Identifying Environmental Chemicals Causing Mutations and Cancer

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Summary. Damage to DNA appears to be the major cause of most cancer and genetic birth defects and may contribute to aging and heart disease as well. The agents that cause this damage must be identified. Many of these agents are natural chemicals present in the human diet as complex mixtures. The tens of thousands of man-made chemicals that have been introducted into the environment in the last few decades must also be tested for their ability to damage DNA. Existing animal tests and human epidemiology alone are inadequate for this task because of time, expense, and the difficulty of dealing with complex mixtures. Newly developed short-term tests, most of them assaying for mutagenicity, are discussed as key tools in identifying environmental mutagens and carcinogens.

Damage to DNA by environmental mutagens (both natural and man-made) is likely to be a major cause of cancer (*1*, *2*) and genetic birth defects and may contribute to heart disease (*3*), aging (*4*), cataracts (*5*), and developmental birth defects as well. Currently almost one-fourth of the population will develop cancer, and 5 to 10 percent of children are born with birth defects. Damage to the DNA of germ cells can result in genetic defects that may appear in future generations. Somatic mutation in the DNA of the other cells of the body may give rise to cancerous cells by changing the normal cellular mechanisms, coded for in the DNA, that control and prevent cell multiplication. Exposure to mutagens occurs from natural chemicals in our diet, from synthetic chemicals (such as industrial chemicals, pesticides, hair dyes, cosmetics, and drugs), and from complex mixtures (such as cigarette smoke and contaminants in air and water).

A variety of data supports the hypothesis that environmental factors are a major cause of cancer (*1*, *2*). Epidemiological studies show different rates of incidence for certain types of cancer in different parts of the world. For example, in Japan there is an extremely low rate of breast and colon cancer and a high rate of stomach cancer, whereas in the United States the reverse is true. When Japanese immigrate to the United States, within a generation or two they show the high colon and breast cancer rates and low stomach cancer rates characteristic of other Americans. Known environmental mutagens that can cause human cancer include cigarette smoke tar, ultraviolet light, x-rays, and asbestos (*6*), and the list of human chemical carcinogens is steadily lengthening (*1*).

A high percentage of carcinogens is also likely to be able to reach and mutate the germ cells (*7*), as well as the somatic cells, and the costs of this to society may be more than is generally realized (*8*).

This article is not intended as a thorough review of what is clearly an enormous literature. However, I cite some recent reviews that describe the many important contributions that I am unable to discuss here and that contributed to our present ideas.

Identifying Mutagens and Carcinogens: Limitations of Epidemiology

Identifying the mutagens and carcinogens that cause cancer in people is difficult owing to a 20- to 30-year latent period between initial exposure to a carcinogen and the appearance of most types of human cancer. This is illustrated in the case of cigarette smoking (Fig. 1). Men started smoking cigarettes in large numbers in about 1900, but the resulting increase in lung cancer did not appear until 20 to 25 years later. Similarly, women started smoking in appreciable numbers about the time of World War II, and now the lung cancer rate for women is climbing rapidly. This same 20-year lag has been shown to apply for most types of cancer caused by the atomic bomb (leukemia and lymphoma show up earlier) and for cancer in factory workers exposed to a variety of chemicals. Cigarette smoking has been much easier to identify as a cause of cancer than most environmental carcinogens because there is a clear control group of nonsmokers, and because smoking causes a characteristic type of cancer (of the lung) that is infrequent in the control group. This is not the case with most environmental chemicals, and thus it is extremely difficult convincingly to identify the causal agent by epidemiology unless the background cancer incidence is increased by at least 100 percent (that is, a doubling of risk).

Thus human epidemiology, though its use continues to be essential, cannot be our primary tool in detecting individual carcinogens because of the difficulties in connecting cause and effect (*9*), the great expense involved, and the fact that people would already have been exposed for decades by the time a particular cause of cancer was identified.

Human genetic defects are not easy to attribute to a specific cause, and a considerable increase in birth defects might go unnoticed. Moreover, many consequences of a general increase in gene mutations in the germ line might be subtle, such as decreased intelligence.

