromosomes during replication.

▶ Tests with intact organisms. The *Drosophila* test used by Seymour Abrahamson of the University of Wisconsin tests for damage to a specific, sex-linked recessive gene in the fruit fly. A second

Industry Reacts to OSHA Proposals

American industry has reacted sharply to the Occupational Safety and Health Administration's (OSHA) proposed regulations governing carcinogens in the workplace. The industry view of the proposed regulations is probably typified by Richard Fleming of Air Products Company, who terms them "scientifically unsupportable, administratively unsound, legally wrong, and economically infeasible." The intensity of industry reaction apparently results from recognition that other regulatory agencies will pattern their own regulations on OSHA's. More than 90 companies and 30 trade associations have thus banded together in the American Industrial Health Council (AIHC) to try to modify the regulations into a more acceptable form. AIHC's key proposals:

▶ A nine-member "data evaluation and classification" panel should be selected by the National Academy of Sciences to determine which substances are truly carcinogenic. AIHC argues that "identification and classification of carcinogens is too important and too complicated to be left to government regulators alone.

▶ Negative evidence from epidemiological studies in humans should be given more weight than positive results in animal bioassays.

▶ Results obtained in animal studies should not be used indiscriminately to predict effects in humans.

▶ Short-term tests are so unreliable as predictors of human response that they are unsuitable as a basis for regulatory decisions.

▶ Acceptable risks should be established for individual chemicals rather than the zero-risk concept implicit in the proposed OSHA regulations. "We do not, and cannot, have a risk-free society," AIHC argues, "and it is not useful to propose regulation rooted in such an idea." Social and economic benefits of potentially hazardous chemicals should thus be evaluated in the establishment of acceptable exposure levels.

▶ The potency of potential carcinogens should be considered in setting exposure levels.

▶ Personal protective devices for individual workers should be permitted as an alternative to engineering controls.

▶ Substances or mixtures containing small amounts of suspect carcinogens should be exempted from regulation. The sensitivity of detection methods has increased so much that it is possible readily to detect quantities of carcinogens so small that their presence is of no consequence.

▶ Research laboratories and construction activities present completely different sets of problems and should have different regulations from factories.

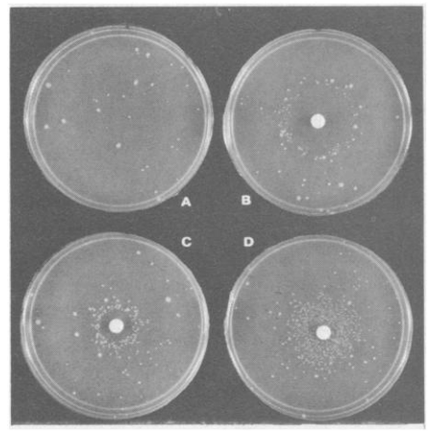
It is hard to judge how much impact the counterproposals will have on the final regulations. Some of AIHC's proposals appear to be little more than bargaining chips designed to be thrown out in any eventual compromise. In particular, the arguments that negative epidemiological studies in humans should have more weight than positive results in animals and that results in animals don't apply to man will probably be dismissed out of hand as insupportable. A similar fate probably awaits the objection to short-term tests when validation studies are completed. There is also resistance to formation of a data evaluation panel because it would, in the words of Food and Drug Administration Commissioner Donald Kennedy, "merely create an additional layer of bureaucracy." More credence will be given to the other proposals, but it seems likely that the final OSHA regulations will not differ substantively from the current proposals.—T.H.M.

test, developed by Andrew D. Kligerman and his associates at Cornell University, looks for genetic damage in cells from the gills, intestines, and kidneys of a small fish, called the central mudminnow, after the entire fish has been exposed to the test chemical. These and similar tests are appealing because the intact organisms are able to metabolize the test chemicals.

▶ Tests that look for genetic damage and mutations in cultured mammalian cells. One test devised by Hans Stich of the University of British Columbia looks for unscheduled DNA synthesis, which occurs when cellular enzymes attempt to repair damage to DNA. The sister chromatid exchange (SCE) test developed by Samuel Latt of Harvard University and refined by Sheldon Wolff and his colleagues at the University of California at San Francisco checks for a scrambling of chromosomes in cultured human lymphocytes and other cultured cells. A test for point mutations in cultured mouse lymphoma cells was developed by W. Gary Flamm, now at the Food and Drug Administration, and Donald Clive of Burroughs Wellcome Company; a similar test with cultured Chinese hamster ovary cells was developed by Abraham Hsie of the National Center for Toxicological Research. All these tests seem to be very sensitive.

▶ Tests for in vitro transformation. These focus on the changes in morphology and growth characteristics that accompany transformation of healthy mammalian cells into malignant ones. Such changes can be recognized in culture or by injecting the cells into laboratory animals, where transformed cells grow into tumors. Two of the best-studied in vitro transformation tests use a mouse cell line and hamster embryo cells, respectively. The first was developed by Charles Heidelberger of the University of Southern California (USC); the second was developed by Joseph A. DiPaolo of NCI and refined by Roman Pienta of NCI's Frederick Cancer Research Center (FCRC). Other investigators are attempting to develop similar tests with epithelial cells, which are the source of most human tumors.

