Chapter 4

The State-of-the-Art of Genetic Monitoring

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Chapter 4
The State-of-the-Art of Genetic Monitoring

With the advent of molecular biology, the field of human genetics has undergone an extraordinary metamorphosis. Progress in molecular biology and human genetics clearly has transformed society on many levels—medical, social, economic, legal, and ethical. Because biotechniques are having such profound and practical impacts on daily living, it is important to appreciate the nature of the technologies in order to understand potential applications, such as genetic monitoring and screening by employers. The techniques used in genetic monitoring v. genetic screening for the most part are distinct, although the two areas tend to merge in the detection and diagnosis of cancer.

The term "genetic disease" is used broadly in this and the following chapter, referring to those conditions for which the major causative factor is genetic. There are over 3,000 diseases known to be caused by a single-gene defect and chromosomal anomalies are found in over 1 in 700 live births (44). In addition, research has demonstrated that genetic viability affects many aspects of health, ranging from heart disease to cancer (2,19). It has long been speculated that genetically determined variation in susceptibility may predispose some workers to occupational disease while others in the same environment seem to be unaffected (26,52,65). Additionally, certain environmental agents are known to mutate previously normal somatic cells that could, in some cases, cause disease.

Recognition of genetic factors in disease presents new opportunities for detection, prevention, and treatment. Because of uncertainties about the exact nature of the relationship between genes and environment, genetic monitoring and screening of otherwise healthy populations remain problematic.

Medical screening in the workplace involves evaluating employees before they begin work. It can range from a cursory questionnaire to an oral history to a full preemployment physical. (See chs. 3 and 9 for industry practice.) Genetic screening is a process that considers attributes or indices of altered DNA that may put an employee at high risk for developing disease, whether work-related or not. An extensive discussion of the state-of-the-art in genetic screening for inherited disorders appears in chapter 5. Monitoring, on the other hand, involves the periodic evaluation of employees for either the effects of a toxic substance or its byproducts (60). Genetic monitoring evaluates the genetic damage caused by such substances. In short, genetic monitoring ascertains whether an individual's genetic material has altered over time. Basic human genetics information necessary to understanding this chapter is contained in appendix A. Several documents have presented background material on human genetics and the techniques often used in this field (68,69,70,71, 72,73). The state-of-the-art in genetic monitoring, methodological and reliability issues in monitoring, and the interpretive value of monitoring are also discussed.

MUTATION AND HEALTH EFFECTS

Over the past 15 years an increasing number of health effects have been attributed to mutations caused by toxic agents (26). These mutational effects occur at a rate significantly above the normal background rate found in human cells. The relationships between genes, mutations, and disease are becoming clearer with the development of molecular techniques that enhance both the quantitative and qualitative evaluation of mutation.

The diseases most associated with genotoxic substances are various forms of cancer. Several types of mutational changes (i.e., point mutations, chromosomal rearrangements) have been associated with the early stage of tumor development, as well as with the following steps of tumor promotion and progression (36).

The emphasis on the relationships between genotoxins and cancer may be due to the fact that most studies have focused on somatic cell changes and because germline effects may take generations to appear. Genetic effects on human germ cells are imprecise. More research is needed. Most validation efforts undertaken in genetic monitoring have been designed to quantify the correlation of mutagenesis with carcinogenesis (46). Thus, genetic monitoring involves, for the most part, search for mutations in the somatic cells.

There are two classes of genotoxic agents commonly found in the workplace--chemicals and radiation. The differences between these agents are described below.

Mutagenic Effects of Radiation v. Chemicals

It has been documented for over 40 years that radiation at high doses causes significant carcinogenic and genetic effects. Less clear and certainly more controversial are the effects of low-level doses. The effects of radiation on chromosomes are described in the 1983 Office of Technology Assessment (OTA) report (72) and will not be discussed again here. However, two new topics are worth examination. The first is the extent to which research findings have affected the setting of limits for exposure for chemicals v. radiation. Second is the continuing debate about the effects of low-level radiation.

The finding that ionizing radiation induces chromosomal aberrations (CAs) may help elucidate the means by which certain chemicals alter DNA. Radiation-induced damage can be observed in cells within a few hours following exposure. In general, chemically induced lesions, however, are not converted into aberrations until the cells containing them undergo DNA replication (19). Meanwhile, some chemically induced damage may be repaired long before replication. Because of the lack of good baseline information on chromosomal damage as an effect of chemical mutagens, enthusiasm differs on recommending cytogenetic surveillance for exposed individuals. This has led some to assert that chemical exposures should be evaluated differently from radiation exposures (24).

For example, groups such as the International Commission on Radiological Protection use different rationales in setting limits for exposures to radiation than does the American Conference of Governmental Industrial Hygienists, which sets standards for chemical exposures. There are basic scientific and philosophical differences underlying these discrepancies (24).

External radiation dosimetry can be done on a continuous basis, providing a cumulative dose reading as well as the possibility of periodic readings. Dosimeters of this type are not available for chemical exposures. Thus, relation of dose to genetic monitoring results is much more difficult with chemicals (39).

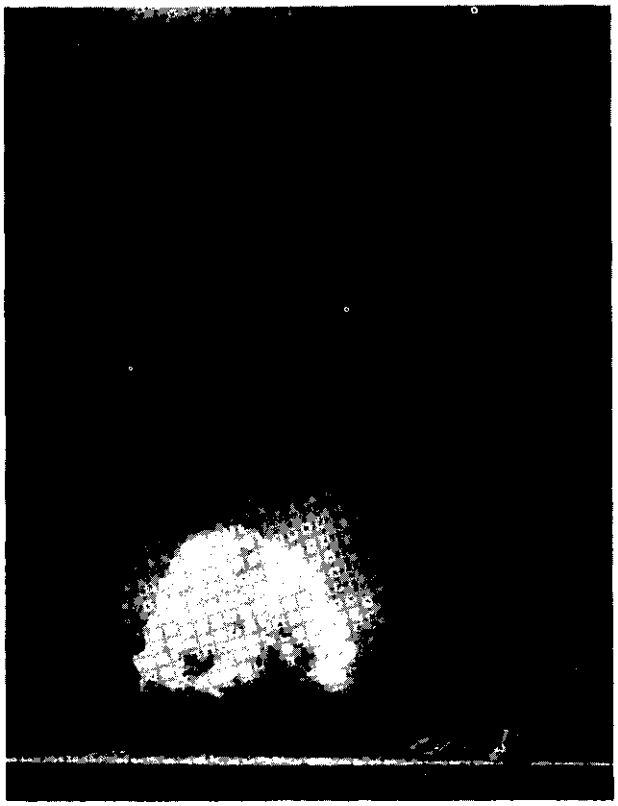
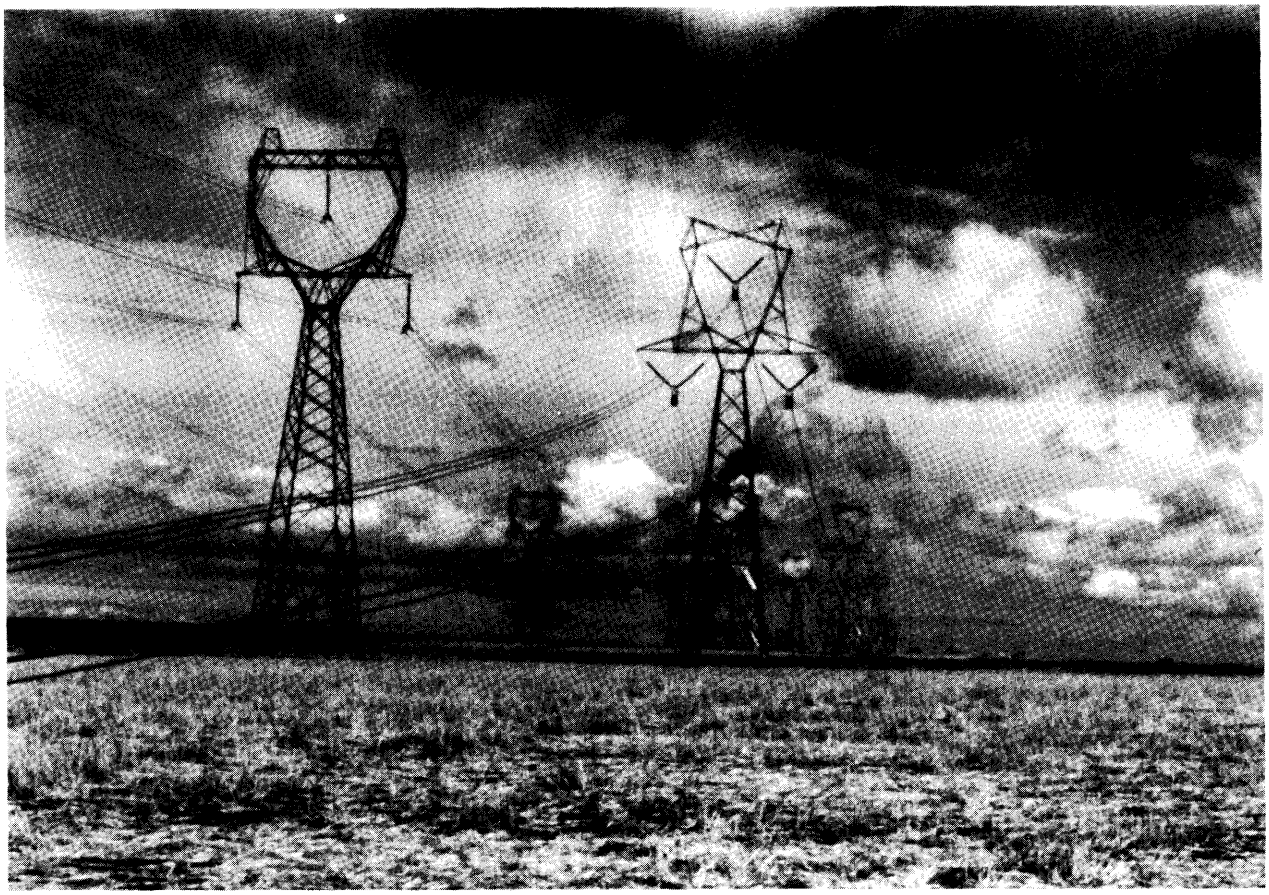