Human Exposure to New Chemicals in the Environment

Clearly, many more chemicals will be identified as human mutagens and carcinogens. Currently over 50,000 synthetic chemicals are produced and used in significant quantities and close to 1000 new chemicals are introduced each year (*10*). Only a small fraction of these were tested for carcinogenicity or mutagenicity *before* their use. In the past this problem was largely ignored, and even very high-production chemicals with extensive human exposure were produced for decades before adequate carcinogenicity or mutagenicity tests were performed. Such chemicals now known to be both carcinogenic and mutagenic include vinyl chloride (produced at a rate of about 6 billion pounds per year in the United States in 1977) and 1,2-dichloroethane (ethylene dichloride, about 10 bil-

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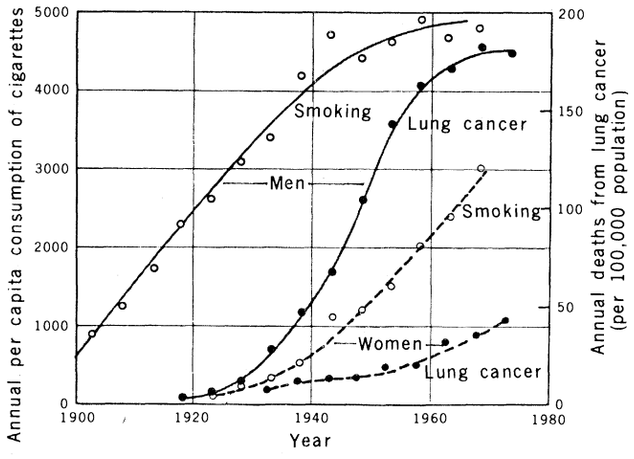

Fig. 1. Relation between cigarette smoking and lung cancer. Cigarette smoking and lung cancer are unmistakably related, but the nature of the relationship remained obscure because of the long latent period between the increase in cigarette consumption and the increase in the incidence of lung cancer. The data are for England and Wales. In men (solid line) smoking began to increase at the beginning of the 20th century, but the corresponding trend in deaths from lung cancer did not begin until after 1920. In women (broken line) smoking began later, and lung cancers are only now appearing. [From Cairns (*52*), courtesy of *Scientific American*]

lion pounds per year) (see Fig. 2), and a host of high-production pesticides.

The increase in production and use of chemicals has been particularly great since the mid-1950's, as is illustrated by the two high-production compounds shown in Fig. 2. This flowering of the chemical age may be followed by genetic birth defects and a significant increase in human cancer during the 1980 decade (because of the 20- to 30-year lag) if many of these chemicals with widespread human exposure are indeed powerful mutagens and carcinogens.

Some of these environmental carcinogens accumulate in human body fat; almost all of us are continuously being exposed to low, but disturbing, doses of these carcinogens. Table 1 shows some chlorine-containing chemicals found in human body fat; almost all are known carcinogens in rodents. Table 2 shows the levels of some of these same carcinogens in human mothers' milk.

Table 1. Chlorinated hydrocarbon residues in human fat (average of 168 Canadian samples). Almost all of them are carcinogens. [Adapted from Mes *et al.* (*55*), courtesy of Springer-Verlag New York, Inc.]

Compound	Amount* (μg/kg wet weight)	Percentage of samples containing residues
PCB	907	100
Hexachlorobenzene	62	100
BHC (lindane)	65	88
Oxychlordane	55	97
Trans-nonachlor	65	99
Heptachlor epoxide	43	100
Dieldrin	69	100
p, p'-DDE	2095	100
o,p'-DDT	31	63
p,p'-TDE	6	26
p,p'-DDT	439	100

*Values are means.

The few chemicals that were assayed for in these studies are only some of those that have accumulated in people. Carcinogens such as toxaphene (*11*), Kepone, mirex, and other major chlorinated pesticides are also accumulating in the food chain, as are a wide variety of industrial chemicals as yet untested for carcinogenicity (*12*). Some of these, like the polybrominated biphenyls (PBB's), are likely to be carcinogens (*12*).

Organic chemicals containing chlorine and bromine are not used in natural mammalian biochemical processes and may not have been usually present in the human diet until the onset of the modern chemical age. A very high percentage of chlorinated and brominated chemicals are carcinogens in animal cancer tests and thus represent a highly suspect class of chemicals.

Is There a Safe Dose of Mutagens and Carcinogens?

There is considerable debate as to whether there is a safe dose for carcinogens and whether we should be concerned about the exposure of the general population to the many low doses of environmental carcinogens. There is no firm answer to this question since with very low dose levels of a chemical it is impossible to get statistically significant information from cancer experiments in which only a few hundred animals are exposed (*13*). Several arguments suggest that thresholds, or completely safe doses of carcinogens, are not likely to be the general case (*13*), and it seems prudent to assume, until shown otherwise experimentally, that small increments of an environmental carcinogen will increase risk linearly.