The potential of the short-term tests can be illustrated by their use in helping to resolve the recent dispute over the carcinogenicity of saccharin. Because of the seeming contradictions of animal bioassays of saccharin, the U.S. Congress commissioned its Office of Technology Assessment (OTA) to review the literature on saccharin and to obtain whatever other information might be available in a short time. As part of the study, McCann arranged for a battery of 12 short-term tests to be conducted on the sweetener; these tests, several of which have already been discussed, were representative of the most extensively documented tests available at the time.



In the Ames test, chemicals to be examined are placed on a filter paper disk in the center of a dish containing nutrients and mutants of *Salmonella typhimurium*. Mutagen-induced revertants appear as a ring of colonies around each disk. (A) Spontaneous revertants; (B) the food additive furylfuramide; (C) aflatoxin B_1; (D) 2-aminofluorence. (C) and (D) also contain a liver microsomal activation system. [Source: Bruce Ames, University of California]

Saccharin tested positive in three of the systems. Wolff found that it induces SCE in both cultured human lymphocytes and Chinese hamster cells, with a clear-cut dose-response relationship. Clive found that saccharin is a weak mutagen in tests on cultured mouse lymphoma cells. And Hsie found that it induces chromosome aberrations in cultured Chinese hamster ovary cells. In a test that wasn't complete at the time of publication of the OTA report,* Heidelberger and Suktab Mondal of USC found that saccharin acts as a promoter of transformation in cultured mouse cells; that is, it shifts the dose-response curve of carcinogens so that they are more potent at lower concentrations. Results from the other eight tests were negative. OTA reviewed all the animal and short-term tests and concluded that positive results from them provide "presumptive evidence of risk to humans." Considering all the data, the study's authors concluded that saccharin is definitely a carcinogen, but a weak one whose effects may become apparent only with very high doses or after long exposure.

The informal saccharin study illustrates how short-term tests will probably be used in the future. By using a battery of tests, many scientists now argue, chemicals that escape detection in one system can be identified in another. The Ames test, for example, does not do very well with chlorinated chemicals, pesticides, and metals. These are, however, readily picked up by in vitro transformation tests or others. Already, investigators at commercial testing facilities such as Litton Bionetics and SRI International are using batteries of three or four tests to screen chemicals for their customers.

David Brusick of Litton says his company now uses a battery of four tests that generally includes the Ames test, a test for gene mutation in mouse cells, the SCE test, and an in vitro transformation test. Positive results in any one of these, he argues, indicate that the chemical deserves further study. Investigators at Litton have tested more than 3000 chemicals with one or more of the short-term assays, he adds, and they are confident that the battery will identify at least 95 percent of rodent carcinogens. NIEHS, NCI, and other groups are developing similar screening systems.

The short-term assays seem likely to be woven into the fabric of governmental regulation rather quickly. Already, the Occupational Safety and Health Administration (OSHA) has published a highly controversial proposal establishing three categories for industrial chemicals used in this country—proved carcinogens, suspected carcinogens, and nonsuspect chemicals. OSHA would require industry to provide extensive protective measures for workers with potential exposure to a proved carcinogen and would require that there be no exposure at all if "suitable" substitutes exist. Chemicals would be placed in the proved category if they are found to be carcinogenic in two animal bioassays or in one bioassay and two or more short-term tests.

Exposure to chemicals in the suspect category would be reduced to levels "low enough to prevent acute or chronic effects." Chemicals would be placed in this category if they were found to be carcinogenic in one animal bioassay or in short-term tests. OSHA recently published a preliminary list that would place 261 chemicals in the proved category and 196 in the suspect category.

The Environmental Protection Agency (EPA) will already consider data from short-term tests on pesticides if they are voluntarily submitted by the manufacturer. New pesticide regulations suggest that exposure should be limited if a pesticide is found to be either mutagenic or

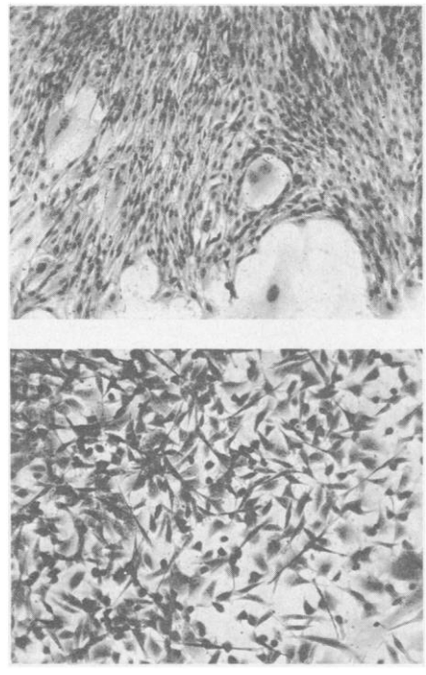
*Office of Technology Assessment, *Cancer Testing Technology and Saccharin* (Government Printing Office, Washington, D.C., 1977).

carcinogenic. The pesticides office of EPA is thus drawing up a series of guidelines that will require short-term tests for mutagenicity; if one or more of these tests is positive, the chemical will also be required to be tested for carcinogenicity in a bioassay. In the present form, says EPA's Richard Hill, the guidelines would require anyone who wishes to register a pesticide to perform eight assays selected from among 13 possibilities. The eight chosen would have to include tests from each of three areas: DNA damage, point mutations, and chromosome aberrations.