Photo credit: Los Alamos National Laboratory Records Center/Archives

The first atomic bomb detonation—July 16, 1945.

More data exist on the genetic and health effects of radiation, based largely on studies of Japanese atomic bomb survivors, as well as biological experimentation. The data on radiation have been collected over several generations and have led to the consideration of radiation as a somatic and germinal genotoxic agent. Chemical standards, on the other hand, are based on far fewer data, and tend to consider acute, rather than long-term and germinal effects of exposures. Such analysis has led, in many cases, to differences in evaluating exposures.

Internal biological doses are often determined for workers exposed to radiation whereas most limits for workplace chemicals are established in terms of airborne concentration or external exposures (24). Finally, radiation standards assume that biological damage caused by low doses of radiation is cumulative and is not repaired as rapidly as damage caused by chemicals, whereas standards set for chemical exposure are based on the assumption that biological damage caused by exposures to low doses is not cumulative and is frequently repaired. While the



credit: Casazza Schultz ocia

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effects of low-level, long-term exposure to chemicals are hardly understood, the debate in this area relative to ionizing radiation still rages.

Until the health effects of radiation and chemical exposures are better understood, genetic and biological monitoring of exposed populations can only provide a gross indication of health risks. Health effects may not appear for as many as 30 years following initial exposure.

Low-Level Radiation

In the past few years, science has offered sharply conflicting opinions about the dangers of low levels of radioactivity. This divergence is due, in part, to the different assessments of radiation doses received by those studied, and to insufficient understanding about how small doses of radiation increase cancer risk (55).

The debate has been rekindled, in part, because of the nuclear accidents at Three Mile Island and Chernobyl, and current concerns about the effects of radon (74). Concern about the carcinogenic effects of radiation in employees of the nuclear weapons and nuclear power industries has also fueled the debate (14,55). And, most recently, an association between low-dose exposure to radiation and leukemia in the offspring of men employed in a nuclear facility was reported (20).

In a study of mortality among workers at a nuclear fuels production facility, the rate of cancer was found to be normal or below normal except for leukemia (16). This, and similar studies, have led some to the conclusion that very low doses of ionizing radiation are not harmful after all, or might even have net benefits, a phenomenon called "hormesis" (57). This net benefit is attributed to an overprotective response involving enhanced DNA

Table 4-1—Major DNA Lesions Produced by Chemical Interaction and Their Genotoxic Consequences

Primary lesion	Description	Consequence
Alkylation	Covalent adduct formed, involving the genotoxic agent and a DNA base or phosphodiester bridge	Alteration of base pairing, loss of the base, stimulation of error-prone repair
Intercalation	Noncovalent stacking of the genotoxic agent between adjacent base pairs in the DNA helix	Alteration of DNA transcription, replication, or repair
Cross-linkage	Formation of two covalent bonds between bases within (intrastrand) or between (interstrand) DNA strands	Dimer formation, alteration of replication
Breakage	Scission of either a single or both strands of the DNA helix	DNA rearrangements forming chromosomal aberrations after mitotic cell division

SOURCE: R.W. Hart and D. Brusick, "Assessment of the Hazard of Genetic Toxicity," *Toxic Substances and Human Risk,* R.G. Tardiff and J.V. Rodricks (eds.) (New York, NY: Plenum Press, 1987).

repair of arising mutations that more than compensates for the harmful effects of radiation.

Critics of this theory argue that radiation-induced mutations have not been proven to be beneficial (in fact, the preponderance of evidence is quite the opposite (79)). Although an adaptive response was detected after exposure to very low doses of ionizing radiation, the protective effects remain to be determined (35). Also, studies reporting no increase in cancer after radiation exposure have not waited long enough before drawing conclusions. Leukemias typically start to appear about 2 years after a dose of radiation, compared with about 15 years for other cancers. Forty years after Hiroshima and Nagasaki, most cases of leukemia have already occurred, whereas other cancers are still being reported (55). Thus, studies that report "only" an increase in leukemias could be reporting the beginning of the trend toward more cancer reporting years away.

In late 1989, a panel of the National Research Council (NRC) concluded that exposure to low levels of radiation, such as that from x-rays or radon, is at least three to four times more likely to cause fatal cancer than is commonly believed. This dramatic about-face from previous NRC reports is due to a reevaluation of dose data from atomic bomb populations. Reconstructions of the original bomb designs revealed much lower radiation doses than originally thought. Also, as the surviving population has aged, more fatal cancers have developed than expected, including cancers of the breast, lung, stomach, ovary, throat, colon, and bladder, as well as leukemia, the standard "canary in the mine" (43).

The effect of the NRC conclusions is to at least quadruple estimates of the number of radiation deaths expected among workers in the nuclear power and nuclear weapons industries, those who frequently undergo radiation therapy and x-rays for diagnosis, and those who are routinely exposed to radioactive elements in certain natural gases, building materials, or tobacco. These revised estimates, however, do not change the difficulty or impossibility of doing definitive epidemiological studies of low-level radiation effects (63).

TECHNOLOGIES FOR GENETIC MONITORING

In simple terms, a mutagen is a substance capable of inducing a heritable change in the genetic material of cells. The changes can be detected at the molecular or chromosomal level through measurement of sister chromatid exchange (SCE), unscheduled DNA synthesis, point mutations, CAs, formation of DNA adducts, and oncogene activation, described in this section. Much progress has been made in measuring these endpoints and understanding the role of these processes in the induction of mutagenesis. Table 4-1 summarizes the major DNA lesions produced by genotoxic substances. In many cases, mutagens are also carcinogens, so at high exposure levels, the most common manifestation of genetic damage is in the form of cancer (75). Box 4-A describes some of the connections between genetic damage and cancer.