The Utility and Limitations of Animal Cancer Tests

A key method for detecting carcinogens is the animal bioassay, usually done with rats and mice. Almost all of the dozen or so organic chemicals known to cause cancer in humans also cause cancer in experimental animals when adequately tested (*1*). A fairly small percentage of chemicals in general appears to be carcinogenic. The National Cancer Institute (NCI) has just completed testing about 200 suspicious industrial compounds to which people are exposed in appreciable amounts. Many more such tests need to be done. The utility of animal cancer tests for cancer prevention, however, is limited by several important factors.

Feasibility: Time and expense. Animal cancer tests are too expensive (currently about $250,000 per chemical for a thorough test) and take too long (about 3 years) to be used for the testing of the many thousands of chemicals to which humans are exposed. In fact, reports for adequate cancer tests are published on only about 150 previously untested chemicals each year. There are not enough pathologists to read the slides even if it were decided to test only the thousand or so new chemicals introduced into commerce each year, not to mention the 50,000 untested commercial chemicals already in use and the even greater number of chemicals in the natural world. Animal tests are also not practical as bioassays for identifying the carcinogens in the many complex chemical mixtures that surround us, such as natural chemicals in our diet, cigarette smoke, and impurities in water and air and complex industrial products. A further limitation is that chemical and drug

Table 2. Some pesticides in the milk of 1400 women. [From Harris and Highland (*56*), courtesy of Environmental Defense Fund]

Compound	Number positive (%)	Mean of positives (μg/kg fat)*	Maximum (μg/kg fat)
DDE	100	3521	214,167
DDT	99	529	34,369
Dieldrin	81	164	12,300
Heptachlor epoxide	64	91	2,050
Oxychlordane	63	96	5,700
β-BHC	87	183	9,217
PCB's	30†	2076	12,600

*4.5 percent = mean fat content. †99 percent detectable PCB's; (30 percent = >1100 μg per kilogram of fat) (1038 women, Environmental Protection Agency, 1977).

companies need to have a method for weeding out hazardous chemicals while they are still under development and while alternatives can be substituted. Currently, many chemicals undergo a long-term animal test, if they are tested at all, only after millions of dollars have been invested in them.

Sensitivity. An environmental carcinogen causing cancer in 1 percent of 100 million people would result in a million cases of cancer. Detection of a chemical causing cancer in only 1 percent of the test animals would require the use of 10,000 rats or mice and would be extraordinarily expensive. A test group of only 50 mice or rats of each sex at each of two doses is the usual size of the most thorough cancer experiments. This limitation is somewhat overcome, though not entirely satisfactorily, by exposing the animals to as high a dose as possible (the "maximum tolerated dose") which, by increasing the tumor incidence, partially offsets the statistical problems inherent in the small sample size.

The *Salmonella* Mammalian Liver Test: Detecting Chemical Mutagens

Over the past 15 years I and my colleagues have been developing a simple test for identifying chemical mutagens (*14*) and have used it to show that about 90 percent of the organic chemical carcinogens tested thus far are mutagens (*15, 16*). This work, and that of others (*2, 17-20*), has strongly supported the theory that most carcinogens act by damaging DNA. The *Salmonella* test and other short-term tests that have been developed for testing chemicals for their ability to interact with DNA or for mutagenicity (*18*) are being widely used and should help in solving some of the problems that cannot be adequately approached by human epidemiology or animal cancer tests alone. The *Salmonella* test is in current use in over 2000 government, industrial, and academic laboratories throughout the world. A number of companies have made important economic decisions on the basis of the *Salmonella* test (*21*).

Our work started as an offshoot from our basic research on the molecular biology of *Salmonella* bacteria. We were studying how genes are switched on and off in bacteria in response to the presence of the amino acid histidine in the growth medium and the effect of mutations which perturbed this control mechanism. We were using a large collection of histidine-requiring bacterial mutants, mostly made by Philip E. Hartman of Johns Hopkins University. In 1964 we began to develop a test system for detecting mutagens using these mutants. During the course of this work, and other work on the theory of frameshift mutagenesis, we became convinced that the essential property of most carcinogens was their mutagenicity (*22*). The ideas and work of the Millers, Boyland, Magee, the Weisburgers, and other workers (*20*), had made a major contribution to the understanding that many carcinogens must be converted by enzymes in liver or other tissues to an active (electrophilic) form that is the true carcinogen (and mutagen). Also of great importance was the in vitro work of Malling, Rosenkranz, and the Millers on the use of liver homogenate for the activation of dimethylnitrosamine, diethylnitrosamine, and aflatoxin combined with our tester strains, or *Escherichia coli*, as indicators of DNA damage (*22a*). We thus added mammalian liver tissue to the test to provide a first approximation of mammalian metabolism.