EPA's Office of Toxic Substances must promulgate guidelines for premarket testing of new chemicals within 30 days after publication of an inventory of existing chemicals, an event that will probably occur in January or February of 1979. The agency does not have the authority to require specific tests, says Blake Biles, but it will publish guidelines showing what types of test results it would like to receive and can hold up production until it gets what it considers to be adequate information. A preliminary draft of the guidelines would require: six oral, dermal, and inhalation tests in rats, dogs, and rabbits; 90-day tests in rats; a battery of six short-term mutagenicity assays; and teratogenic and reproductive tests in rodents. This battery is expected to cost $50,000 per chemical. The Food and Drug Administration and the Consumer Products Safety Commission are also reviewing short-term assays and seem likely to require their use in future testing programs.

Many investigators think these agencies are jumping the gun—or are being forced to jump it—on short-term assays because their applicability to carcinogenicity has not been confirmed. Most of the assays have been studied with only a small number of chemicals and their sensitivity and specificity are still not known; many investigators fear that guidelines that are too rigid for use of the tests will inhibit incorporation of future findings. Regulators are seduced by the ease of performance and apparent low cost of the tests, argues Legator, but these may be chimeras. The most expensive test, he adds, "is one that doesn't give meaningful results."

Two efforts are being made to provide more information. NCI is studying five in vitro transformation tests, three mutation tests, and five strains of *Salmonella*. The participants, says study coordinator Virginia C. Dunkel, will refine and standardize procedures for conducting the tests and will perform assays on 50 chemicals for which the results of animal bioassays are already known and another 50 chemicals that are currently undergoing bioassay.



(Top) Normal hamster embryo cells in culture. (Bottom) Transformed cells. Both ×60. The transformation of the cells in culture after application of a chemical indicates that the chemical may be a carcinogen. [Source: Roman J. Pienta, Frederick Cancer Research Center]

NIEHS is cooperating with England's Medical Research Council and Imperial Chemical Industries Ltd. in a much larger study that will include about 25 short-term assays and 50 laboratories in the United States, Mexico, Canada, Europe, Japan, and the U.S.S.R. Each test will be performed at least once on each of 42 chemicals, says de Serres; most of the chemicals are pairs that include one known carcinogen and a closely related chemical that is not a carcinogen. Results from both of these validation studies will not be available for at least another 18 months—well after some of the proposed guidelines have gone into effect.

A few investigators have some preliminary results which suggest that the validation studies may not be as successful as most investigators hope. The Ames test, in particular, has drawn fire. William Lijinsky of FCRC, for example, recently subjected 30 polycyclic hydrocarbons and 60 nitrosamines to the Ames test. He found that the concordance between mutagenicity in the Ames test and carcinogenicity in animal studies was only about 55 percent for the hydrocarbons and 70 percent for the nitrosamines. He thus argues that he cannot accept the Ames test as a useful predictor of carcinogenicity.

Similarly, Legator and his colleagues examined 274 chemicals that have been tested in both rodents and the Ames test and found a concordance of results for only 210, or 76 percent. They observed that the extent of concordance depends on the chemical class to which the compounds belong. A high concordance was observed for highly electrophilic molecules such as halomethanes and nitrogen mustards, whereas a very low concordance was observed for compounds such as antimetabolites, polychlorinated cyclic compounds, azonaphthols, symmetrical hydrazines, and steroids, all of which have complex metabolic routes. Since the extent of concordance varies so widely among the different classes, Legator concludes that it would be extremely dangerous to use the Ames test as a basis for regulation. Critics of such studies, however, argue that it was already known that the Ames test is inadequate for certain classes of chemicals and that this problem can probably be avoided if a battery of tests is used.

Despite these reservations, it seems clear that the use of short-term assays will continue to accelerate unless the results of the validation studies prove to be really disastrous. (Even then, the tests would retain their value for identifying mutagens.) Yet even if the validation studies show that these tests do what is claimed for them, there are still problem areas that are not covered by assays. Perhaps the biggest gap, says de Serres, is the lack of a short-term assay for nondisjunction, the occurrence of abnormal numbers of chromosomes as in Down's syndrome. Another problem is that the mutagenesis tests detect only very specific types of reverse mutations. What is needed, de Serres argues, are more general, forward assays that detect all types of genetic damage. Better assays also need to be developed to test for transmissible mutations in germ cells; existing tests are cumbersome and costly.

Other areas of concern are teratology, neurotoxicity, and behavioral effects. Some assays are available in these areas, but they too are costly and time-consuming. A complete battery of tests for one chemical, asserts V. K. Rowe of Dow Chemical Company, would cost more than $1 million. But until effective assays are developed for each of these areas of concern, there can never be complete assurance that the regulation of toxic substances is adequate.

—THOMAS H. MAUGH II