Exposure to genetically toxic agents initiates a process which is illustrated in figure 4-1. The damage will be resolved in one of three ways: cell death, successful DNA repair, or viable mutation. It is difficult to establish the causal relationships between the mutation and cancer because of the long latency of human cancer. Nonetheless, the rationale behind the use of genetic damage assays as indicators of exposure is that events observed initially and at high frequencies are the start of a process that may ultimately produce abnormal growth (neoplastic changes) in a smaller subset of cells.

Box 4-A--Genetics and Cancer

Cancer is a genetic disease arising from genetic damage of diverse sorts-recessive and dominant mutations, large rearrangements of DNA, and point mutations-all leading to distortions of either the expression or biochemical function of genes. The growing field of cancer genetics aims to uncover the genetic alterations responsible for uncontrolled growth of cancer cells. Many types of human cancer occur in familial as well as sporadic forms, Discrete genetic changes have been associated with different types of neoplasm, and are thought to initiate or cause progression of cancer. Chromosome studies in more than 10,000 cases of neoplasms have reported specific anomalies. The identification of genetic changes, therefore, presents the major diagnostic challenge in cancer.

Both dominant and recessive forms of cancer have been found. The genetics of the common cancers-breast, colon, and lung—are beginning to fit a pattern. Approximately 5 percent of cancer cases constitute a hereditary cancer syndrome in which a dominant gene predisposes to cancers of the breast, ovary, brain, gastrointestinal system, and white blood cell precursors. These are referred to as 'cancer families' (see ch. 5 for further discussion). Apart from these, each type of cancer appears to have a small group of cases that fits the pattern of a major predisposing gene and a much larger group that seems to be largely environmental in origin.

In addition, examples such as xeroderma pigmentosa imply that there is a connection between susceptibility to cancer and impaired ability of cells to repair damaged DNA. It is a reasonable expectation that if cancer is related to alterations in somatic cell genes, then the rate at which those changes occur could serve as a barometer of changes in the gem-dine that may not be expressed for many generations to come.

Certain cancers, e.g., lung, laryngeal, bladder, and testicular, have repeatedly been linked to environmental exposures. The effects on chromosomes of such chemicals as arsenic, asbestos, chromium, nickel, and vinyl chloride are well-documented. Substances that cause chromosomal abnormalities are called ''clastogens. The reader is referred to the 1983 Office of Technology Assessment report for more detail on the specific effects of those agents. Chapter 5 describes recent advances in detecting predisposition to some common cancers.

SOURCES: Office of Technology Assessment, 1990, based on J.M. Bishop, "he Molecular Genetics of Cancer," *Science* 235:305-311, 1987; F. Mitelman and J.D. Rowley, ''Genes, Chromosomes and Cancer: A New Forum for Research in Cancer Genetics," Genes, *Chromosomes & Cancer 1:1-2, 1989;* J.J. Nom and F.C. Fraser, *Medical Genetics: Principles and Practice* **(Philadelphia%** PA: Lea & Febiger, 1989); R. Parshad, K.K. Sanford,, K.H. Kraemer, et al., ''Carrier Detection in Xeroderma Pigmentosum," *The Journal of Clinical Investigation 85: 135-138,* 1990.

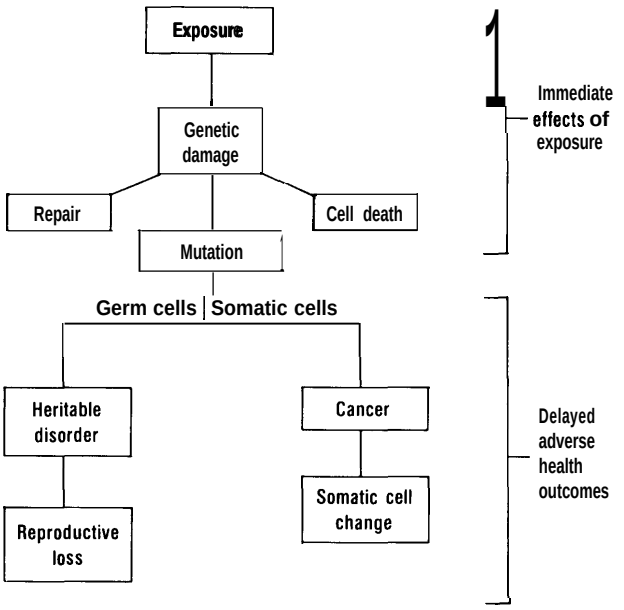
Thus, the purpose of monitoring tests is to detect biologically significant exposures **early,** even though the results are currently unsuitable as a basis of quantitative risk assessment. Advances in testing at the molecular level (discussed below) will most likely provide better predictors of neoplasia, as the relationships between mutation and neoplasia become better understood.

Environmental agents can increase the risk of genetic disease and cancer in exposed populations. Humans are exposed to over 25,000 toxic compounds that are potentially or demonstrably mutagenic in lower organisms (44). The fundamental problem of evaluating genetic risk from environmental exposures rests with the ability to identify a chemical as a somatic or germ cell mutagen in humans (67). Because this cannot be done ethically or legally in humans, most studies rely on animal models. Problems arise in trying to extrapolate from animal studies to human populations because of genetic differences and dose-response relationships. Yet, the whole process of making carcinogenic risk estimates is based on the assumption that there is a qualitative and quantitative correlation between the results of animal mutagenicity and carcinogenicity tests and expected effects in humans. Most Federal and State regulations are based on this premise (17): that is, if mutagenic activity is observed for a chemical, even in bacteria, it is possible that it or its metabolizes could be carcinogenic.

Reduction of risk requires, among other things, sensitive methods for detecting harmful agents. Mutagenesis can be measured in many ways, the most conventional methods are cytogenetic and biochemical. Molecular methods, however, are increasingly being developed and will shed further light on the nature of mutagenesis and its relationship to carcinogenesis (see also ch. 5).

A previous OTA report discusses in greater detail technologies for detecting heritable mutations (73). The reader is referred to that publication for elabora-

Figure 4-1—Biological Consequences of Exposure to Mutagenic Agents



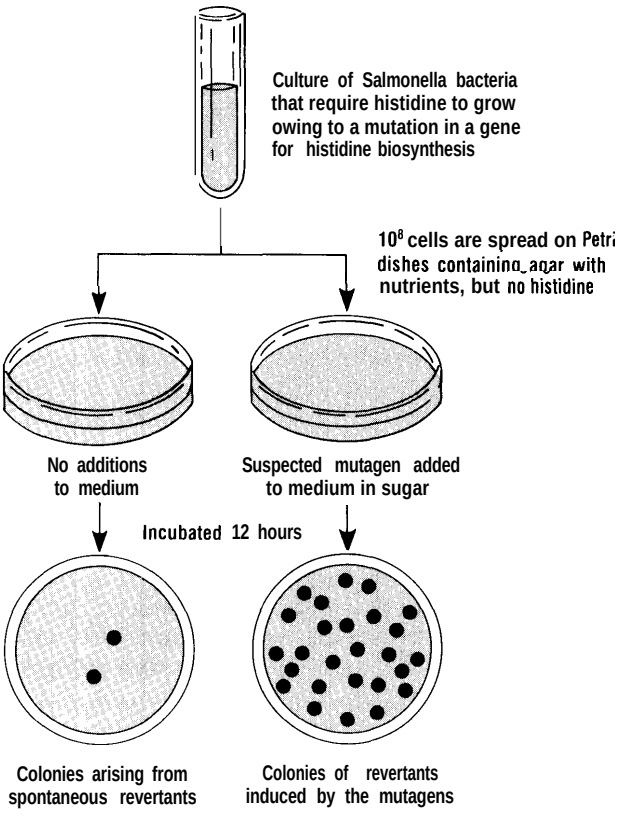
SOURCE: Office of Technology Assessment, adapted from J.B. Ward, "Issues in Monitoring Population Exposures," *Carcinogens and Mutagens in the Environment Volume II, The* Workplace, Hans F. Stich (cd.) (Boca Raton, FL: CRC Press, 19S5).

tion of techniques and methodological considerations relevant to tests for mutagenicity. Other than a brief discussion of biological monitoring, the remainder of this section will address tests used specifically for monitoring occupational populations for genotoxic effects. The focus is on detection of genetic changes, not just the presence of chemicals, in workers.