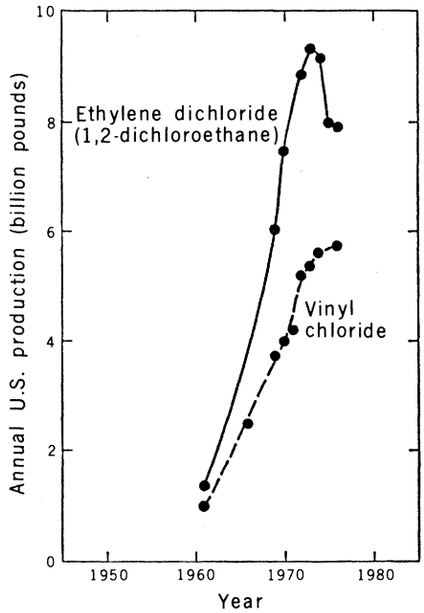

Fig. 2. Production of two mutagens and carcinogens with widespread human exposure: ethylene dichloride and vinyl chloride (production data from "Top-50 Chemicals" issues of *Chemical and Engineering News*). Approximately 100 billion pounds (5×10^{10} kilograms) of ethylene dichloride and over 50 billion pounds of vinyl chloride have been produced since 1960. Ethylene dichloride is a volatile liquid that is the precursor of vinyl chloride and is also used extensively as a fumigant, solvent, gasoline additive (200 million pounds per year), and metal degreaser. Ethylene dichloride was first shown to be a mutagen in *Drosophila* in 1960 (*53*), and later in barley and *Salmonella* but these data have been ignored. The first adequate cancer test in animals has just been completed by NCI (September 1978) and shows ethylene dichloride to be carcinogenic in both sexes of rats and mice. Vinyl chloride gas is used to make polyvinyl chloride (PVC; vinyl) plastic. It was shown to be a carcinogen in rats and in people in the mid-1970's, and a mutagen in *Salmonella* and other systems shortly afterward. Vinyl chloride production results in the dumping of enormous quantities of a waste product, EDC-tar, that is a complex mixture of chlorinated hydrocarbons; this is mutagenic in *Salmonella* and has been detected as an ocean pollutant (*54*).

The test is done by combining on a petri plate the compound to be tested, about 1 billion *Salmonella* bacteria of a particular tester strain (several different histidine-requiring mutants are used), and homogenized liver from rodents (or human autopsy); after incubation at 37°C for 2 days, the number of bacterial revertant colonies is recorded. Each colony is composed of the descendants of a bacterium that has been mutated from a defective histidine gene to a functional one. Normally, one tests doses of a chemical in a series of plates—the plate test—and quantitative dose-response curves are generated (Fig. 3). These curves are almost always linear, which suggests that, at least in this system, thresholds are not common. Mutagens can be detected at low doses, in some cases in nanogram amounts.

Validation of the test with 300 chemicals. We validated the test for the detection of carcinogens as mutagens by examining about 300 chemicals reported as carcinogens or noncarcinogens in animal experiments (*15, 16*). About 90 percent (158 out of 176) of these chemical carcinogens were mutagenic in the *Salmonella* test. The *Salmonella* test system has subsequently been independently validated, with similar results (*19*). The percentage of carcinogens detectable would, of course, depend on how nearly any particular list of carcinogens was representative of those existing in the real world. For this reason we also examined the organic chemicals known as or suspected of being human carcinogens and found that almost all [16 out of 18; benzene and diethylstilbestrol (DES) were the exceptions] were mutagens in the test (*15*). Nevertheless, it is important to reemphasize that the success rate is markedly lower (*23*) for some classes of carcinogens, such as hydrazines and heavily chlorinated chemicals (*15*). Even with further test improvements, promoters and some carcinogens (for example, griseofulvin and steroids) may never be detected as such because they probably do not act through a direct interaction with DNA (*15*).

