as the role of DNA repair processes in carcinogenesis and genetic susceptibility to cancer.

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ENVIRONMENTAL CHEMICALS CAUSING CANCER AND GENETIC BIRTH DEFECTS

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Damage to DNA appears to be the cause of most cancer and genetic birth defects and may contribute to aging and heart disease as well. A major part of this DNA damage is caused by environmental chemicals, both natural and man made. Many more chemicals will be added to the current list of human carcinogens and mutagens. Since the late 1950s we have been exposed to a flood of chemicals--from flame retardants in our children's pajamas to pesticides accumulating in our body fat--that were not tested for carcinogenicity or mutagenicity before their use. In the past this problem has been largely ignored; even high-production chemicals, with extensive human exposure, have been produced for decades without adequate carcinogenicity or mutagenicity tests. A few of these chemicals are now being tested in animals, but for most of them the human population is serving as the test animal. Because the 20- to 30-year lag time for chemical carcinogenesis in humans is almost over, the incidence of cancer may increase steeply if too many of the thousands of new chemicals to which humans have been exposed turn out to be powerful mutagens and carcinogens (Figures 1 and 2). We must identify the agents that have caused the cancer and genetic birth defects of today (many of these are natural compounds present in our diet as complex mixtures) and test the many man-made chemicals that have been introduced into the environment in the last few decades. Existing animal tests and human epidemiology *alone* are inadequate procedures for this task for a number of reasons including time, expense, and the difficulty of dealing with complex mixtures.

Over the last 14 years we have developed a simple method for identifying chemical mutagens, and we have shown that almost all chemical carcinogens are mutagens (1). This test combines on a petri plate special strains of *Salmonella* bacteria (as indicators of reverse mutation) and mammalian liver homogenates (rodent or human autopsy--to provide mammalian metabolism) (Figures 3 and 4) (4-6). We have validated the test for detection of carcinogens as mutagens by testing over 300 chemicals and have reported that almost all chemical

ISBN 0-12-125650-3

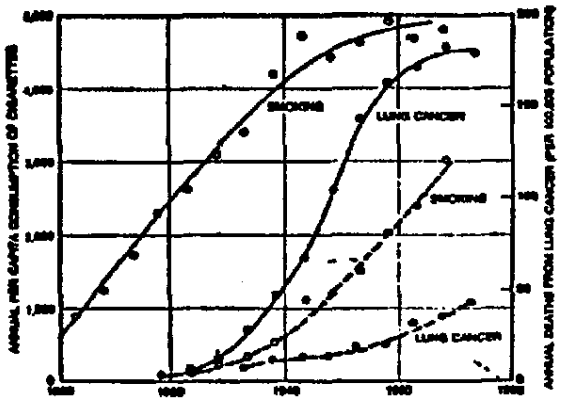

FIGURE 1. Cigarette smoking and lung cancer are unmistakably related, but the nature of the relation remained obscure because of the long latent period between the increase in cigarette consumption and the increase in the incidence of lung cancer. The data are for England and Wales. In men (solid line) 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 (dotted line) smoking began later, and lung cancers are only now appearing. (From J. Cairns, "The Cancer Problem." Copyright 1975 by Scientific American, Inc. All rights reserved.)

Even if we could identify new hazardous chemicals by human epidemiology, people will already have been exposed for decades, and the discovery may be too late. This happened with vinyl chloride: it was in millions of spray cans and in foods packaged in PVC (polyvinyl chloride) containers. The 20- to 30-year lag time needed for cancer to develop from the exposure to the tremendous increase in chemicals that started during the late 1950s and early 1960s (Figure 2) is almost over. Thus, we may expect a steep increase in human cancer if many of these thousands of new chemicals are indeed powerful mutagens and carcinogens with widespread human exposure.

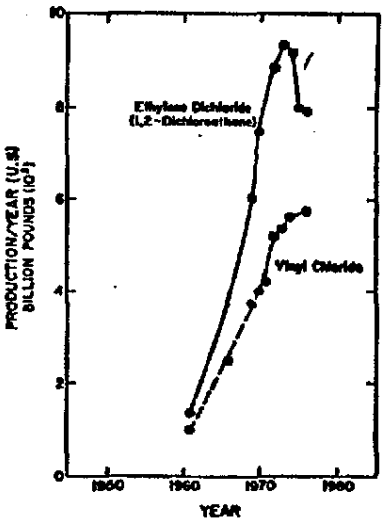

FIGURE 2. Production of two mutagens-carcinogens with widespread human exposure: ethylene dichloride and vinyl chloride (production data from "Top-50 Chemicals" issues of *Chemical and Engineering News*). Approximately 100 billion lb of ethylene dichloride and over 50 billion lb of vinyl chloride have 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 lb/yr), and metal degreaser. Ethylene dichloride was first shown to be a mutagen in *Drosophila* in 1960, and later in barley and *Salmonella*, but this fact has been ignored. The first adequate cancer test in animals has just been completed by the N.C.I. (November 1977) and is positive in both sexes of both 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-1970s, and a mutagen in *Salmonella* and other systems shortly afterwards. (From B. N. Ames, "Environmental Chemicals Causing Cancer and Genetic Birth Defects," Institute of Governmental Studies, University of California, Berkeley, 1978.)