Tests of Mutagenicity

The more traditional approach to testing for exposure to mutagens has been to measure the chemical itself (or a byproduct) in blood, breath, and urine. Mutagenic activity in urine can be shown by using rapid screening tests developed for bacterial or in vitro cell culture systems. Standard analysis of body fluids for the presence of mutagens was discussed in greater detail in the 1983 OTA report and will not be covered further here. The most common short-term test for mutagenicity conducted on body fluids is the Ames/Salmonella test (see figure 4-2). Because the specificity of the procedure has come under fire in recent years it is discussed in detail in box 4-B.

Figure 4-2—The Ames Test



The Ames testis used to determine whether a chemical can cause mutations in bacteria Mutant bacteria that have lost the ability to synthesize the amino acid histidine and cannot grow in its absence are treated with the test chemical. Potent mutagens can cause mutations that reverse the hismutation, resulting in bacteria that can grow in the absence of histidine.

SOURCE: Office of Technology Assesment, 1990.

Testing for the presence of mutagens in blood and urine is more suited to occupational settings as a complement to ambient measurements, thereby providing an indicator of exposure and absorption. The presence of mutagens only serves as an indicator of recent exposure and provides no information regarding the health effect on the individual.

Biological monitoring, therefore, involves examining the worker for absorption of a toxic substance or its byproduct as an indicator of internal dose. Most work to date has focused on the relationship between internal dose and external exposure, rather than between internal dose and adverse effects (58). Detection of mutagens in urine has been reported in several types of workers including oncology nurses and pharmacists involved in preparing and administering cancer chemotherapeutic drugs (65).

Box 4-B—The Ames Test

Most current studies of **mutagenesis are** based on the pioneering work of Ames et al. The general procedure involves treating a bacterial cell population containing a designated genetic marker with a mutagen. The mutagen kills off a fraction of the cell population with survivors growing back into a larger population. Within this survivor population, a fraction of the cells will have lost the marker. This fraction, expressed as a percentage, is taken as a measure of the mutagenic action suffered by the original population. Since about ***85*** percent of compounds known to be carcinogenic in rodents arc also mutagenic in the Ames test, some have suggested that the Ames test is a belter indirect test of carcinogenicity than a direct test of mutagenicity. In fact, because of the correlation between mutagenicity and carcinogenicity, some statutes, such as the Toxic Substances Control Act (Public Law 94-469), require chemical manufacturers to demonstrate negative mutagenicity of a chemical via the Ames test as a substitute for long-term, more expensive bioassays for carcinogenicity.

The limitations of the Ames test, however, are many. First, only mutations in viable cells are scored. Those m cells killed by the agent are not measurable. While such mutations could be lethal in the particular chromosome containing the marker gene, similar mutations at loci on other chromosomes could produce viable but genetically damaged cells. Furthermore, mutants often possess reduced rates of cell multiplication. Thus, the fraction of mutated cells in the test population will be materially decreased from the original value.

The Ames test also fails to measure large mutations such as deletions, because such lesions have a high probability of extending into vital genes on the marker chromosome and causing the death of the cell. Large mutations are known to be extremely important in the activation of oncogenes and in the induction of genetic disease.

In addition, critics argue that the Ames testis not specific, as large doses of mutagenic agent are required before significant measurements can be made. The low specificity produces a high false positive rate and a less than desirable predictive value. Finally, in order to calculate mutagenic effects for low dosages, it is necessary to resort to extrapolation over a large dosage interval. Whether this extrapolation should be linear or based on a threshold region has been widely debated. So far, regulatory agencies have favored the threshold hypothesis which postulates that there are low dosages with no mutagenic effect.

Efforts have been made to correct for these deficiencies, including use of a plasmid unnecessary for reproduction as the carrier of the marker, use of several markers, and use of lower dosages on a more variable cell population. Some feel that the current reliance on the Ames test and its requisite extrapolation may underestimate the health effects of low doses of some mutagens.

A method by which to recover and analyze the mutated genes could facilitate the molecular analysis of mutagenesis in intact organisms as well as in cultured cells. This approach uses chromosomally integrated shuttle vector genes that are integrated into the mammalian cell's chromosomes and replicated in synchrony with the chromosomal DNA. Pure clones of mammalian cells containing the mutant genes can then be isolated, recovered, and sequenced.

SOURCES: Office of Technology **Assessment,** 1990, based on B.N. Ames, J. McCann, and E. Yamasaki, "Methods **for Detecting Carcinogens and Mutagens** *with the Salmonella/Mammalian-Microsome Mutagenicity* Test" Mutation *Research 31(6):347-364, 1975; RI...* Davidson and C.R. Ashman, "Chromosomally Integrated Shuttle Vectors and Molecular Analysis of **Mutagenesis in Mammalian Cells," Somatic Cell** *and Molecular Genetics 13(4):415-417, 1987;* J.J. Nom and F.C. Fraser, Medical Genetics: *Principles and Practice (Philadelphia, PA: Lea & Febiger,* **1989);** T.T. Puck and C.A. Waldren, "Mutation in *Mammalian* Cells: Theory and Implications,"Somatic Celland ***Molecular*** Genetics 13(4):405-409, 1987; R.W. Tennant, B.H. Margolin, M.D. *Shelby, et al.,* "*Prediction* **of Cbemical Carcinogenicity in Rodents From In** Vitro Genetic Toxicity Assays," Science 236:933-941, 1987; C.A. Waldren and T.T. Puck, "Steps Toward Experimental Measurement of Total Mutations Relevant to Human Disease,' Somatic Cell *and Molecular Genetics* **13(4):411-414, 1987.**

Biological monitoring techniques are frequently used for chemicals known to have adverse health effects, as well as mutagenic effects. For example, the recognition of the neurotoxic and narcotic properties of toluene--a product of crude oil used as a solvent in oils, resins, rubber, and paints, and as a basic material in many synthetic chemicals-has lead to the development of biological monitoring methods for assessing toluene uptake. Short-term exposure to high concentrations of toluene can cause drowsiness, dizziness, and headaches. Breath, blood, and urine tests can be used to check and control levels of exposures (11).

Studies of Effects on Sperm

Traditionally, most studies of chromosomal abnormalities are performed on cultured white blood cells. But to assess the effect of mutagens on reproduction, analysis must be done on germ cells. Knowledge about adverse effects of toxic exposures on reproduction is limited, but some credible associations have emerged.

The potential for occupational exposure to have an adverse effect on sperm was shown when workers exposed to dibromochloropropane had markedly reduced sperm counts and a decrease in number of offspring (34,78). Sperm count can be affected by a multitude of factors, so direct causal relationships between decreased counts and particular exposures are difficult to establish. Some studies, however, have shown that certain physical abnormalities of sperm are produced by environmental exposure, such as atypical shape, nondisjunction of the Y chromosome, and abnormal motility (54).

Abnormal sperm morphology has been associated with exposure to lead and carbaryl (32,80). Adriamycin, a cancer drug effective against a broad spectrum of neoplasias, has been shown to cause reduced sperm count and increased CAs in mouse germinal cells (3). Solvents such as ethylene glycol ethers, pesticides such as ethylene dibromide, metals such as mercury and arsenic, and alkylating agents such as ethylene oxide, have demonstrated spermatotoxic effects in animals (18).

Cytogenetic Indicators

Results from extensive animal and human studies show an empirical association between chromosomal damage and mutagenic-carcinogenic agents. CAs and SCEs are the principal cytogenetic indicators used to estimate exposures to carcinogens. The efficiency of these indicators can potentially be improved by the application of developing computer image analysis for the scoring of CAs (35). It has not been determined whether these indicators of exposure are predictors of disease risk except as a diagnostic tool for some tumors, so the clinical significance for individual workers is unclear.