Thus, a very high percentage of carcinogens tested are mutagens, and most mutagens appear to be carcinogens. We

found that few (13 out of 108) "noncarcinogens" tested were mutagenic, and these few may in fact be weak carcinogens that were not detected as such because of the statistical limitations of animal carcinogenicity tests (*15, 16*). Only a small percentage of chemicals in general appears to be mutagens (*23a*).

Mutagens subsequently found to be carcinogens. There have been a number of instances of chemicals initially detected as mutagens being found subsequently to be carcinogens [see also (*17, 24*)]. I will discuss five of these that involved extensive human exposure.

1) Furylfuramide (AF-2) was a food additive used extensively in Japan from 1965 until recently as an antibacterial additive in a wide variety of common food products such as soybean curd and fish sausage (*17, 25*). It showed no carcinogenic activity in tests on rats in 1962 and on mice in 1971. In 1972 and 1973, however, Japanese scientists found that it caused chromosomal aberrations in cultured human cells and was also highly mutagenic in a strain of *Escherichia coli* bacteria. It was later found to be an extraordinarily potent mutagen in our *Salmonella* test, so much so that one could easily demonstrate the mutagenicity of a slice of fish sausage put on a petri plate (*17*). It was later found to be mutagenic in yeast and *Neurospora* and in a variety of other short-term tests, and more recently to mutate embryos when even low doses were fed to pregnant Syrian hamsters (*25*). More thorough animal tests for carcinogenicity were initiated, and these tests have recently shown that AF-2 is, in fact, a carcinogen in rats, mice, and hamsters. As a consequence, the Japanese government prohibited the use of AF-2 as a food additive, and all products containing AF-2 were removed from the market (*17*).

Since AF-2 had already been tested for carcinogenicity in two animal systems and found negative, it is unlikely that further animal tests would have been conducted if it had not been shown to be mutagenic; any deleterious effects on the Japanese population would probably not have been evident for decades. The Japanese people did consume relatively large amounts of furylfuramide for 9 years, and it is still too early to assess the consequences of this exposure.

2) Ethylene dichloride, a 10-billion-pound-a-year chemical (see Fig. 2) first shown to be a mutagen in the fruit fly, *Drosophila*, and then in barley, and in *Salmonella*, was recently tested by NCI and found to be a carcinogen in both sexes of rats and mice.

3) Ethylene dibromide (1,2-dibromoethane) (*10*), is a widely used (400 million pounds per year in the United States) industrial chemical and gasoline additive, which was detected as a mutagen in *Neurospora* in 1969 (*25a*) and then in several microbial systems (including *Salmonella*; see Fig. 3). In 1973 it was found to be a potent carcinogen in rats and mice.

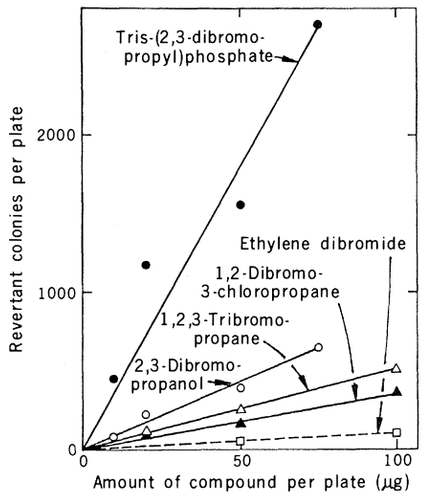

Fig. 3. Linear dose responses obtained with the *Salmonella* test. The flame-retardant tris-BP, its metabolite dibromopropanol, and the pesticide dibromochloropropane were in the presence of rat liver homogenate. All compounds were tested on *Salmonella* strain TA100. The amount of the industrial chemical ethylene dibromide added was ten times that indicated on the scale. [From (*27*)]