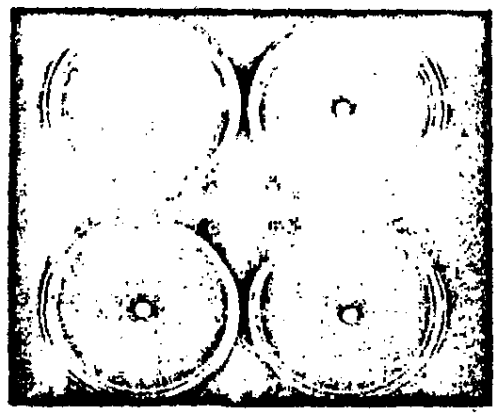

FIGURE 3. The "spot test." Each petri plate contains, in a thin overlay of top agar, the tester strain TA98 and, in the cases of plates *C* and *D*, a rat liver microsomal activation system (S-9 Mix). Mutagens were applied to 6-mm filter-paper discs, which were then placed in the center of each plate: (*A*) spontaneous revertants; (*B*) the Japanese food additive furylfuramide (AF-2) (1 μg); (*C*) the mold carcinogen aflatoxin B_1 (1 μg); (*D*) 2-aminofluorene (10 μg). Mutagen-induced revertants appear as a ring of colonies around each disc. (*Reprinted from Ref. 1.*)

The food additive furylfuramide was 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. It showed no carcinogenic activity in tests on rats in 1962 and on mice in 1971. In 1973, Japanese scientists found it to be highly mutagenic in a strain of *Escherichia coli* bacteria (it was also found to be extraordinarily potent in reverting our *Salmonella* tester strain TA100). They subsequently examined it in higher (eukaryotic) organisms and found it to be mutagenic in yeast and *Neurospora*, and to cause chromosome breaks in human white blood cells. Animal tests for carcinogenicity, more extensive than the previous ones, were initiated, and these tests have recently shown that AF-2 is, in fact, a carcinogen. As a consequence of this finding, the Japanese government prohibited the use of AF-2 as a food additive, and all products containing AF-2 were removed from

the market. Since AF-2 had already been tested for carcinogenicity in two animal systems and found negative, it is unlikely that further tests would have been conducted if it had not been shown to be mutagenic.

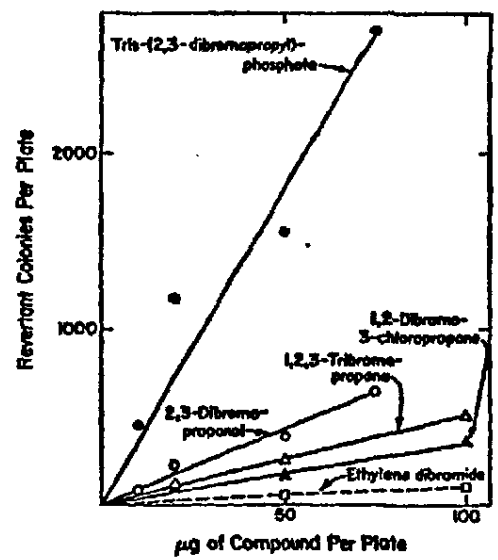

FIGURE 4. The flame retardant *tris*(2,3-dibromopropyl) phosphate and the pesticide dibromochloropropane were in the presence of rat liver homogenate. All compounds were tested on *Salmonella* strain TA100. The amount of the industrial chemical ethylene dibromide added was ten times that indicated on the scale. (Reprinted from Ref. 2.)

Since this and the Prival *et al.* (3) studies, *tris* has been shown to be a carcinogen in rats and mice. Fifty million children have been exposed. Dibromochloropropane (DBCP) has now been shown to have sterilized a large number of factory workers.

carcinogens tested are mutagenic in this test (157/175) and most "non-carcinogens" (95/108) are negative. A number of the "false positives" generated by this study appear to be explainable as consequences of statistical limitations of animal carcinogenicity tests (4,7).