Studies of some occupational exposures reveal associations between exposures and chromosomal effects (12,65). The results of cytogenetic techniques that use blood cultures to study the in vivo response of people exposed to mutagens are compelling but inconclusive. The main conceptual basis for the application of cytogenetic tests to measure chromosomal damage is that damage to the genetic material of cells represents initial events in a process that may eventually lead to disease. Cytogenetic methods can detect human exposures at biologically significant levels in populations, but the interpretation of findings for the individual remain uncertain.

Detectable mutations result from gross changes in chromosome structure and can be visualized under the microscope. The disruptive effects of mutagens on chromosome structure, organization, and behavior have long been studied by geneticists. The relationship between CAs, spontaneous abortions, and birth defects is well-documented. But, the connections between chromosomal damage and disease are unclear except in a small number of cancer cases. Again, cancer is the disease most commonly hypothesized to be associated with induced CAs because of their presence in lymphoproliferative disorders such as leukemia, and in solid tumors (62). Most analysts agree that interpretation of cytogenetic results at the individual level is questionable and recommend that until the relationship between cytogenetic damage and disease is better understood, interpretation should be maintained at the population level. In addition, cytogenetic monitoring of human populations is expensive and time-consuming (12).

Chromosomal Aberrations

One of the few direct methods for measuring gross changes in DNA involves visualization of the chromosomes through the light microscope. The viewer might see overt breakage and rearrangement of the chromosomes within the cell as well as more subtle changes involving the exchange of material between chromatics of a chromosome. The type of

alteration produced by physical and chemical agents depends on the lesions induced in the DNA and, therefore, on the chemical structure of the genotoxic substance (12).

CAs are usually induced by agents that can directly break the DNA duplex such as different types of radiation chemicals that imitate the effects of radiation. CAs therefore serve as a biological dosimeter in individuals exposed to ionizing radiation. The same is not true for cases of chemical exposure, however, since most chromosome-breaking (clastogenic) chemicals require metabolic activation and are dependent on a critical time in DNA replication. CAs have been demonstrated for a large number of chemicals in vitro, but relatively few chemicals have been convincingly shown to increase CAs in vivo (15). On the other hand, some investigators have reported that in vivo cytogenetic assay is a very accurate assay system to identify carcinogens from non-carcinogens (4).

For chemical exposures, chromosome analysis is, for the most part, a low sensitivity method. This stems from the low frequency of CAs, thereby requiring that large numbers of individuals and cells be studied to detect a statistically significant increase in CAs. Detecting effects at low exposure levels or in small groups is not informative (15). Application of this method to ionizing radiation, on the other hand, is well-established. It continues to be applied routinely to all suspected cases of radiation exposure in several countries, most notably by the National Radiological Protection Board in the United Kingdom (7).

Recently, two studies have demonstrated that cancer developed more frequently among individuals having CAs (22,64). These data suggest a direct relationships between CAs and development of disease.

Sister Chromatid Exchange

The study of SCEs is an indirect indicator of mutation, although the biological significance is unknown. Unlike CA measurements, SCE can be a sensitive marker for the measurement of DNA damage and repair (76). Sister chromatics are the two daughter strands of a duplicated chromosome. SCEs are events that occur when apparently equivalent sections of the sister chromatics of the same chromosome are exchanged during cell division (mitosis). SCEs occur in cells at a normal rate, but

Photo credit: U.S. Council for Energy Awareness

A Nuclear Power Worker: Controversy continues regarding the carcinogenic effects of radiation in employees of the nuclear weapons and nuclear power industries.

appear to be elevated when exposed to agents that damage DNA. Of importance from a practical standpoint, SCEs appear to result only as an effect of chemical mutagens, not radiation. They are most efficiently induced by substances that form covalent adducts to the DNA, distort the DNA helix, or interfere with DNA precursor metabolism or repair (33).

Detecting SCEs in peripheral blood lymphocytes is one way of monitoring chemically induced chromosomal damage and is less costly than tests of CAs because SCEs are easily scored. Because CA and SCE represent different types of genetic damage, however, it would be misleading to replace one assay with the other.

SCE analysis has the potential for being useful in both screening and monitoring, because in addition to the tendency toward increased SCE as a result of exposure to genotoxic chemicals, certain inherited conditions demonstrate increased SCE (13). On the other hand, caution must be taken to protect against confounding factors such as cigarette smoking, alcohol consumption, drug intake, chemotherapy, infections, and vaccination, as all have been shown to induce SCEs.

As mentioned previously, chemically induced lesions are often repaired and therefore would not show up in the SCE assay. The frequencies of SCEs, therefore, can fall off rapidly with time after an acute exposure, and the time at which the SCEs are scored becomes a confounding variable in interpretation. Nevertheless, elevated SCE frequencies may pro-

vide a good indication of prior exposure to chemical mutagens. Although, because the effect of chemicals on induction of SCEs varies, calibration curves have to be derived for each agent for SCEs to be quantitative predictors of mutation induction (13).

To date, many studies of the effects of occupational chemicals on SCE frequencies have been conducted; often with contradictory results (76). The contradictions could be due to unidentified confounding factors related to lifestyle of those tested. Some evidence also exists that SCE frequencies do not necessarily increase with level of exposure. At relatively low levels, certain chemicals, such as benzene, mainly affect DNA repair at the replication point, inhibiting, rather than inducing the formation of SCEs (76). Thus, without accounting accurately for exposure levels, separate studies could yield conflicting results. Finally, for a given exposure, it is not known whether higher or lower frequencies of SCEs is better, i.e., a sign of damage or a sign of repair (47).

At present, many known carcinogens produce SCEs, but no systematic sampling of chemical agents has been conducted to determine whether correlations for certain chemicals are truly predictive of health risk (66).

Micronuclei Assay

One consequence of the induction of CAs is the formation of micronuclei, which result from the exclusion of fragments of/or whole chromosomes from nuclei formed at mitosis. The presence of micronuclei can be taken as an indication of the previous existence of CAs. Micronuclei are far easier to score than CAs at metaphase (although less frequent) and provide a simple means for estimating induced genetic damage. In addition, micronuclei persist for varying lengths of time after their formation so they can be detected in nondividing descendants of cells. Early studies of the effects of ionizing radiation on mitosis showed that the frequency of micronuclei was dependent on radiation dose (19).

One of the most dramatic presentations of micronuclei has been demonstrated in worker populations exposed to cytostatic drugs such as cyclophosphamide, a chemotherapeutic agent. Increased numbers of micronuclei were observed in lymphocytes of groups of workers from industry and hospitals where the drug is processed and administered (81).

Table 4-2—Main Confounders and Limitations of Occupational Cytogenetic Studies and Ways To Control Them

	Control efforts
Confounders	
Exposure conditions:	
Identification of correct chemical exposure	Factory record checking
Estimate of dose of exposure	Industrial hygiene survey
Individual variations:	
Genetic factors	Unknown before analysis
Lifestyle factors	Match with controls
Health factors	Check medical records
Limitations	
Culture conditions:	
Culture time	CAs: First division metaphrases SCEs: Second division metaphases
Culture medium and chemicals	Keep constant
Time between sampling and culture	Keep constant
Persistence of mutagens in the blood sample	In vitro experimentation
Analysis and scoring:	
Scorer variation	Coded slides, one scorer
Interpretation of damage scored	Strict scoring criteria

SOURCE: M. Sorsa and J.W. Yager, "Cytogenetic Surveillance of Occupational Exposures," *Cytogenetics*, G, Obe and A. Basler (eds.) (Berlin, West Germany: Springer Verlag, 1987).

Limitations of Cytogenetic Tests

In cytogenetic studies, at least two major types of technical variations exist. The first includes factors associated with differences in slide reading, culture conditions, and concentrations of test chemicals. The second involves sampling times and differences in cell populations being tested (65).

Test Limitations

Scoring, or counting of the cells, is also an extremely important element in cytogenetic toxicology. Slides must be randomized and coded to avoid scorer bias. Accurate results depend on slides prepared at a specific time during the analysis to ensure that the proper time in the lifecycle of the cell is reached. Consistent scoring criteria and statistical analyses must be maintained to obtain reliable and valid results. Table 4-2 summarizes some of the major limitations and confounders of occupational cytogenetic studies.