4) The flame-retardant "tris-BP" [tris(2,3-dibromopropyl)phosphate], a related dibromo chemical which was the main flame retardant in children's polyester pajamas, is a mutagen in the *Salmonella* test; so are its metabolic product, dibromopropanol, and its impurity, the carcinogen (and human sterilant) dibromochloropropane (DBCP) (Fig. 3) (*26, 27*). During the years 1972 to 1977, 50 million children wore sleepwear that contained this material, at about 5 percent of the weight of the fabric. We argued that tris-BP would pose a serious hazard to children because nonpolar (relatively fat-soluble and water-insoluble) chemicals such as tris-BP are generally absorbed through human skin at appreciable rates (*26, 27*). Since its detection as a mutagen in *Salmonella*, it has been shown to be active in a number of short-term tests: it is a potent mutagen in *Drosophila*, it interacts with human DNA, and it damages mammalian chromosomes. The compound was tested recently at NCI and was shown to be a carcinogen in both rats and mice. It has also been shown to cause cancer in skin-painting studies on mice (*28*) and, like DBCP, to cause sterility in animals. It is no longer being used in sleepwear. We have recently shown that a mutagenic metabolite of tris-BP, dibromopropanol, is present in the urine of children wearing tris-treated sleepwear (*29*).

5) Hair dyes also contain mutagens. In a study at our laboratory (*30*) about 90 percent (150 out of 169) of commercial oxidative-type (hydrogen peroxide) hair dye formulations were mutagenic, and of the 18 components of these hair dyes (mostly aromatic amines), eight were mutagenic. Many semipermanent hair dyes tested were also mutagenic. Hair dye components are known to be absorbed through the skin, yet very few of the hair dyes, their components, or their peroxide reaction products have ever been tested adequately for carcinogenicity. A variety of these ingredients have now been shown to be mutagenic in other short-term tests. Several of the chemicals are being tested at NCI and now appear to be carcinogens. About 25 million people, mostly women, dye their hair in the United States, and the hazard could be considerable if these chemicals are mutagenic and carcinogenic in humans. A recent epidemiological study suggests that there may be a considerable excess of breast cancer in postmenopausal women who have dyed their hair over a long period (*31*), though more definitive work needs to be done.

Identifying mutagenic components in complex mixtures. The sensitivity of the *Salmonella* test makes it useful for rapidly obtaining information about mutagenic components of complex mixtures such as natural products, air and water pollutants, pyrolyzed material, body fluids, and the impurities in industrial chemicals (*16, 17*). For example, a detailed study has been made of the mutagenic activity of cigarette smoke condensate and 12 standard smoke condensate fractions (*32*); mutagenicity could be detected in the condensate from less than 0.01 cigarette. The *Salmonella* test system has been used to examine human urine, and mutagens have been found in the urine of cigarette smokers but not (at the level of sensitivity used) in the urine of nonsmokers (*33*).

Natural Carcinogens and Mutagens in the Diet

Any major effect of man-made chemicals as carcinogens and mutagens should become apparent in the next several decades. Much of the cancer occurring today, in addition to that caused by cigarette smoke and radiation (such as ultraviolet light which induces skin can-

cer), appears likely to be due to the ingestion of natural carcinogens in our diet (*24*). For example, fat intake has been correlated with breast and colon cancer (*2, 24*), and many plants used in the human diet have developed a wide assortment of toxic chemicals (probably to discourage insects and animals from eating them) that may be mutagens and carcinogens (*2, 17, 24, 34, 35*). In addition, powerful nitrosamine and nitrosamide carcinogens are formed from certain normal dietary biochemicals containing nitrogen, by reaction with nitrite (*24, 36, 37*). Nitrite is produced by bacteria in the body from nitrates that are present in ingested plant material and water (*2, 24, 36*). A number of molds produce powerful carcinogens such as aflatoxin and sterigmatocystin; these molds can be present in small amounts in foods such as peanut butter and corn (*2, 24*).

In several studies now in progress the *Salmonella* test is being used to identify natural carcinogens. Bruce and his colleagues in Toronto have found a powerful mutagen in human feces (*24, 37*) that appears to be a nitrosamide-type compound formed from a component of dietary fat and nitrite. This compound could be a cause of colon and breast cancer. Bruce has purified the compound using *Salmonella* mutagenicity as a bioassay, and has also found that ingestion of vitamin C or vitamin E lowers the amount of the mutagen present in feces (*24*).

Sugimura and other workers in Japan have discovered by means of the *Salmonella* test that when fish or other foods containing protein are broiled, mutagenic chemicals are formed (*17, 24, 38*). They have also found that broiling protein or many of the amino acid components of protein produces potent mutagens. Several of these mutagens have been identified chemically, with the *Salmonella* test being used as a rapid bioassay, and have been found to be extremely active in another short-term assay (transformation) with animal cells (*24*). Animal cancer tests are being done on the pure substances. Identifying the active chemicals by the use of animal cancer tests would have been impractical because of the time involved. Commoner *et al.* (*39*) have reported that fried hamburgers also show mutagenic activity in the *Salmonella* test.