The test is particularly useful for the detection of mutagens/carcinogens in complex mixtures (such as cigarette smoke, water, air pollution, food, and urine) (8-10). We have recently developed a simple method for examining human urine in our test system and have shown that cigarette smokers have mutagens in their urine and non-smokers do not (10). The test is also useful in the development of drugs and industrial chemicals where large numbers of chemicals must be screened. Most of the major drug and chemical companies in the world are now using the test system.

We are exposed to a very large number of chemicals that are mutagens and carcinogens, many of them quite useful for society, and it is clearly impractical to ban them all, yet foolish to ignore their potential danger. We must have some way of setting priorities for regulation of these chemicals, and this requires an assessment of human risk. We (with C. Sawyer, N. K. Hooper, A. Friedman, and R. Peto, following the lead of Meselson and Russell [11]) have shown by the quantitative analysis of animal cancer tests that there is over a million-fold range in the strength of carcinogens, and this knowledge, combined with knowledge on human exposure, may enable an assessment of human risk to be done in a more rational manner. Because few chemicals (or mixtures) in the environment have been tested in animal cancer tests, we need additional ways of obtaining information as to the mutagenic and carcinogenic danger of chemicals.

We believe that short-term tests such as *Salmonella* will play an essential role in priority setting: a key issue, however, is what they can tell us about potency. We have preliminary studies on the relation between mutagenic and carcinogenic potency. There are a number of reasons why one should not expect a very close quantitative correlation between mutagenicity in bacteria and carcinogenicity in animals. Nevertheless, there is over a million-fold range in mutagenic potency in the *Salmonella* test and a similar range in carcinogenic potency and even a rough quantitative correlation would be very useful in human risk assessment. There is an indication, from Meselson and Russell's work (11) and our own that there is a quantitative correlation, not only for carcinogens in the same chemical class, but also across a broad range of classes. Further work will show how general this correlation is. There appears to be a correlation between mutagenic potency in *Salmonella* (and at least one short-term test using human cells [12]). Thus it appears likely that potency in a

battery of short-term tests (many good ones have now been developed) may be able to be used as an aid in human risk assessment.

We believe that a major area of public health can best be attacked by prevention: identifying environmental mutagens/carcinogens, making a rough estimate of human risk based on potency and amount of human exposure, and minimizing human exposure to the more dangerous of these agents. It seems likely that we will soon know how dearly we will have to pay in increased cancer and birth defects for the modern world of industrial chemicals, pesticides, food additives, and plastics. It appears likely, however, that the new methods and discoveries from basic biology that have been developed over the last decade and that are being developed at present will help in making future decisions more rational.

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WORKSHOP SUMMARY: Chemical Damage and Mutagenesis in Mammalian Systems

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From the diverse contributions of the six speakers at this Workshop three major themes can be resolved. The first concerns the possibility of the existence of different repair modes for different classes of DNA damage in mammalian cells. Both Strauss and Roberts produced biochemical evidence that suggests that cells may have available a number of alternative repair pathways to modify a specific type of damage. Thus it is possible to produce evidence that the excision repair processes which handle the arylalkyl residues may be discriminated from those which modify thymine dimers produced by UV irradiation (Dipple and Roberts, 1977). Although chemical adducts can be excised they may also be bypassed by the replication machinery, the mechanism of bypass probably involves branch migration (Higgins, Kato and Strauss, 1976). The consequences to the cell of the continued existence of adducts is not known but they may be diluted out of the system if replication can continue, in addition they are still available as substrates for excision repair.

Support, at the biological level, for the existence of differential repair modes was provided by Maher and Arlett. Maher showed that defects in excision repair, particularly in xeroderma pigmentosum (XP) cells are very serious for the lethal response when cells are treated with UV or chemicals which produce 'UV-like' distortion in the DNA. Indeed, the more severe the defects in excision the more severe are the effects on cell killing or mutation. Other chemicals which do not produce 'UV-like' damage show no differential killing effects in XP cells. These data indicate a specificity of recognition of the lesion by a particular repair process. These observations were complemented by those of Arlett who showed that by exposing an array of human mutants with specific sensitivities to DNA damaging agents little cross sensitivity was observed. Further, the human DNA damage-sensitive syndromes can be used to identify the mode of action of other agents (Arlett, 1977).

A second major theme was concerned with the complexities of the technology involved in performing mutation studies with cultured mammalian cells. Fox illustrated the difficulties which are encountered in these experiments by showing that the shape of the mutation dose response curves, which are vital for the proper evaluation of the data, are critically dependent upon experimental design. The linear dose-response curves produced by the so-called replating technique (Fox and