Test Interpretation

Baseline data for the effects of various chemicals on SCEs and micronuclei formation are inadequate. In particular, quantitative data on the normal back-

ground frequencies of micronuclei are unavailable, making it difficult to set standards for exposed populations. Theoretically any increase detected between preemployment data and post-exposure data would suggest that exposures are too high.

Micronuclei are associated with increasing age and smoking (29). All measurements must establish a background level of alterations that is seldom, if ever, zero. The background incidence of all genetic events varies with time and between individuals. To date, there is no international standard for the conduct of human cytogenetic surveillance studies, although guidelines have been developed by the International Commission for Protection Against Environmental Mutagens and Carcinogens (31). The U.S. Environmental Protection Agency has also provided guidelines for cytogenetic evaluations (51).

Application of Cytogenetic Tests to Occupational Exposures

Elevated cytogenetic abnormalities of the three types previously described may be associated with occupational exposures to ionizing radiation or some chemicals, particularly where long-lived alterations are involved. The nature and longevity of the alterations vary from one agent to another. For some chemicals such as benzene, the alterations may persist for years and probably represent a cumulative exposure. For others, such as vinyl chloride, the alterations disappear quickly after reduction of exposure; thus cytogenetic assays can monitor exposure only over a short period of time.

Over 100 cytogenetic studies have been reported from various occupational exposure groups (65). Among the occupational chemicals with best documented positive cytogenetic tests are ethylene oxide, styrene, benzene, and alkylating anticancer agents. Occupational cytogenetic studies of arsenic, benzene, epichlorohydrin, ethylene oxide, lead, cadmium, zinc, and vinyl chloride were described in the 1983 OTA report. They are not discussed again here, but summaries of those findings appear in table 4-3, which lists the most common occupational exposures that induce cytogenetic abnormalities.

Since the 1983 OTA report, increased CAs have been reported in individuals exposed to phosphine, a common grain fumigant (21), and a range of pesticides, including organophosphorous, organochlorinated, and carbamate groups (56). Animal studies have demonstrated elevated SCE and CA frequencies in rat cells exposed to a common household insecticide known as DDVP (37).

Table 4-3-Occupational Hazards Reported To Increase the Frequency of Cytogenetic Abnormalities

Alkylating anticancer agents (CA, SCE)	DDT (CA)
Arsenic and arsenic compounds (CA)	Epichlorohydrin (CA)
Asbestos (CA, SCE)	Ethylene oxide (CA, SCE)
Benzene (CA)	Mineral oils (CA)
Benzidine (CA)	Nickel refining (CA)
Bis(chloromethyl)ether (CA)	Organophosphorous insecticides (CA, SCE)
Cadmium/lead/zinc (CA)	Pentachlorophenol (CA,SCE)
Chromium (CA, SCE)	Rubber industry (CA, SCE)
Coal gasification (CA)	Shale oils (SCE)
Coal tars (CA)	Styrene (CA)
Coke production (SCE)	Sulphite (wood pulp) (CA)
Diesel fumes (CA)	Trichloroethylene (CA, SCE)
Dimethylformamide (CA)	Vinyl chloride (CA, SCE)

KEY: CA = chromosomal aberrations; SCE = sister chromatid exchanges.
SOURCES: J. Ashby and C.R. Richardson, 'Tabulation and Assessment of 113 Human Surveillance Cytogenetic Studies Conducted Between 1965 and 1984," *Mutation* Research 154:1 11-133, 1985; M. Sorsa and J.W. Yager, "Cytogenetic Surveillance of Occupational Exposures," *Cytogenetics, G. Obe* and A. Basler (eds.) (Berlin, West Germany: Springer Verlag, 1987).

As mentioned previously, chemical agents are more likely to induce SCEs than CAs, which are more likely to be induced by ionizing radiation. A few notable exceptions exist. Workers exposed to vinyl chloride exhibit increased CAs and are at risk for developing hepatic angiosarcoma, a form of liver cancer. Workers exposed to benzene also show elevated CAs and are at increased risk for developing leukemia (7). Steelworkers with a history of coke oven exposure have an increased SCE frequency as well as significantly elevated CAs (8) and are at increased risk of developing lung cancer.

Frequently, both elevated SCE and CA frequencies are demonstrated for a particular genotoxic agent. In approximately 30 percent of studies conducted, however, there is disagreement between these two endpoints for the same chemical, indicating that the fundamental way in which a particular chemical interacts with the DNA to produce SCEs is likely to be different from the mechanism that produces CAs (72).

The conclusions of the 1983 OTA report pertaining to the appropriate use of cytogenetic assays for occupational testing still hold true and are summarized as follows:

- the appropriateness of cytogenetic tests for occupational monitoring needs to be considered on a case-by-case basis for each chemical;
- a monitoring program should be instituted when in vitro and animal tests have proved that the chemical in question is mutagenic or carcinogenic;
- no occupational studies, to date, directly relate cytogenetic abnormalities to increased individual risk for disease; and
- at the present time, cytogenetic monitoring is insufficient to predict health risks for an individual although it may have predictive value for a group.

For industrial practice this implies that when workers are in the vicinity of an established genotoxin, exposures should be reduced to a level that does not affect their chromosome morphology or DNA. In a sequence of cytogenetic studies on vinyl chloride exposed workers, a reduction of CAs was detected when the exposure level to the agent was decreased (l). To date, data on CAs are routinely used by regulatory agencies as contributing information for setting safe exposure standards. In view of the lack of a threshold level where there is zero risk, there is no safe level of exposure. Thus, this should apply to all workers, not just to those determined by some test to be susceptible (39).

It is likely that new populations of workers who have been exposed to significant levels of a genotoxin are yet to be discerned: data are now available for only 500 of the more than 100,000 major environmental and industrial chemicals (39). In the case of major synthetic genotoxins it is likely that improved hygiene measures will be undertaken before a surveillance study is begun, as was the case in vinyl chloride and ethylene oxide, where the greatest cytogenetic damage was observed at the early stages of surveillance before hygienic measures reduced exposure levels for later sample dates (2).

Analysis of Mutagenesis at the Molecular Level

Until recently, most tests for mutagenicity have been merely indicators of exposure, only providing evidence that exposure has occurred. This limitation is being removed as more techniques at the molecular level are being developed, refining the ability to document exposure and, in some cases, providing qualitative information about the nature of the mutation. As the nature of mutation becomes more clearly defined, the connection between mutation and disease will also become better understood. This section describes the more common molecular approaches to analysis of mutagenesis.

HPRT Lymphocyte Selection System

One method used for detecting gene mutations is a T-cell assay that uses the hypoxanthine-guanine phosphoribosyltransferase (HPRT) gene as a mutation indicator, because there is an easy selection method to distinguish those cells in which mutations have inactivated the HPRT gene. Thus, the assay determines the frequency of T-cells carrying the HPRT-inactivating mutations. Mutation frequencies are elevated in people exposed to such mutagens as chemotherapeutic drugs, cigarette smoke, and ionizing radiation (38). In fact, HPRT mutations inhuman T-cells can be detected in atomic bomb survivors 40 years after the presumed mutational event (23). The test is extremely sensitive, permitting study of effects of very low doses of environmental mutagens (40).

At the laboratory investigation level, individual mutant T-cells have been cloned and their HPRT genes analyzed to identify the specific sequence changes they have undergone. As a means to better understand mutagenesis, this approach is useful, but is obviously not practical for populations. A second laboratory approach involves electrophoresis to separate mutated strands of DNA, amplification of the mutated DNA through the polymerase chain reaction (PCR) (see ch. 5), and determination of their specific DNA sequence. This approach has lead to the establishment of an "HPRT Mutational Spectra Repository" that is collecting data on HPRT mutations together with information on types of environmental exposures experienced by the individuals whose cells manifested the mutations (38). The use of this technique requires that an individual's spontaneous mutation rate be determined as well as the rate of changes induced by environmental mutagens.

It is unclear whether HPRT mutations are related both to exposures and a subsequent cancer; HPRT may just be a sentinel event in a pathway not related to a specific cancer (59).