Glycosides of quercetin, a mutagenic flavonoid, are present in considerable amounts in our diet from a variety of sources (*17, 24, 34*), and, by means of hydrolysis, bacteria in the human gut readily liberate the mutagen. The contribution to human cancer of mutagenic flavonoids and anthraquinone and other plant mutagens present in our diet remains to be evaluated (*17, 24, 34, 35*).

Other Short-Term Tests for Measuring Mutagens

With the development of the *Salmonella* test and the demonstration that, in general, carcinogens are mutagens, there has been a tremendous surge of interest in the other short-term test systems for measuring mutagenicity. Many such systems have been developed (*2, 17, 18*) and have made major contributions to the field. Some, including the use of animal cells in tissue culture which can be examined both for mutagenic and cytogenetic damage, have been validated by testing a number of carcinogens and noncarcinogens. In addition, a number of the older systems, such as mutagenicity testing in *Drosophila*, have been improved. (The first mutagens known, such as x-rays and mustard gas, were identified in *Drosophila* before they were known to be carcinogens.) An important advance is the development of several tests that have as an end point the "transformation" of animal cells in tissue culture to cells that can form tumors (*18*) when injected into animals. A number of tests with rodents also are being developed that will enable investigators to examine mutagenic damage in cells in the whole animal (*2, 18, 25*).

No single short-term test, however, is perfect. Because each system detects a few carcinogens that others do not, the idea of a battery of short-term tests is now favored by many investigators (*18*). It is becoming apparent that positive results from a battery of these short-term test systems are meaningful; these systems, as well as complementing animal cancer tests, provide useful toxicological information about mutagenicity.

Mutagens should be treated with respect not only because of their probable carcinogenicity, but also because of other biological consequences of DNA damage, such as genetic birth defects. There are now simple methods for looking at the effects of chemicals on the germ line (*7, 18*), such as testing for defective sperm in rodents or people (*7*) or for sterility. Because of the discovery that the carcinogen and mutagen DBCP causes sterility in both rodents and male workers (*40*) and causes chromosomal abnormalities in the sperm of chemical plant workers (*40, 41*), and because of the finding that a high percentage of carcinogens damage the germ line (*7, 8*), interest in these methods should increase.

Carcinogenic Potency and Human Risk Assessment

With the large number of environmental carcinogens and mutagens, both man-made and natural, it is clearly impractical to ban or eliminate every one of them. Priorities must be established for dealing with these chemicals, and this requires an assessment of human risk, a complex problem.

A knowledge of carcinogenic potency would be an important aid in human risk assessment. Following the lead of Meselson [see (*42*)] and collaborating with R. Peto on the theoretical aspects, we (*43*) have been working on the potency problem for several years and are nearing completion of quantitative analyses of the several thousand published animal cancer tests in which chemicals were fed continuously for an appreciable fraction of the lifetime of the animal. We have found that the potency of carcinogens (the TD_{50}, the daily dose required to produce cancer in half of the animals, or more precisely to reduce the probability of being tumor-free by one-half) can vary by well over a millionfold. This range of potency must be considered in assessing the hazard of chemicals for man and demonstrates the need for carcinogens to be considered in more quantitative terms. These results should be useful for determining the following.

1) Which chemicals, among the thousands of carcinogens to which people are exposed, are likely to present the greatest human hazard and thus require the most immediate attention. Setting priorities also requires, of course, an estimate of the amount of human exposure to a given chemical.

2) Better ways of calculating unacceptable levels of carcinogen exposure for workers or for the general population.

3) The significance of negative animal cancer tests. Each particular cancer test has a particular thoroughness (sensitivity) that depends on the dose level of the chemical, the numbers of animals used, and other factors and can detect only those carcinogens having potencies above a certain level. Because cancer tests vary in thoroughness, we have expressed the results of a negative cancer test by assigning the chemical a maximum potency value rather than using the quantitatively meaningless term "noncarcinogen."

4) The extent to which carcinogenic potency is species-, strain-, and sex-specific and similar in long-lived species, such as monkeys, and short-lived rodents. Our analysis so far indicates that,

in general, potency values for a given chemical do not vary much between males and females or, with a few significant exceptions, between rats and mice.

We believe that the potency scale can be applied to such current problems as human exposure limits for volatile carcinogens in the workplace [threshold limit values (TLV's)]. The TLV's might be safer if carcinogenic potency were taken into account in determining them. Under the present standard-setting system, workers are allowed to breathe in amounts of certain volatile halogen-containing solvents [for example, DBCP (*40*) or ethylene dichloride (*41*)] that are approximately the TD_{50} doses (on a milligram per kilogram basis) for rats and mice. The establishment of a priority list for investigations of these and other chemicals with wide use and an appreciable carcinogenic potency is an urgent necessity, as is the examination of workers and other exposed populations for the effects of these chemicals.

Correlating *Salmonella* and Animal Test Data

Although a start can now be made on human risk assessment on the basis of animal cancer tests, few of the chemicals to which people are exposed in the environment have actually undergone cancer testing in animals. Furthermore, many of the completed tests lack the quality needed for making a quantitative analysis of the data. Thus the question remains: Can short-term tests provide any quantitative information about human risk?

There is more than a millionfold range in mutagenic potency in the *Salmonella* test and there is also a similar range in carcinogenic potency (*15, 17*). Although one would certainly not expect a precise quantitative correlation between mutagenicity in a bacterial liver test and carcinogenicity in animals, even a rough quantitative correlation would be useful in human risk assessment. Work done by Meselson and Russell (*42*) on 14 chemicals suggests there is a good correlation of the two potencies, not only for carcinogens in the same class but also across a broad range of classes, although some nitrosamines did not fit this general relationship.

We are comparing (*43, 44*) the potency of chemicals in causing tumors in rats with potency in the *Salmonella* test (with a rat liver homogenate being used for activation). The results so far are promising, and additional work will show how general this correlation is (*45*). It is feasible to obtain *Salmonella* mutagenicity data on all those carcinogens for which one can calculate a carcinogenic potency. Our analysis should, in any case, give some indication of the chemicals for which it is necessary to use homogenates of tissue other than the liver in mutagenicity tests, and of areas where the test needs improvement.

A number of laboratories are examining the extent to which species differences in carcinogenic potency of chemicals can be correlated with differences in mutagenic potency by using homogenates of liver or other tissues from the different species. Other short-term tests that are currently being developed can also be calibrated against our carcinogenic potency index to see how well they correlate. The quantitative agreement between *Salmonella* and another short-term test [inhibition of DNA synthesis in human (HeLa) cells in tissue culture] has been examined and appears good (*46*). If several short-term tests can be shown to provide rough quantitative results consistent with those from animal cancer tests, a battery of short-term tests could then be used for helping to establish priorities among the many mutagens, both natural and man-made, that have never been tested in animal cancer tests and to which there is significant human exposure.

The Prevention of DNA Damage

The problems of cancer, genetic birth defects, and other consequences of DNA damage can be usefully attacked by prevention. The following approaches (I will not discuss regulatory policy) are suggested.

1) Identification of mutagens and carcinogens from among the wide variety of environmental chemicals to which humans are exposed. All approaches must be used: human epidemiology for cancer and genetic birth defects, animal tests for cancer and for genetic birth defects, and short-term mutagenicity and transformation tests.

2) Premarket testing of new chemicals to which humans will be exposed.

3) Provision of more readily available information on both natural and man-made chemicals capable of causing cancer and mutations (including their relative danger where this is known) for use by the state and federal governments, industry, unions, consumer groups, and the public at large (*47*).

4) Establishment of priorities for trying to minimize human exposure to these chemicals. Priority lists could take into account, among other factors, amount of human exposure to each chemical and the potency of the chemical in animal cancer tests (*48*). Where adequate animal cancer data will not soon be available, potency information from several short-term tests might be substituted provided that the tests have been validated for this purpose. It seems unlikely that animal cancer tests will soon catch up with the many mutagenic substances being discovered (*17*). Soon more sophisticated and sensitive ways of measuring DNA or other damage caused by mutagenic chemicals in people might play an essential role in risk assessment (*7, 49*).

5) Investigation of modifying factors in carcinogenesis such as vitamins C and E (*24, 37*), selenium (*50*), genetic factors (*50*) (for example, skin color in ultraviolet carcinogenesis), promoters (*2, 24*), and viruses (*2*), which could have a great impact in prevention.

Mutagens and carcinogens must be treated with respect (*51*); priorities must be set, alternatives examined, and human exposure minimized. We have seen, and will continue to see, the folly of using humans as guinea pigs.

References and Notes

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