DNA Adducts

One type of DNA alteration involves the binding of exogenous and xenobiotic materials to DNA to form additional products, or adducts (42). Radiolabeling, immunochemical, and physical methods can detect adducts at extremely low concentrations. Adducts can form in many tissues but are not stable since they are easily removed by DNA repair systems. DNA adducts have special significance in view of their potential to force replication or repair errors and thus be chemical progenitors of genetic alterations that can be passed on to offspring (5).

The toxicological significance of adducts is unclear, but they can be used as markers of exposures to specific toxicants. Current evidence suggests an association between the onset of specific types of toxicity (e.g., mutation, cancer, developmental effects) and the concentration of DNA adducts (42). Recent studies have suggested that for DNA adduct formation, it may be more meaningful to relate tumor response to the target organ concentration of DNA adducts than to applied dose (6).

Adducts exist at variable background levels between individuals differing by age, race, sex, and interference factors. Adducts can be measured using blood, semen, urine, buccal mucosa, or skin biopsy specimens (42).

The relationship between DNA adducts and tumor initiation depends heavily on the nature of the chemical exposure. The use of DNA adducts as molecular dosimeters will provide better information about individual differences in absorption, distribution, biotransformation, cell proliferation, and DNA repair and detoxification between high- and low-dose exposures and between tissues (42). Further research must correlate specific toxic effects of specific DNA adducts with the induction of gene mutation or tumor formation before they will be useful beyond dosimetry studies. It may be simplistic to assume that specific adducts will ever be good predictors of tumor formation, since there are so many other intermediary steps and modifying factors between adduct formation and the development of a detectable tumor (63).

Most studies of DNA adducts in humans have been in populations where the exposure was to a ubiquitous compound producing delayed clinical effects, making the cumulative exposure unknown and the identification of unexposed control populations impossible.

Photo credit: American Cancer Society

DNA from lung cancer patients who smoke cigarettes shows a DNA adduct pattern that intensifies with the amount and duration of smoking.

However, certain cohorts reveal reliable dose relationships. Testicular and ovarian cancer patients receiving cisplatin, a platinum-based chemotherapeutic agent, show a dose-response relationship for adduct formation (50). Roofers and foundry workers have tested adduct positive for benzopyrene (25,61).

Lung cancer patients have tested positive for adducts, probably from a variety of hydrocarbons (49). In fact, DNA from cancer patients who smoke cigarettes shows an adduct pattern that intensifies with the amount and duration of smoking. Traces of this adduct pattern can persist for at least 14 years (53). Furthermore, while DNA from heart and lung tissue shows the highest adduct levels, the bladder, kidney, aorta, and liver of longtime smokers showed the same pattern of adducts, indicating widespread damage.

Results in most studies show individual rates of metabolic activation of carcinogens and repair capacities. The same chemical exposure, therefore, can produce wide variability in the numbers of adducts. More baseline data are needed before adducts will be a reliable form of risk assessment. In addition, there is an appreciable amount of background DNA adducts that needs to be more carefully assessed in all individuals.

Most of the assays for detecting adducts resulting from occupational exposures are sufficiently sensitive and will be improved by three methods currently

under investigation: tandem mass spectrometry, 32P-postlabeling, and accelerator mass spectrometry, which provide the additional advantage of detecting low levels of interactive genotoxic agents (63).

In general, protein adducts, as compared to DNA adducts, are stable for the lifetime of the protein and can be used as indicators for recent exposure. They can be found in the hemoglobin of red blood cells and in sperm. They are considered a form of biological monitoring rather than a test of mutagenicity because they allow for direct measurement of the relationship between external exposure and internal dose. The unique features of this approach are the sample size and the ease of obtaining red blood cells. As in other tests, however, there will be considerable differences between chemicals and their effect on adduct formation and data must be collected on each chemical.

Determination of DNA Repair

Determining DNA repair in lymphocytes can indirectly estimate some types of damage to genetic material. DNA repair systems probably arose as evolutionary consequences of DNA damage resulting from ultraviolet radiation and naturally occurring mutagens. The method detects damage susceptible to excision repair, but some other mutagenic lesions may not be detected. DNA repair is an ongoing normal cellular process; monitoring methods detect elevated levels of DNA repair activity. Increased DNA repair activity probably reflects recent exposure to a genotoxic compound.

Sensitivity of the DNA repair assay to detect abnormality from low-dose exposure has not been demonstrated. Since this assay as used routinely cannot determine whether the damage is correctly repaired or not, the biological significance of detectable induced repair cannot be determined (35).

DNA Quantification

Two cytometric methods to measure the DNA content of individual cells could provide a means for identifying workers who are at increased risk in occupational groups exposed to certain carcinogens. Most recently these methods-called simple filter microfluorometry and quantitative fluorescence image analysis-have been applied to groups exposed to bladder carcinogens (27). Collectively the methods are referred to as absolute nuclear fluorescence intensity, or ANFI. The value of ANFI is based on the finding that tumors contain cells with abnormal, elevated amounts of chromosomes, and therefore, DNA. These aneuploid cells may be cancerous or premalignant.

Photo credit: U.S. *Environmental Protection Agency*

Increased chromosomal aberrations have been reported in individuals exposed to phosphine, a common grain fumigant, and a range of pesticides.

In the ANFI technique, cellular DNA obtained from exposed populations is treated with a fluorescent stain. Quantitative spectrofluorometry is then used to detect excess DNA. The intensity of the fluorescence is proportional to the DNA content of the cell. Fluorescence in excess of a standard norm may be a useful diagnostic criterion. In fact, DNA changes have been observed in asymptomatic patients prior to biopsy-confined clinical disease (27).

The real power of the technique could likely be its ability to detect disease in asymptomatic individuals. If tumors can be detected while still noninvasive and nonmetastatic, then screening could become valuable for treatment success (77). In 1981, a study was conducted of 1,385 chemical production workers exposed to aromatic amines, primarily 2-naphthylamine, to assess the predictive value of this technique. Of a cohort of 67 individuals tested positive via ANFI, 33 have been diagnosed histologically positive for bladder cancer (48).

Serum Oncogene Proteins

Oncogenes, or cancer-causing genes, are discussed in greater detail in chapter 5 because of their importance in detecting early stages of cancer. Oncogene detection, however, may become in-

creasingly important in monitoring situations because of the effect of genotoxic agents on the induction of oncogene activity. Oncogenes can be activated by translocations, breaks, and deletions caused by clastogens. The presence of activated oncogenes can be identified by molecular methods such as restriction fragment length polymorphisms (see ch. 5) or by screening of serum for oncogene-related proteins in conjunction with PCR sequencing.

This approach was recently used in a study of workers exposed to polychlorinated biphenyls (PCBs) (9). PCBs are a group of chlorinated aromatic hydrocarbons found in the past in transformer and capacitor fluids, plastics, pump oils, hydraulic systems, printing ink, flame retardants, pesticides, and copy paper. They have well-documented acute and chronic health effects on skin, neurophysiology, and reproduction.

Municipal workers exposed to PCBs in cleaning of a transformer were tested for oncogene-related proteins in their serum. While the connection between exposure to PCBs and elevated serum oncogene proteins was not substantial, the relationship between cigarette smokers exposed to PCBs and elevated proteins was remarkable, indicating a strong effect of smoking on oncogene activity. Serum oncogene protein detection may offer a tool for early diagnosis of cancer.

METHODOLOGIC CONSIDERATIONS

Before a decision can be made on the value of any genetic test, it must be valid and reliable. In considering the application of genetic monitoring to detect job-related illness, the additional criteria of cause and effect between a particular trait (or genetic change) and occupational illness must be evaluated.

The 1983 OTA report presented a full discussion of the concepts of validity, reliability, predictive value, and relative risk (72). Because these fundamental criteria have not altered since that report, basic aspects are only summarized here. Similarly, general criteria for evaluating the acceptability of genetic tests linked to environmental exposure have been discussed elsewhere (41,72). Certain variables such as age, sex, race, and lifestyle will continue to confuse establishing causal linkages between exposures and subsequent disease. If the tests are valid and reliable, establishing procedural safeguards and designing well-conceptualized test protocols can avert erroneous and misleading conclusions. Table 4-4 presents some of the pitfalls encountered in any epidemiology study, whether genetic or not, attempting to identify hazardous agents in the workplace.

Table 4-4-Pitfalls of Classical Epidemiological Studies in Identifying Hazardous Chemicals in the Workplace

Difficulty identifying suitable study populations:
. inadequate size
. unreliability of death or birth medical records
● lack of reliable incidence data
Long latency period in onset of effects (excluding in utero exposure for major anomalies):
● complicates data collection
● prevents detection of effects of new exposures
. requires assessment of current risks based on much earlier exposures
Lack of sensitivity:
● normal incidence of specific diseases can obscure increased rates
. multiple exposures confound attempts to establish cause-effect relationship
. effects of ubiquitous exposure are difficult to detect
● large populations are required to detect common effects
Substantial population exposure to agent prior to detection:
● dilution of exposed population
● failure to consider power of study

SOURCE: M. Legator, University of Texas Medical Branch, Galveston, TX, written communication, August 1990.

Validity, Reliability, and Predictive Value

The validity of genetic testing (i.e., the probability that a test will correctly classify true susceptible and true nonsusceptible individuals) should be evaluated before any test is placed into routine use. Few tests are 100 percent valid because of the influences of variable test performance and genetic and environmental factors. Sensitivity and specificity are the two characteristics subsumed under validity. Sensitivity is the frequency with which the test will be positive when the genotype in question is present. Specificity is the frequency with which the test will be negative when the genotype in question is absent. Sensitivity and specificity are usually inversely related.

In addition to validity, reliability under conditions of routine use must also be demonstrated. That is, tests of the same specimen must repeatedly give the same result whether performed by several different laboratories or by the same laboratory on several occasions.

Predictive value of the test is determined by sensitivity and specificity, as well as the prevalence of the trait or genetic damage in the population. Prevalence is the percentage of the population that is affected with a particular disease at a given time. When the prevalence of a particular traitor genetic damage is low in the population, even a highly specific test will give a relatively large number of false positives because many persons being tested will not have the endpoint.

Procedural Safeguards and Difficulties

In undertaking any testing of exposed populations—whether cytogenetic, biochemical, or DNA-based tests—good sampling and data collection are essential. Individual factors that can affect test results include such confounding variables as sex, age, race, ethnic group, work history, diet, genotype, medication, alcohol and drug consumption, and smoking. These factors play a role in the induction of CAs and must be considered when drawing conclusions about the effects of genotoxic agents. For example, smoking and alcohol consumption have been shown to increase the frequency of CAs and SCEs (30,45). Thus, they must be controlled for any population study.

In all cases, certain precautions should be taken before employing these techniques in wide-scale population monitoring. They are:

- documentation of clastogenicity of the chemical in question in vitro and in vivo;
- determination of the duration and level of exposure;
- establishment of an appropriate matched control population;
- determination of the history and habits of the individuals to be tested (i.e., smoker, medication and drug use, other exposures, nutrition) (12); and
- determination of test variability and sample size requirements to detect a true difference (lo).

The greatest difficulties in monitoring may not be technical but procedural. Eliminating biases, obtaining suitable control groups, and obtaining good records may be the greatest obstacles, made especially difficult with chemical carcinogens because of the long latency period between exposure and resultant malignancy (a problem with retrospective cohort studies). Adequate protocols (enough subjects used and cells scored) must be used to ensure that the results are reliable.

Box 4-C-A Battery Approach To Determining Exposure-Disease Associations

The use of biological markers in a battery of tests over time allows for the resolution of more detail in exposure-disease associations. For instance, instead of waiting to identify a worker who has developed bladder cancer and had been exposed to benzidine, a researcher might: 1) ascertain the worker's predisposition by determining whether he or she is a slow or fast acetylator (see ch. 5); 2) determine early biologic effect by measuring the amount of the H-ras oncogene expression product, p-21 protein in the urine; 3) quantify the degree to which bladder cells are in a premalignant aneuploid state by using quantitative fluorescence image analysis; and 4) evaluate the prognosis of early tumors by measuring the glycosaminoglycans on bladder cell surfaces. Additionally, the current contribution of cigarette smoking, a confounder to the benzidine-bladder cancer association, can be assessed by evaluation of macromolecule adducts to a representative cigarette smoke component such as 4-aminobiphenyl. The implications of this example are that the exposure-disease association can now, in some cases, be resolved into detailed and quantifiable components. This resolution has implications for understanding basic mechanisms and for intervention.

SOURCE: P.A. Schulte, "Methodologic Issues in the Use of Biologic Markers in Epidemiologic Research" .4merican .Journal of *Epidemiology 126(6):1006-1016, 1987.*

The Battery Approach to Genetic Monitoring

The most sensible approach to genetic monitoring, if validated, would be to employ a battery of relevant and sensitive tests, rather than rely on any one test for valid and reliable information. Genetic monitoring is based on epiderniological methods, using the observation of immediate effects such as sperm morphology, urine mutagenicity, and cytogenetics. Immediate effects can be measured in tandem and more long-term health outcomes, such as appearance of neoplasia and reproductive effects, should follow in the study design. It should be borne in mind that the frequencies of immediate effects will always be higher than frequencies of adverse health outcomes (28).

In an ideal study, types and durations of external exposures should be determined as best possible. Mutagenicity assays, such as the Ames or HPRT tests, could be conducted to determine if mutagenic agents were present. Cytogenetic analysis examining overall CA rates and SCE or micronuclei frequencies could be conducted as indicators of mutation. Tests of sperm morphology could be done to estimate potential germline mutations. Molecular studies, such as DNA adduct formation, DNA quantification, or serum oncogene protein detection can serve as direct measures of mutagenicity and toxicity. Combined, these tests can provide a qualitative association between occupational exposure and abnormalities in endpoints. This approach is likely to be extremely costly.

If the tests are conducted in parallel, sensitivity increases while specificity decreases. If they are conducted in sequence, sensitivity decreases while specificity increases. The investigator would have to decide which characteristic was more desirable given the exposure and the circumstances.

The intelligent use of a combination of tests may yield a finer resolution of exposure-disease associations. Box 4-C gives an example of such an approach.

SUMMARY AND CONCLUSIONS

Occupational exposures to certain substances can alter genetic makeup through structural damage to both genes and chromosomes. Genetic damage, regardless of cause, appears as recessive and dominant mutations, large rearrangements of DNA, point mutations, and loss of genetic material, leading to distortions of either the expression or biochemical function of genes. But not all mutations cause disease. In addition, most occupational exposures are likely to cause principally nonheritable damage to somatic cells, rather than germline or heritable damage. The relationship between mutation and health effect is often indirect and not well understood. Cancer, a disease of somatic cells, is the most common class of genetic disease correlated with genotoxic substances.

Until the health effects of exposures are better understood, monitoring can only provide a gross indication that genetic changes have occurred and that adverse health effects could follow. The rationale behind the use of assays of genetic damage stems from historical evidence that events observed initially and at high frequency could be the start of a process that ultimately produces neoplasm in a smaller number of cells.

New molecular assays of mutagenicity, such as HPRT and oncogene protein detection, are providing greater specificity and will augment tests already in use, such as the Ames test. New methods may provide better estimates of the health effects of low doses of some mutagens, as well as providing qualitative data on the nature of mutation. Detecting activated oncogenes and DNA adducts has the potential of predicting disease in asymptomatic individuals. The use of genetic monitoring methods in epidemiologic studies will continue to be plagued by some of the pitfalls associated with classical approaches to determining hazardous exposures in the workplace. The greatest difficulties may not be technical but procedural-eliminating biases, obtaining controls, and keeping good records. Methods for determining types and levels of exposures must be improved, and certain methodological and procedural safeguards should be adhered to. In addition, the employment of more specific and sensitive tests, rather than relying on any one test for valid and reliable results, will lead us closer to understanding the relationships between exposure, mutation, and disease.

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